

V International Oat Conference & VII International Barley Genetics Symposium



Proceedings

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Al Slinkard
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Brian Rossnagel



POSTER SESSIONS Volume 1

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Conference**

&

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POSTER SESSIONS Volume 1

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The co-chairs of the Conference Organizing Committee, Brian Rossnagel and Bryan Harvey, wish to express their sincere appreciation to their colleagues in the Crop Development Centre and the Department of Crop Science & Plant Ecology and other groups from the University of Saskatchewan community who so graciously volunteered and committed their time and effort to making the conference a success:

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The Organizing Committee of the V International Oat Conference and the VII International Barley Genetics Symposium wishes to particularly acknowledge the significant financial contribution from the Quaker Oats Company of Canada for this combined conference. In addition, the moral support and encouragement from Quaker some five years prior to the conference was critical in our original decision to invite the International Oat group to hold the Oat Conference in Saskatoon and to do so in conjunction with the Barley Symposium. The support from Quaker has enabled the local organizing committee to produce a well-rounded program of excellence for conference attendees, a lasting value in the three-volume conference proceedings and all at an affordable cost for all participants. This tremendous support, as well as the Quaker Oats Company's long-term support of our University of Saskatchewan oat research and development effort, is gratefully acknowledged.

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Preface

At the VI International Barley Genetics Symposium, held in Lund, Sweden, in 1991, it was agreed that the next symposium would be held in Canada, at Saskatoon, during 1996. At the IV International Oat Conference, held at Adelaide, Australia, in 1992, Saskatoon was also chosen as the venue for the V International Oat Conference to be held during 1996. With the agreement of the respective international committees, a decision was made to hold the two conferences together from July 29 through August 6, 1996.

The joint conference was organized as a fully integrated meeting, with no concurrent sessions. All oral and poster presentations were scheduled at facilities at the University of Saskatchewan campus in Saskatoon. Keynote and other invited speakers were asked to address various topics of relevance to both crops. Considerable time was set aside for poster presentations, workshops and social activities to allow for maximum one-on-one attendee interaction.

To avoid significant mailing costs, the Local Organizing Committee decided that the joint conference proceedings would be made available to the participants at the time of registration. These joint proceedings are in three volumes. The first volume includes 47 papers submitted by invited speakers. The other two volumes include 279 short papers submitted by those presenting posters. At the time of printing these proceedings, more than 425 persons were registered to attend the conference.

The Local Organizing Committee consisted primarily of staff members from the Crop Development Centre and the Department of Crop Science and Plant Ecology from the University of Saskatchewan, as well as colleagues from other groups from the university community. Significant financial sponsorship for the operation of the conference came from 51 different organizations; 39 from Canada, 7 from the United States and 5 from outside North America.

Support specifically for the development and production of the joint proceedings came from the Quaker Oats Company of Canada Limited and the Brewing and Malting Barley Research Institute. This assistance enabled the Local Organizing Committee to provide each conference registrant with copies of the proceedings as part of their registration package.

— G. J. Scoles & B. G. Rossnagel
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Section 1: Malting and Brewing Quality

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Protein stability in malting barley. N.-O. BERTHOLDSSON, Nilsson-Ehle Laboratory, Svalöf Weibull AB, S-268 81 Svalöv, Sweden.

Introduction. One characteristics of importance for malting quality is grain protein concentration (Pconc), which is controlled by genes to a large extent. It is also influenced by environmental factors such as drought and N nutrition (Coles *et al.* 1991) and some cultivars are more affected than others. Today, knowledge about stability in Pconc is obtained very late or maybe not at all in the normal breeding programme. In this study a screening method is presented by which it is possible to select for stability and low Pconc soon after the genotype is fixed.

Materials. Since many years different malting barley cultivars have been tested in the European Brewery Convention Barley Trials (EBC) with 7-15 sites in each of four regions, Northern, Central, Western and Southern Europe. By use of data from these trials a material of 19 cultivars was selected according to a stability index that was calculated as the mean of protein variances over sites for each region and year and then a new mean was calculated from standardised variances over regions and years. (Fig. 1).

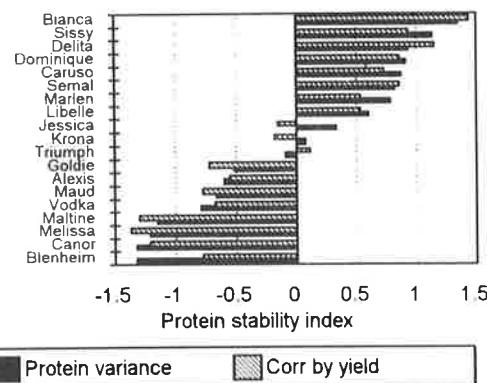


Fig. 1. Protein stability index (corrected and non-corrected for yield variability) calculated as the standardised variance of Pconc data from EBC-trials in Europe 1988-1993. The index is calculated from cultivar means of at least four regions or years. Correction for yield is made by covariance. (Low index = low variance = high stability)

Methods. The selected material and some other Swedish cultivars and lines were sown in randomised Hill-plots with 8 replicates (12 plants/hill within 10 cm diameter, 28 cm distance between plots) in 1994 and 1995. Effects on yield, grain size and Pconc were studied in three nitrogen treatments (low =LN, medium=MN and high=HN) and two drought treatments (early drought=ED and late drought=LD). For the ED treatment a stationary rain shelter was placed above the soil bed from sowing until anthesis. The shelter was thereafter placed over the LD material where it was left until maturity. When needed all, but drought treated plots, were irrigated through a drip water system (Bertholdsson 1995). LD plots were, however, irrigated also once during the drought period. The plots were harvested at time of seed maturity and grain yield, grain weight and Pconc were determined.

Eight cultivars with low and high protein stability were also sown in normal field plots with 3 replicates and different nitrogen regimes in 1994 and 1995. Crop development was followed by light transmission measurements (Ceptometer,

Delta-T Devices) and in 1995 also by light reflectance measurements (MSR 16, CropScan). Leaf area index (LAI) was calculated from the Ceptometer data obtained at different sun angles (Lang 1987). From the CropScan data a green active leaf area was calculated as the reflectance at 813 nm/661 nm (Petersen 1989). At the time of heading and at maturity an area of 0.25 m² was cut by hand for determination of total biomass and N conc. in straw and grains. The remains were machine harvested.

Results. Both in 1994 and in 1995 much of the variance of the calculated protein stability index could be explained by the variance in Pconc from 4 of the 5 treatments (Fig.2). Inclusion of the LN treatment gave a lower correlation coefficient (r). The Pconc. is corrected for different yields by covariance. R was lower in 1995 ($r=0.62$), mainly because of two cultivars that showed a different variance this year

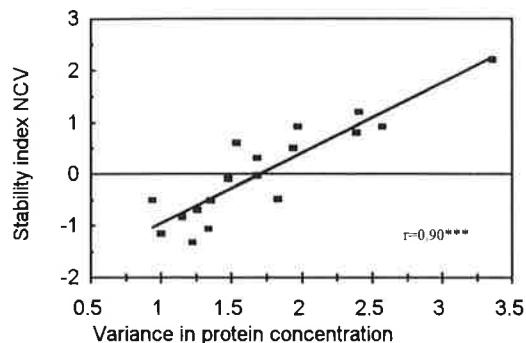


Fig.2. Correlation between protein stability index calculated from EBC-trials in Northern, Central and Western Europe and variance in Pconc from soil-bed studies with medium and high nitrogen and drought before and after anthesis in 1994.. SEE=0.5.

R was somewhat lower ($r=0.87$) if the variance was calculated without correction for yields or if the Southern region was included in the calculations ($r=0.77$). If ED or LD were excluded instead of LN, r were 0.45 and 0.72, respectively, and if MN or HN were excluded r were 0.81 and 0.77, respectively. Hence this indicates that the drought treatments are essential when testing for protein stability.

Along with the reference material some Swedish cultivars and breeding lines were also tested (Fig.3). Of the new breeding material in 1994 SW8588 showed low and stable Pconc. SW8487 was also stable but too high in protein. In 1995 some other lines were tested, but most of them proved to be very unstable

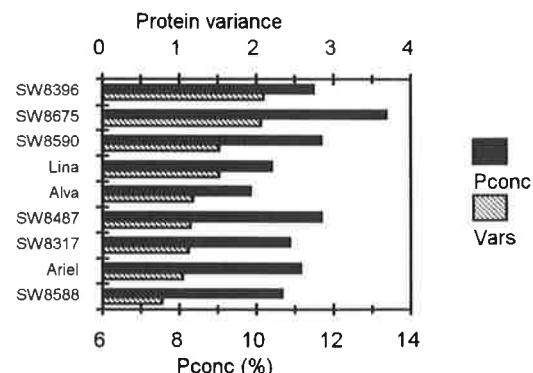


Fig.3. Protein and protein variance of some Swedish breeding material

Preliminary results from the field study indicate that stable cultivars show a prolonged vegetative growth with more biomass and nitrogen uptake before heading and higher harvest index and nitrogen harvest index than the more instable cultivars. They also mature later. LAI and reflectance index, i.e. the active leaf area, mainly differ during grain filling. (Fig. 4).

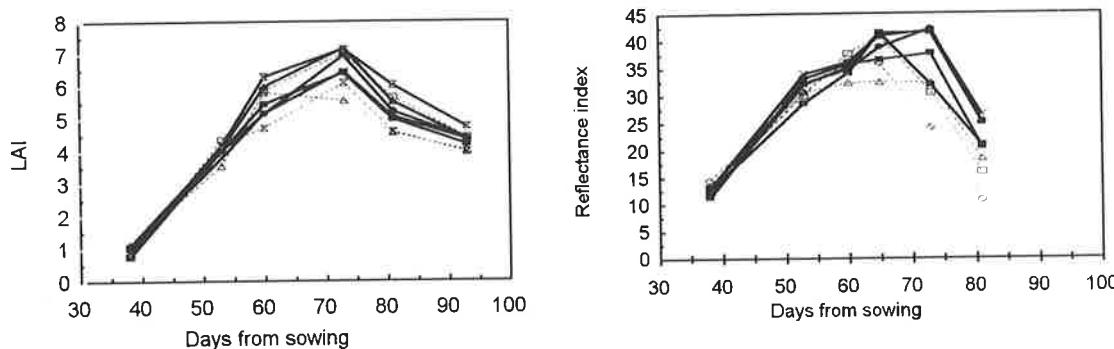


Fig.4. LAI and reflectance index (813 nm/661nm) of 8 barley cultivars in 1995. LAI was calculated from light transmission data and reflectance index from MSR-data. The three cultivars with dotted lines are found to be instable in Pconc in European field trials. Day of heading occurred between day 60 and 65 from sowing. The instable cultivar were 2-5 days earlier than the stable.

Discussion. This study shows that it is possible to mimic the variation in Pconc observed in different environments through in a single trial with Hill-plots subjected to two drought treatments and two nitrogen regimes. The drought treatments must be included and this is furthermore stressed by the reflectance index that was shown to be lower in instable cultivars during grain-filling. The reflectance index is very sensitive to stress and the weather was unusually very dry and warm during this period in 1995. It should be noted that the weather in 1994 and 1995 was unusually warm during grain filling which may have been favourable for the test method. Therefore the soil-bed studies will be repeated during 1996. Anyhow, earlier studies have shown that the year x genotype interaction is much lower in the soil-bed set up than in the field (Bertholdsson 1995). So far the results are promising and the method might open a possibility to assess for protein stability in a single test. Studies are now carried out to see if also variations in e.g. grain size and the content of β -glucan can be assessed in these tests.

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THE RELATIONSHIPS BETWEEN BARLEY ENDOSPERM TEXTURE, PROTEIN COMPOSITION AND MALTING QUALITIES.

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ABSTRACT.

Barley isogenic lines varying in the presence of B and D hordein alleles were examined in relation to structural and biochemical differences. No correlation could be established between the presence or absence of B Hordein alleles to nitrogen, gel protein content, or the potential malt quality of the grain. The presence of D Hordein was positively correlated with increases in gel-protein levels. Textural differences were also associated with malt quality as measured by micromalt analysis, but were not correlated with variation in the hordein composition of the grain.

INTRODUCTION.

Hordein, the major storage protein of barley grains, consists of four groups of polypeptides called B, C, D and γ -hordeins¹. Differences in the amount, properties, composition and distribution of hordein have been related to variation in malting performance. In particular, D and B hordeins are major components of gel protein, and negative correlations between the amounts of D hordein and gel protein and malting quality have been reported^{2,3}. Interactions between gel proteins and starch or cell wall components may also be important⁴.

MATERIALS AND METHOD.

Micromalt analysis was conducted on grains after 96hrs germination according to Institute of Brewing and European Brewing Convention recommended methods at PBI-Cambridge. Microscopical examination of the material was conducted using Calcofluor as a stain for cell wall degradation. Scanning electron microscopy (SEM) of samples were conducted on grain fractured under liquid N₂ and examined using a Joel JSM848.

RESULTS AND DISCUSSION.

Isogenic lines exhibiting differences in the presence or absence of D-hordein and B-hordein composition (Table 1), were assessed for protein distribution and endosperm structure at maturity, and post micro-malting. A positive correlation was established between gel protein levels and the presence of D-hordeins (Table 1), supporting previous observations that D hordein is an important component of gel proteins³⁻⁴.

No correlation was seen between variation in hordein composition and either the milling energy or the total protein content of the grain. Scanning electron microscopy (SEM) of the lines showed variation in textural characteristics, with some exhibiting soft endosperm characteristics (e.g. Fig. 1a; line 45: note the relatively clean starch granules (S) with little protein (P) associated with the granule surface), while others exhibited hard endosperms (e.g. Fig 1b; line 9: note the high level of starch-associated proteins). Again, no correlation could be established between the presence or absence of B- and D-hordeins and grain textural characteristics (as observed by SEM). This implies that the

differences in starch-associated proteins, which are important in determining textural properties, are independent of hordeins composition.

Table 1: Variations in protein levels in the isogenic lines examined.

Isogenic line	B hordein allele	D hordein	Gel Protein (g/g)	Milling energy (mg)	Texture
9	A	-	0.06	751.2	Hard
10	A	+	0.37	755.3	Hard
13	A	-	0.03	733.4	Medium
14	A	+	0.35	771.9	Medium
15	B	-	0.07	710.3	Soft
16	B	+	0.43	772.4	Soft
19	D	-	0.36	785.1	Soft
20	D	+	0.61	769.0	Soft
43	B	-	0.21	680.8	Soft
44	B	+	0.53	698.9	Soft
45	B	-	0.34	761.0	Soft
46	B	+	0.66	656.8	Medium

Micromalt analysis of the samples showed no clear correlation between malting characteristics and hordein composition, but a general association between high HWE levels and the presence of the B allele of B hordein was observed (Table 2). Differences in wort viscosity, wort β -glucan and soluble nitrogen ratio (SNR) levels were not correlated with variation in hordein composition.

Table 2: Micromalt analysis of the isogenic lines examined.

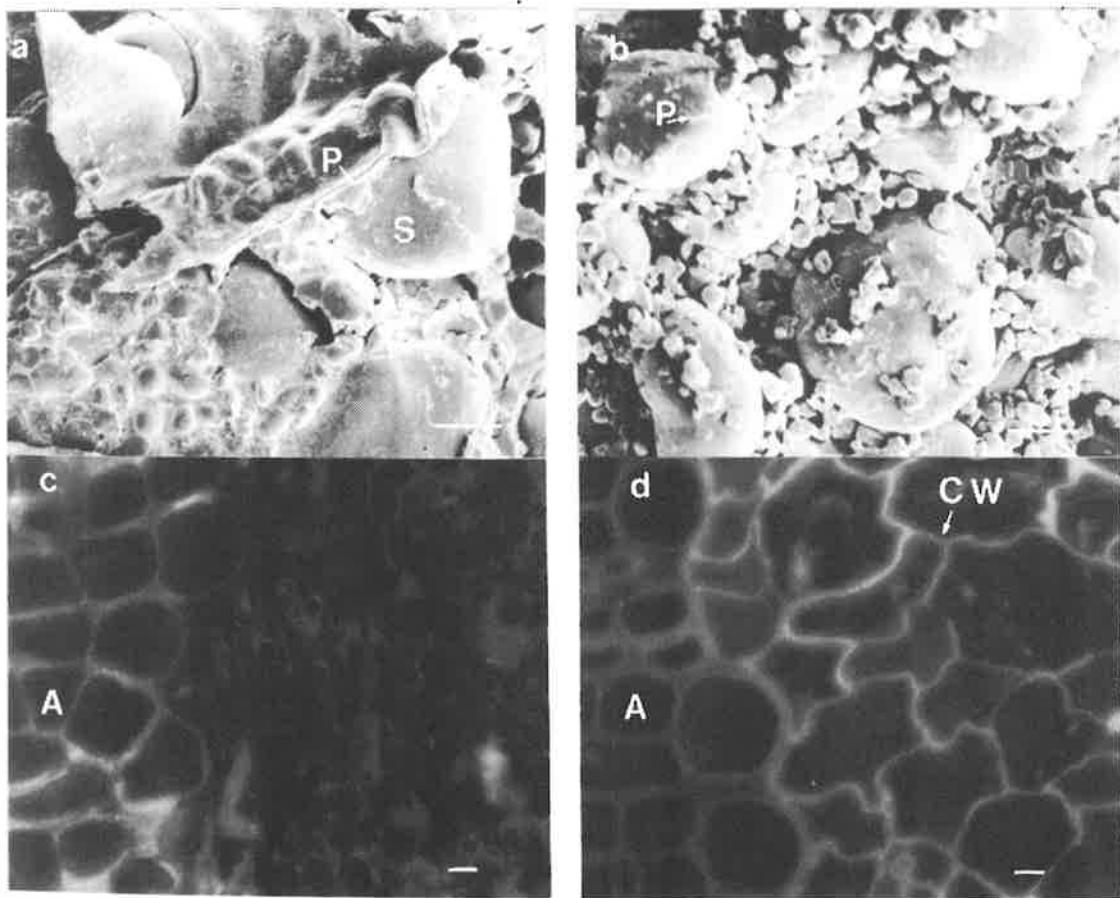
Isogenic line	Hot Water Extract (L°/Kg)	Wort Viscosity	Wort β -glucan (mg/L)	SNR
Check malt	321	1.38	-	37.3
9	297	2.30	567	20.8
10	297	1.99	573	24.8
13	299	1.89	309	27.3
14	299	1.83	294	26.3
15	303	1.69	276	28.3
16	300	2.14	432	25.5
19	294	1.97	414	24.8
20	295	1.72	432	29.9
43	307	1.70	246	28.6
44	301	1.63	262	27.3
45	313	2.10	348	27.5
46	305	1.68	594	24.9

However, differences in endosperm texture were correlated with the variation in malting performance of the samples. Lines exhibiting soft endosperms showed substantial modification during malting (e.g. line 45, Fig. 1c), whereas little modification was observed in the lines with hard endosperm characteristics (e.g. line 9, Fig. 1d).

CONCLUSION.

Our results support the previously demonstrated correlation between gel-protein content and D hordein amount but suggest that correlations previously recorded between barley malting quality and hordein composition may need re-evaluation. The lack of correlation between hordein composition, milling energy and the textural properties of the grain suggests that other protein groups (possibly starch associated) contribute to variations in barley texture. Differences in the starch-protein interactions within the grain appear to be separate from gel-protein levels but are associated with variation in endosperm texture and final malt potential of the grain.

Figure 1: **a** and **b** SEM images of mature grains (isogenic lines 9 and 45 respectively). Note the increased level of starch associated proteins (P) in line 9 and the clean starches (S) of line 45. **c** and **d**. Calcofluor staining of sections of malted grains (lines 45 and 9 respectively) showing retention of cell wall integrity (CW) in line 9. A = Aleurone layer. Scale bar = 10 μ m.



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PRELIMINARY CHARACTERISATION OF TWO FORMS OF BARLEY β -AMYLASE

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INTRODUCTION.

Barley β -amylase (α -1,4-glucan maltohydrolase; EC 3.2.1.2) catalyses the liberation of β -maltose from the non-reducing ends of starch and related 1,4- α -glucans. The complete degradation of starch requires the action of α -amylase, β -amylase, limit dextrinase and α -glucosidase, and the combined action of these enzymes is described as diastatic power (DP). In studies examining the relationships between DP and its component enzymes, only β -amylase is positively correlated with diastatic power (For review see Evans *et al* 1995). This supports the view that β -amylase may be rate limiting in the breakdown of starch during malting and mashing (Bamforth & Quain 1989).

β -amylase is synthesised during grain development as a 59.6kDa protein (Yoshigi *et al* 1994). Germination results in carboxypeptidase action removing up to 4 kDa from the C-terminus of the protein to leave the 56 kDa malt form of β -amylase (Lundgard & Svensson 1987).

Two distinct electrophoretic forms of β -amylase have been distinguished on the basis of charge, and designated Sd1 (acidic form) and Sd2 (basic form) (Allison 1973). The characteristic acidic shift of the Sd1 form is evident in both the mature barley grain (Allison and Ellis 1973) and the proteolytically cleaved malt form of β -amylase (Allison 1973). This variation has been mapped to the *Bmy1* locus on chromosome 4H, demonstrating that this represents allelic variation (C. Li, personal communication). A survey of 105 barley varieties using IEF suggests that cultivated barley is restricted to these two β -amylase alleles (Eglinton *et al* 1995).

β -amylase sequence data has been determined from cDNA clones and limited protein sequencing of CNBr fragments (Kreis *et al* 1987, Yoshigi *et al* 1994). However the barley varieties used for these studies (Hiprolly and Haruna) are both of the Sd2 β -amylase type. The primary aim of this study is to characterise the differences between the Sd1 and Sd2 forms of the β -amylase enzyme.

MATERIALS AND METHODS.

Isoelectric focusing (IEF) was performed on an LKB electrophoresis system using a nonlinear gradient of pH 4.5-8 as previously described (Eglinton *et al* 1995). β -amylase banding was detected using starch staining (Sargent and Walker 1978) or Western blotting probed with a specific anti- β -amylase polyclonal antibody (Evans *et al* 1995).

Protein purification was performed essentially as previously described (Lundgard & Svensson 1987). Sd2 seed and malt β -amylase were purified from cv. Schooner, and cv. Franklin was the source of Sd1 seed and malt β -amylase.

Reversed Phase-HPLC was conducted on a Hewlett-Packard 1090 instrument fitted with a diode array detector and a 4.6mm C-18 column (Vydac). Solvent A was 0.05% trifluoroacetic acid (TFA), solvent B was 0.045% TFA in acetonitrile, and the flow rate was maintained at 0.6ml min⁻¹. Before N-terminal sequencing, β -amylase was further purified on a gradient of 2.5%

B min⁻¹. β -amylase digests were separated on the same system using a gradient of 0.6% B min⁻¹ and fractions collected manually.

Prior to enzymic digestion, β -amylase was reduced and alkylated with iodoacetic acid. Buffer exchanges were made with 10K micro-concentrators (Filtron). Digestion was performed in 25mM TrisHCl, 4.5M urea, pH 7.0 for 18 hours at 25°C with Lys-C (Promega) at an enzyme to substrate ratio of 1:50. Digestion was stopped by adding 0.1% TFA to reduce the pH to 4.

Amino acid analysis was performed on a Hewlett-Packard 1090 HPLC with reference to internal standards as described in the HP AminoQuant Series II operators manual. N-terminal protein sequencing was performed on a Hewlett-Packard G1005A protein sequencer using the HP 3.0 sequencing routine, based on Edman chemistry.

RESULTS.

Crude extracts of β -amylase exhibit significant charge heterogeneity when subjected to IEF (Figure 1). The four purified β -amylase enzymes migrate as single bands on SDS-PAGE and elute as single peaks on RP-HPLC. They also exhibit the same charge heterogeneity as seen in their respective crude extracts (data not shown).

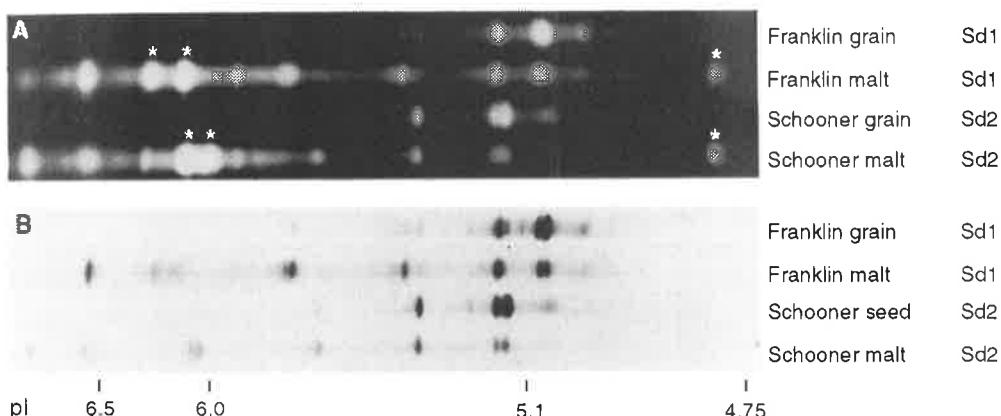


Figure 1A: IEF gel of crude extracts from grain and green malt stained for β -amylase activity. Alpha-amylase bands were identified by thermal inactivation studies and are denoted by an *. B: Western blot of an IEF gel probed with specific anti- β -amylase polyclonal antibodies.

Protein sequencing of the Sd1 and Sd2 seed forms of β -amylase revealed that the enzyme is N-terminally blocked to Edman degradation. Analysis of the two malt forms of β -amylase revealed the absence of the first two amino acids (methionine and glutamic acid) allowing the protein to be sequenced. The Sd1 malt β -amylase was sequenced to 105 amino acids without deviation from the deduced Sd2 sequence.

Analysis of Sd1 and Sd2 malt β -amylase digested with Lys-C by RP-HPLC revealed the high level of homology between the two forms of the enzyme, with chromatograms of the two digests almost identical. Three peaks unique to the Sd1 digest were sequenced and each contained a single amino acid substitution. The three sequence changes were: Glu-165, Val-257, Ala-430 (Franklin) compared to Asp-165, Gly-257, Val-430 (Haruna and Hiprol).

A further two peptides from the Sd1 digest contained a non-standard amino acid at residues 270 and 433. The corresponding amino acids in the Sd2 sequence are both lysine residues, which are cleavage sites for the Lys-C enzyme. The peptides were longer than expected, consisting of two adjacent Lys-C fragments, because cleavage did not

occur at positions 270 and 433. During protein sequencing the retention time of the PTH derivative of the non-standard amino acid was consistent with mono- or dimethyl lysine (Crankshaw and Grant 1993). Amino acid analysis of the four purified β -amylases correlate well with the amino acid composition deduced from the cDNA clones, except the lysine values which range from 62% in Franklin malt β -amylase, to 81% of the expected value in Schooner seed β -amylase. The method used for amino acid analysis is unable to detect methylated lysine.

DISCUSSION.

The Sd1 and Sd2 seed β -amylases were resistant to Edman degradation, confirming a previous report that the Sd2 form is N-terminally blocked (Yoshigi *et al* 1994). The loss of the two N-terminal amino acid residues in the malt forms of β -amylase suggests the enzyme is subject to aminopeptidase activity during germination, in addition to the carboxypeptidase processing discussed previously (Lundgard & Svensson 1987). The removal of a glutamic acid residue during germination is consistent with the characteristic basic shift observed after the conversion of the seed form to the malt form of β -amylase.

Peptide mapping and protein sequencing of the Sd1 and Sd2 β -amylases revealed three amino acid substitutions with respect to the published Sd2 sequences. These substitutions are essentially conservative, and do not alter the net charge of the Sd1 enzyme. Therefore the amino acid changes do not explain the observed difference in pI between the Sd1 and Sd2 forms of β -amylase.

A non-standard amino acid has been identified in the Sd1 β -amylase enzyme at positions 270 and 433 which correspond to lysine residues in the Sd2 cDNA clones. The retention time of the PTH derivative of the non-standard amino acid was consistent with methyl lysine, however mass spectrometry or NMR is required to confirm the identity of this residue. Supporting evidence for the putative assignment of modified lysine comes from amino acid analysis which yielded significantly lower than expected values for lysine. The presence of methyl lysine in β -amylase may also affect the pI of the protein.

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Spring or winter barley genotypes for high malting quality? P. FACCIOLO, G. DELOGU and A.M. STANCA, Experimental Institute for Cereal Research, Section of Fiorenzuola d'Arda, I-29017, Via S.Protaso, 302, Fiorenzuola d'Arda (PC), Italy

Introduction. Barley is, and has been since the dawn of civilization, the cereal par excellence in the production of malt for beer and other alcoholic beverages. The transformation of barley into malt and of the latter into beer is a process that is markedly dependent on the initial raw material, i.e. on the barley grown that gives rise to the malt. Malting quality depends on a host of several organic factors: the analytical data of a single character are insufficient to judge the grain quality initially or malt quality thereafter (Cattivelli et al., 1994; Lewis and Young, 1995). The breeding activity on spring barley has been intense in Europe and good dual purpose varieties, possessing excellent malting quality combined with high yield potential, have been released (Sage, 1989). In contrast to spring barley, the dual purpose winter types are very rare and winter malting barleys do not yield as much grain per unit of area as do barleys for animal feed.

This fact can be attributed to: the breeding work which has received less attention than spring barley from breeders. So barley breeders are interested to develop new high yielding winter malting varieties to compete with the yield of feed varieties. Another point of debate is the belief that spring malting varieties give higher standard of malting quality than winter types.

On the basis of these considerations winter and spring malting varieties in autumn and spring sowing have been evaluated in different environments with the final aim to assess whether or not the two barley types in winter or spring sowing change their quality performance.

Materials and Methods. Six winter (Magie, Marinka, Kaskade, Timura, Torrent and Tipper) and nine spring (Alexis, Atem, Aura, Carina, Gimpel, Gitane, Porthos, Triumph and Gavotte) malting barley varieties have been evaluated in replicated field trials in autumn and spring sowing for two years.

The field experiments have been carried out at three Italian locations with three replications and plot size of 6 m². The plots were mechanically planted and harvested by using traditional experimental equipments.

Standard agronomic practices have been applied. Grain yield (t/ha) has been determined and a grain sample (500 g) of each plot has been stored in cold chamber. In the winter, after harvesting, the grain samples have been used for laboratory analyses as well as for malt and wort preparations. The following determinations have been done on the grain: sieving fraction (%), 1000 kw (g), protein content (%), falling time (sec).

20 g of grain have been micromalted and malt was ground. 10 g of ground malt have been used to produce wort from which refractometer reading (ZEISS's scale: H₂O = 14) and hot water extract (H.W.E.) were determined.

These determinations have been done following the procedures developed by Gothard et al. (1980) at the Plant Breeding Institute (Cambridge, UK). The data of each trait have been statistically analyzed by using a factorial method.

Results and Discussion. Grain yield of winter malting varieties has been of the same amount of the spring malting varieties grown in high (Fiorenzuola) and low (Udine and Grosseto) fertile locations either in autumn or spring sowing (Fig. 1a). The mean grain

yield of the winter varieties or of the best of them (Magie, 7.4 t/ha) in autumn sowing has been lower of 15% than that of the feeding two rowed barley (Arda, 8.5 t/ha) grown at the same locations. The values of 1000 Kw were higher in winter barleys than in spring ones. No winter varieties in spring sowing for this trait performed better than in autumn sowing (Fig. 1b). Also the sieving fraction showed no differences between spring and autumn sowing with high values of winter varieties (Fig. 1c and d). The protein content of winter varieties varied from 16% of Kaskade and Timura in spring sowing at Grosseto, to 9.6 of Tipper at Fiorenzuola in spring sowing. The spring varieties showed values between 10.1% at Udine in autumn sowing and 13.4% at Grosseto in spring sowing (Fig. 1e). Because this trait is considered one of the most important quality trait it can be evidenced that spring or autumn sowing either with winter or spring varieties did not strongly affect the protein content. Moreover the highest value has been reached at Grosseto with winter varieties in spring sowing.

The winter barley in spring sowing increases the value of falling time indicating that in this environmental conditions all varieties accumulate more β -glucan (Fig. 1f). On the other hand the spring varieties gave the same result in both sowing time with the highest value in Fiorenzuola and the lowest in Udine.

The refractometer reading of spring genotypes was of the same amount in both sowing time with the highest value in Udine (43.03) and with the varieties Alexis (43.3) and Gimpel (43.5). By considering the winter genotypes in autumn sowing the value was of the same amount of the spring varieties in Udine and Grosseto while in spring sowing the same genotypes in the same locations gave the lowest values. Again the best result was reached in Udine (42.3) and the lowest in Grosseto in spring sowing (39.9) (Fig. 1g). Among varieties, Kaskade showed the highest value (43.0). The percentage of hot water extract (Fig. 1h) reflects the trend of the refractometer reading.

The results here reported indicate that: a) winter malting varieties show an yield potential of the same amount of the spring varieties in autumn sowing; b) their yield is lower than feed varieties of 15% and 20% when compared with two rowed or six rowed field barley respectively; c) the poorest malting quality has been shown by winter types in spring sowing particularly in low yield environment. However because high quality standard have been obtained in autumn and spring sowing it can be concluded that much more attention necessitates in winter breeding programme for malting quality to develop new high competitive winter dual purpose genotypes.

Acknowledgements

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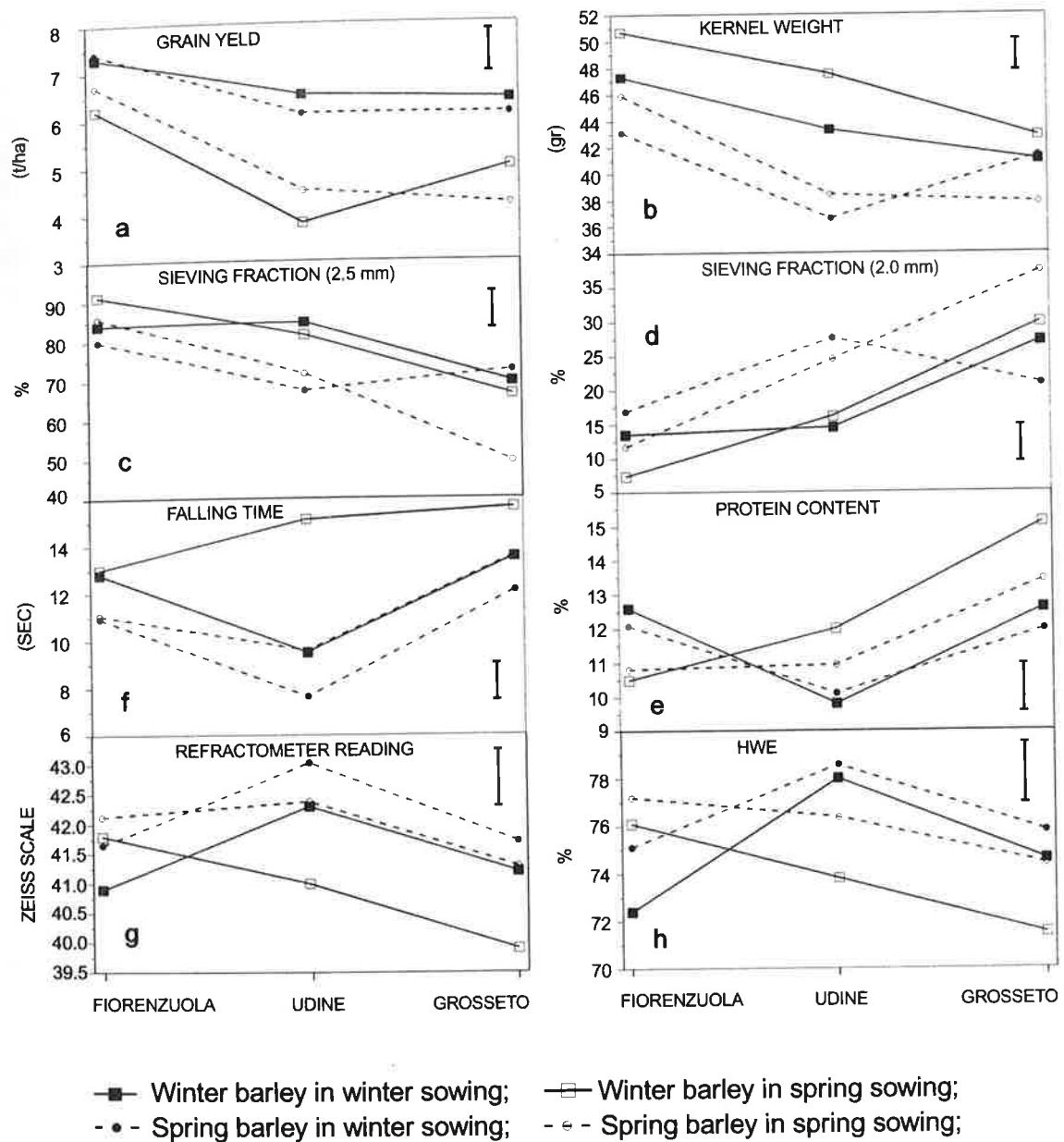


Fig. 1. Grain yield and malting quality traits of winter and spring barley varieties in autumn and spring sowing grown at three different locations. Bars indicate LSD (0.05)

Non Starch Polysaccharides in Malting and Brewing : Arabinoxylans.

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Introduction. Arabinoxylans (AX) compose 4-8% of the kernel and are major constituents of barley aleurone and endosperm cell walls. AX contain a backbone structure of D-xylopyranose residues linked by β -(1 \rightarrow 4)-glycosidic bonds with units such as L-arabinofuranose attached as branches by β -(1 \rightarrow 2)- or β -(1 \rightarrow 3)-linkages. They are partially water soluble, high molecular weight polymers which contribute to viscosity. Both AX and β -glucan content are significantly related to beer viscosity; AX may produce undesirable effects normally attributed to the β -glucans in brewing process.

Two malting barley cultivars were studied to determine the composition of total and insoluble non starch polysaccharide (NSP) fractions and to observe changes occurring during commercial malting and pilot-brewing. This involved determining the AX levels of selected commercial six-rowed malting barley samples, identifying the profile of glycosyl-linkage from the AX of barley, malt and pilot-brewed beer, and, finally determining, quantitatively, the methylated NSP derivatives in the samples.

Materials and Methods. Two barley and two commercially malted samples of the cultivars Robust and Excel were used in this study. Barley was from the 1992 crop year. These cultivars had been part of a blend which was believed to cause sterilized filtration problems during commercial brewing. Barley husk was separated from the grain by air-abrasion. Dehusked samples were ground on a Udy cyclone mill. A modification of solvent washing method of Viëtor et al (4) was used for removal of remaining husk particles. Washed samples were air dried.

Pilot Brewing: The malt samples were ground, mashed, lautered, and boiled in tandem at the North Dakota State University pilot brewery. Beer was filtered through a micromembrane filter, and analyzed for alcohol content and real extract according to the ASBC methods (1). Beer samples were also degassed, distilled, and freeze-dried.

β -Glucan determination: β -Glucan content of barley, malt, and freeze-dried beer was determined using a Megazyme β -glucan assay kit (Warriewood, Australia).

Fractionation of NSP: The glycosyl composition and AX content of barley, malt, and beer were determined using a modification of the method of Carpita and Shea (2). The barley and malt flours were treated with α -amylase, pullulanase, and protease followed by precipitation of Total-NSP fractions with ethanol. For insoluble NSP (IS-NSP) fraction, phosphate buffer extraction replaced ethanol precipitation.

Glycosyl Composition and Linkage Analysis: The acetylated alditol derivatives of Total-NSP and IS-NSP samples were prepared using the method of Han and Schwarz (3) and analyzed on a GC. Normalized % of arabinose and xylose were obtained. Arabinoxylan content was calculated as follows: Total Arabinoxylan = (% arabinose + % xylose) \times 0.88. Glycosyl-linkages were determined using the per-O-methylated method of Carpita and Shea (2). The partially-O-methylated, partially-O-acetylated alditoles (PMAA) derivatives was analyzed using GC-MS for identification and analyzed on the GC with a flame ionization detector for quantification. The quantity of each methylated sugar derivatives was expressed as the mole percentage (mol %).

Results and Discussion. Table I shows the composition of NSP fractions from barley, malt, and beer. Total-NSP minus IS-NSP (others) includes mainly soluble NSP and minor components such as ferulic acid and starch fractions, etc. Total-NSP of Robust decreased significantly ($p < 0.05$) by 71% after malting, compared to 64% in Excel. IS-NSP was also significantly decreased ($p < 0.05$) by 85% for Excel and 92% for Robust.

Table I. Composition of NSP fractions in barley, malt and beer.

Fractions	Robust			Excel		
	Barley	Malt	Beer	Barley	Malt	Beer
Total-NSP	18.20	5.35	1.13	17.25	6.15	1.75
Arabinoxylan	41.00	59.30	0.30	41.31	59.74	0.19
A-X Ratio	0.64	0.69	0.70	0.66	0.76	0.72
IS-NSP	10.65	0.85		11.05	1.65	
Arabinoxylan	53.30	76.02		54.71	75.92	
A-X ratio	0.71	0.56		0.72	0.59	
Others	7.55	4.50		6.20	4.50	
β -glucan	3.99	0.50	0.06	3.92	0.54	0.03

The main constituent sugars of Total-NSP and IS-NSP fractions were glucose, xylose and arabinose. In barley, on average arabinose and xylose accounted for 48% of the composite sugars of Total-NSP. The IS-NSP fraction of barley contained 64% arabinose and xylose. This indicates that, for barley, a large portion of the AX were insoluble (4). The ratio of arabinose/xylose also decreased except in Total-NSP of Robust and Excel flours. The AX content of Total-NSP and IS-NSP increased after malting. Robust malt contained significantly lower ($p < 0.05$) AX in the Total-NSP and IS-NSP fractions after malting.

In beer, the alcohol and real extract were 4.65% (w/w) and 5.075 °P, and 4.14% and 4.606 °P for Robust and Excel, respectively. Total-NSP of all samples decreased dramatically during pilot brewing. The neutral sugar composition in Total-NSP of beer was similar to the composition in Total-NSP of malt. The ratios of arabinosyl to xylosyl residues (0.70 - 0.72) were similar to values obtained by Viëtor et al (4).

There was no statistically significant difference between total AX levels in the barley, malt, and those present in beer. AX are partly degraded during malting. Between varieties, Robust displayed significantly higher ($p < 0.05$) levels of AX in beer, although levels in Excel were similar. β -glucan is, at least in part, responsible for increasing viscosity because of its swelling property. Robust barley had the higher β -glucan content than Excel; the level present in beer after malting, pilot brewing and filtration were also significantly higher ($p < 0.05$).

Glycosyl-linkage: Four major peaks were identified in all barley, malt and beer samples (Table II). These peaks were the methylated glycitol per acetates of t-Glu-p, 4-Xyl-p, 4-Glu-p, and 2,3,4-Xyl-p. A possible structure maybe predicted in which arabinoxylans contain highly-branched regions, mostly consisting of an unsubstituted and a double arabinofuranosylated xylose residue. The highly-branched regions are enriched in both

O-2,3 disubstituted, as well as O-2 and O-3 monosubstituted, xylose residues.

The length of (1→4)- β -xylan in Robust was longer than in Excel. However, the number of xylose branches substituted were similar in the two varieties; twice as many xylose were substituted in C-2 and C-3 positions as were single substituted in these positions. At least one branch of arabinose was ester linked with other compound, possibly ferulic acid, through its C-3 position. Long (1→4)- β -xylan chains in malt were predicted from the ratio of mol %. The configuration of each malt was similar to that of corresponding barley samples.

Table II. Glycosyl-linkage analysis of PMAA (Mol %).

Deduced Linkages	Robust			Excel		
	Barley	Malt	Beer	Barley	Malt	Beer
t-Ara-f	1.46	1.53	1.13	1.24	1.38	1.75
3-Ara-f	T	1.50	0.50	5.76	2.60	3.96
4-Ara-p	2.14	1.60	1.54	2.04	1.52	3.56
t-Xyl-p	1.63	2.12	0.51	1.68	0.78	1.25
2-Xyl-p	7.30	7.20	3.99	5.41	6.49	3.92
4-Xyl-p	21.90	21.60	11.97	16.24	19.46	11.76
2,4-Xyl-p	4.91	5.13	2.90	4.68	5.13	2.82
3,4-Xyl-p	4.91	5.13	2.90	4.68	5.13	2.82
2,3,4-Xyl-p	19.02	17.00	9.01	11.69	15.96	8.25
t-Gal-p	1.68	T	T	2.99	1.58	1.70
2-Gal-p	2.55	2.07	1.52	3.38	2.13	2.07
4-Gal-p	4.10	1.94	0.82	3.14	1.48	0.53
t-Glu-p	12.92	13.73	23.03	22.60	19.05	20.39
4-Glu-p	12.30	17.72	37.75	17.84	16.22	32.91
6-Glu-p	3.17	1.74	2.44	0.99	1.87	2.31

Conclusions. AX in barley, malt and beer may contribute to wort filtration problems due to its high viscosity. The NSP fractions significantly ($P < 0.05$) decreased during malting and brewing. Arabinose and xylose, at a ratio of 0.70 - 0.72, accounted for 70% of Total-NSP in pilot-brewed beer. This highly substituted AX alone or synergistically with β -glucan could result in lautering and/or filtering problems during brewing. As such, their potential impact on various stages of brewing process could be considerable.

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The use of cross evaluation trials and cross prediction for malting quality

improvement. R.C.M. LANCE¹, L.C. MACLEOD³, S.J. LOGUE², and A.R. BARR²

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Introduction. Malting quality improvement requires a large commitment of resources; both in terms of time and financially. Decisions are even more critical if crosses are to be used for anther culture derived doubled-haploids, molecular mapping research, and/or molecular marker assisted selection programs. Gains in efficiency can be achieved if more rational and objective approaches are considered in the choices of parental material and their respective cross combinations. Cross evaluation trials have been used to evaluate the genetic contribution of parents in crosses for improving malting quality and to predict the performance of new cross combinations. The trials can be either i) randomly derived F₃ progeny populations from which estimates of genetic means and variances can be made or ii) bulk populations which give an estimates of genetic means only. Only the latter experiment is described here in more detail. The results obtained enable decisions to be made on i) which crosses to keep, ii) the population size required, iii) which crosses to choose when multiple traits are to be selected simultaneously, iv) which new cross combinations should be made.

Materials and Methods. An F₄ bulk population (Expt. 15/93) was grown at the Charlick Experiment Station near Strathalbyn, South Australia in 1993. The trial design consisted of 61 crosses (involving ~ 22 parents) and 9 controls sown as a randomised block with 3 replicates. Harvested barley samples were cleaned, and micromalted in a Phoenix Systems Micromalting System facility (Sparrow et al., 1987) and malts were analysed for; % Protein, % Extract, and Diastatic Power. (MacLeod et al., 1991; Lance and MacLeod, 1993)

Results and Discussion. The contribution of an individual parent to the mean performance of the progeny of an individual cross can be estimated by assigning appropriate parental coefficients to components of specific crosses reflecting the proportion of the parental contribution to a cross. A matrix of these coefficients is collated, the malting quality parameters measured and then the resulting equation is solved by utilising multiple linear regression techniques. The results are illustrated for the F₄ bulk population: Expt. 15/93 (Table 1). Parents such as ; Haruna nijo, Rubin, Natasha and Franklin were found to contribute to high levels of % Extract, whereas Chebec, WI-2738, Galleon and WI-2692 gave poor results. Haruna nijo, Natasha and Harrington engendered high levels of Diastatic Power to their progeny.

The prediction of the mean performance of a new cross can be determined with the following example by substituting estimates for the intercepts, the % protein level (10%) and parental contributions : eg ((A*B)*B) for Diastatic Power

$$Y_{DP} = \text{intercept}_{DP} + X_{Pr,DP} * (\% Pr) + X_A * (0.25) + X_B * (0.75)$$

where X_A and X_B are the contributions of the A and B parents

Table 1: Estimates of the contribution of individual parents for % Extract and Diastatic Power to crosses adjusted for % protein which was included in the analysis as a co-variate.

	% Extract	Diastatic Power (U)		% Extract	Diastatic Power (U)
Intercept	87.74	264	Intercept	87.74	264
% Pr	-0.74	21.0	% Pr	-0.74	21.0
WI-2674	-1.05	88	Chebec	-1.63	129
WI-2692	-1.74	-35	Cornisch	1.80	-29
WI-2693	-0.14	39	Franklin	2.15	116
WI-2734	-0.22	4	Galleon	-1.83	39
WI-2738	-2.41	66	Harrington	0.32	162
WI-2785	-1.48	27	Haruna nijo	2.64	231
WI-2804	-1.53	91	Natasha	2.20	158
WI-2808	-0.82	33	Rubin	2.29	-115
Amagi nijo	0.82	100	Skiff	-0.35	-4
Bearpaw	1.34	-3	Stirling	-0.88	103
Blenheim	1.18	-28	Zenit	1.74	53

Estimations of the means of all possible 231 cross combinations were made using the estimated parameters (ie. Table 1) for the 22 parents from Expt. 15/93, assuming only single crosses (eg A*B). The numbers of crosses and their respective mean values are shown in Table 2 for each "Cross Type". Crosses were divided into various categories depending on the "origin" of the parents. These were ; Australian (Au), European (Eu), Japanese (Jp) and United States/Canada (USC). A selection of the "Best" crosses was made on the basis that the mean values for both % Extract and Diastatic Power were greater than average of the malting controls.

Table 2: Summary of predicted means of crosses by "Cross Type". The values for % Extract and Diastatic Power were adjusted for the effect of % Protein. Values were then calculated on a standard % Protein of 10%.

Cross Type	All Crosses			"Best" Crosses		
	# of Crosses	Mean	Mean	# of Crosses	Mean	Mean
Au*Au	78	79.5	527	4	80.8	583
Au*Eu	65	80.8	504	11	81.2	584
Au*Jp	26	80.7	583	10	81.5	607
Au*USC	26	80.5	540	2	81.2	595
Eu*Eu	10	82.3	481	1	82.7	579
Eu*Jp	10	82.0	560	5	82.3	608
Eu*USC	10	82.0	517	2	81.5	608
Jp*Jp	1	81.2	640	1	81.2	640
Jp*USC	4	81.8	596	3	81.9	621
USC*USC	1	81.8	553	0		
Total	231	80.5	529	39	81.5	598
Controls		81.3	545		81.3	545

It is pertinent to note that few Au*Au crosses survived and that all of these involved Franklin as a parent. The surviving crosses favoured Au*Eu and Au*Jp with a higher proportion of Au*Jp crosses than Au*Eu. Haruna nijo contributed significantly to the predicted success of the Au*Jp crosses. The crosses of most relevance in terms of combining improvements in malting quality with adaptation, agronomic performance and disease resistance were : Au*Au, Au*Eu and Au*Jp. The predicted mean values for the controls are included to indicate the criteria for the selection of the "Best" Crosses. The predictions for the different "cross type" combinations were that ; i) Au*Au crosses gave low % Extract and DP, ii) the crosses Au*Eu resulted in higher % Extract than Au*Au crosses but in general gave disappointing extract levels, and finally iii) the Au*Jp crosses gave the most promising levels of both % Extract and Diastatic Power.

Cross prediction exercises of the most simplistic type suggest that crossing strategies in which a higher proportion of the "best" parents will lead to populations of even greater promise. A major criteria in the crossing strategy is to combine efficiently high % Extract high Diastatic Power with an agronomic type suited to southern Australian conditions. Crossing strategies involving crosses of the type ; (Au*Eu)*Jp, (Au*Jp)*Eu, , (Au*Jp)*Jp, and (A*USC)*Jp are predicted to be quite profitable. In each of these crosses the Australian contribution is 25%.

Conclusions. Cross evaluation experiments provide objective information on the performance of individual crosses. Bulk Population Experiments can only give an estimate of the mean performance of a cross for a specific character. Crosses can be culled from the program each year if their mean performances are less than control varieties. The performance of new cross combinations can be predicted from the information gathered. Cross Evaluation Experiments based on randomly derived F₃ progeny populations would provide an estimate on the level of genetic variation found within a cross. This information is particularly relevant if the cross is to be utilised for doubled-haploid production for selection of new varieties or for molecular mapping research.

Acknowledgments

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Differences in Malt Quality of Several International Barley Cultivars D. E. LANGRELL and M. J. EDNEY, Grain Research Laboratory, Canadian Grain Commission, 303 Main St. Winnipeg, Manitoba, CANADA R0C 3E0

Introduction. The purpose of this study was to illustrate the genetic variability in international malting barley cultivars, and to demonstrate how this variability is expressed in barley and malt quality parameters.

Barley varieties from Canada, Australia, and the United Kingdom grown in the 1994 season were collected by and exchanged between the collaborators in this study in late 1994 and early 1995. Samples received by the Grain Research Laboratory were analyzed for barley quality, then malted in a Phoenix micromalting system, followed by complete malt quality analysis. Substantial differences were observed in the levels of barley protein, malt extract, soluble protein, and starch degrading enzymes.

Materials and Methods. CANADA - Four Cultivars (Harrington, Manley, AC Oxbow, TR 232) each grown at three locations (Table I). The samples were provided by Dr. Brian Rossnagel CDC-U of S, Saskatoon, Saskatchewan; Dr. Bill Legge AAFC-Brandon, Manitoba; and Dr. Ken May AAFC-Lethbridge, Alberta. The barley seed was from breeder seed stock and was grown on experimental plots in 1994 season.

AUSTRALIA - Samples of 7 different cultivars were obtained from commercial sources, and are designated 1, 2, and 3 indicating their different origins (Table I). Cultivars selected were Grimmett, Tallon, TG-121, Stirling, Schooner, Arapiles, and Franklin. The samples were requested and forwarded by Dr. P.A. Inkerman and Ms. Kerri Fry of the Queensland Wheat Research Institute, Department of Primary Industries, Toowoomba, Queensland, Australia 4350.

UNITED KINGDOM - Four cultivars (Halcyon, Riviera, Chariot, Alexis) were each grown on experimental plots at three locations in the U.K. (Table I). The samples were supplied by Mr. Derek Smith and Dr. Peter Hanson, Plant Breeding International, Trumpington, Cambridge, England CB2 2LQ.

The samples were malted under the following conditions: steeped for 48 hours(8wet,4dry,13 deg C); germinated for 96 hours(15 deg C), and kiln dried for 48 hours (maximum temperature 85 deg C).

Analytical results were carried out in accordance with the methods of the American Society of Brewing Chemists (1), with the exception of the 70 degree extract (3), and the alpha-amylase procedure (2).

Results of analysis are listed in Table I. Only those parameters selected for comparison are listed, however a complete set of barley and malt quality analyses were performed. All analysis referred to here was performed in the Malting Barley Research Section of the Grain Research Laboratory, Canadian Grain Commission in Winnipeg. The results of other collaborators analysis are not listed here. The interpretation of the results is solely that of the authors.

TABLE I RESULTS OF BARLEY AND MALT ANALYSIS

Description	70°				Fine Grind				
	Barley Protein	Fine Extract %	Extract (coarse) %	F/70° Diff.	beta-Glucan ppm	Soluble Protein %	Ratio S/T %	Diast. Power °L.	Alpha-Amylase D.U.
Harrington-Brandon	12.5	79.0	73.9	5.1	457	4.92	38.7	84	36.9
Harrington-Saskatoon	13.2	76.9	72.8	4.1	358	4.66	36.1	95	47.7
Harrington-Oak River	13.6	78.8	70.5	8.3	333	5.41	40.7	105	50.5
Manley-Brandon	13.4	77.2	73.2	4.0	354	4.82	36.0	102	42.3
Manley-Saskatoon	13.5	76.8	75.1	1.7	318	4.29	33.5	107	48.8
Manley-Oak River	14.3	78.7	74.7	4.0	437	5.33	38.9	119	56.0
AC Oxbow-Brandon	12.4	77.8	74.3	3.5	155	5.01	38.0	86	29.3
AC Oxbow-Saskatoon	14.2	78.6	76.0	2.6	171	5.26	38.7	115	48.3
AC Oxbow-Oak River	14.6	78.5	74.1	4.4	204	5.60	40.9	123	52.2
TR 232-Brandon	12.7	78.7	75.4	3.3	240	4.83	37.2	91	42.3
TR 232-Saskatoon	13.2	79.0	72.7	6.3	233	4.87	37.2	105	47.9
TR 232-Oak River	14.0	78.9	72.2	6.7	394	5.20	38.0	115	54.5
Grimett - 1	9.4	79.2	78.0	1.2	184	3.86	41.5	74	35.2
Grimett - 2	9.4	78.2	74.2	4.0	266	3.05	33.9	61	27.7
Tallon - 1	9.3	81.1	78.6	2.5	93	4.30	46.7	81	45.1
Tallon - 2	9.8	80.4	74.1	6.3	211	4.14	43.6	76	41.5
Tallon - 3	9.8	81.9	79.7	2.2	97	4.19	43.6	78	42.8
TG 121 - 1A	8.9	79.3	77.0	2.3	212	3.54	38.5	73	32.8
TG 121 - 1B	10.7	78.6	76.3	2.3	227	4.78	43.1	80	39.1
Stirling - 1	10.3	77.6	74.2	3.4	662	3.43	33.0	89	35.6
Stirling - 2	11.6	77.5	71.6	5.9	596	4.22	36.1	103	34.7
Schooner - 1	11.0	79.0	77.4	1.6	114	4.87	46.8	81	44.5
Schooner - 2	10.4	80.4	79.0	1.4	38	5.00	51.0	78	45.1
Arapiles	9.3	81.3	79.6	1.7	76	4.40	47.8	93	40.1
Franklin - 1	10.4	79.7	77.5	2.2	105	3.60	36.7	94	37.4
Franklin - 2	10.9	78.8	76.0	2.8	55	3.46	36.0	91	36.8
Franklin - 3	10.8	79.5	77.1	2.4	72	3.47	36.9	94	37.2
Halcyon-Melbourne	13.7	77.5	66.1	11.4	675	3.55	28.4	89	32.7
Halcyon-Perth	12.8	77.4	69.7	7.7	617	3.58	28.6	71	32.8
Halcyon-Bottisham	12.8	77.9	72.0	5.9	482	3.26	28.6	78	28.5
Riviera-Balsham	10.6	79.5	76.7	2.8	335	3.31	33.4	66	35.5
Riviera-Perth	8.3	81.4	79.3	2.1	187	2.88	37.4	61	37.3
Riviera-Slate Hall	11.1	78.9	74.9	4.0	310	3.32	31.3	66	35.1
Chariot-Balsham	11.2	80.1	78.4	1.7	171	3.44	32.1	77	39.9
Chariot-Perth	10.9	81.6	77.9	3.7	211	3.36	38.2	73	41.1
Chariot-Slate Hall	10.9	80.1	76.9	3.2	132	3.37	34.7	77	38.7
Alexis-Balsham	11.0	79.7	77.7	2.0	192	3.73	36.2	79	46.7
Alexis-P.B.I.	10.7	79.4	78.4	1.0	216	3.92	39.6	83	46.2
Alexis-Slate Hall	10.7	79.6	78.1	1.5	98	3.45	35.9	79	45.7

Results and Discussion. The results listed in Table I illustrate significant differences among international cultivars in the levels of barley and soluble protein, malt extract, and starch degrading enzymes. Differences in barley protein levels can be attributed partially to genetic traits, but in larger part to environmental factors (i.e. kernel development profile as affected by length and type of growing season.) Extract levels differ primarily because of the varying levels of protein in the barley (i.e. the more protein the less starch and less extractable carbohydrate). Soluble protein is largely a function of total protein, given the same extent of malt modification. Very low or high soluble protein levels are undesirable in the brewing process, as sufficient amino acids are required for yeast nutrition but too much protein in beer can cause problems with beer color and haze formation. The Canadian malts have significantly higher starch-degrading enzyme levels than those of the Australian and UK malts, as Canadian brewers who use starch adjuncts require the malt enzymes to break down gelatinized starch in the mashing tun. Australian and UK brewers more often use sugars or syrups which do not require enzymatic hydrolysis prior to fermentation.

In conclusion, the data illustrates that genetic variability in malting barley cultivars is expressed in barley and malt quality. To the extent that these genetic characteristics are functionally linked, potential certainly exists for international cultivar cross-breeding to yield considerably improved malting cultivars.

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Comparative performance of selected malting barley lines in North Carolina and Idaho. S. Leath¹, D. M. Wesenberg², and B. L. Jones³, USDA, ARS, Department of Plant Pathology, Box 7616 NCSU, Raleigh, NC 27695, USA¹, National Small Grains Germplasm Research Facility, P. O. Box 307, Aberdeen, ID 83210, USA², and Cereal Crops Research Unit, 501 N. Walnut St., Madison, WI 53705, USA³

INTRODUCTION. High quality malting barley has been difficult to produce in the southeastern United States due to a number of factors, including high disease pressure. Recently, a resurgence of interest in beer brewed from locally produced grain has occurred and is associated with the rapid expansion of microbreweries in this region. The objective of this project is to evaluate the agronomic performance and malting quality of modern malting barley lines when produced in the Southeast.

MATERIAL & METHODS. During the 1993-94 and 1994-95 growing seasons eleven winter and 38 spring barley lines were grown at three North Carolina locations. Here data are reported from the tidewater region of North Carolina where both winter and spring types were planted in the fall. Plots were established October 26, 1993 and November 1, 1994 and grown according to standard agronomic practices. Plots were 4' X 8' and were treated twice in the 1993-94 season and once in the 1994-95 season with propiconazole to reduce leaf and head diseases. Data were recorded on winter survival, heading date, leaf rust severity on the flag leaves, test weight, and yield. Plots were harvested June 8, 1994 and May 26, 1995, and grain samples were subsequently analyzed for malting characteristics at the USDA-ARS Cereal Crops Research Unit, Madison, WI.

RESULTS AND DISCUSSION. Of the 49 lines evaluated most survived the winter and produced seed. No winter barley showed any injury and survival of spring barley's ranged from 96% to 15%. Top performing lines headed within one week of local feed barley checks. Leaf rust was severe on most lines with severity ranging from 0-58% and 0-66% in the two seasons with one and two fungicide applications, respectively (Table 2). All lines with acceptable malt quality were susceptible to leaf rust. Top yielding lines were not the top lines for quality. However, data presented for seven two-rowed and five six-rowed barley's (Table 3) indicated that barley's with acceptable malting quality can be produced in this region. The relatively high LSD statistics associated with these data are a reflection of poor winter survival for several poorly adapted selections.

If the market for Southeastern malting barley exists, a program to evaluate and improve barley for acceptable agronomic and malting performance in the Southeast should be successful.

Table 1. Parentage of selected barley's evaluated in North Carolina in the 1993-94 and 1994-95 growing seasons.

Entry	Parentage
86Ab1527	78Ab10327/Moravian III // Lamont
86Ab2317	Harrington/Crystal
86Ab2626	79Ab10740/Lewis
86Ab9763	79Ab10740/Crystal
86Ab9907	79Ab10740/Crystal
85SR431	Morex/ND5377
85SR598	WA854378/Russell

Table 2. Agronomic data for selected spring barley varieties and selections grown in trials in North Carolina in the 1993-94 and 1994-95 growing seasons.

Entry	Winter Survival	Heading Date ¹	Leaf Rust Severity (%)	Test Weight. ¹ (Lb/Bu)	Yield (Bu/A)
<i>Two-rowed</i>					
Crystal	74.2	111	7.4	47.2	71.8
Klages	68.5	112	5.4	44.6	73.4
86Ab1527	70.8	109	14.2	48.0	65.0
86Ab2317	87.0	109	7.5	46.9	72.6
86Ab2626	82.5	110	9.5	48.2	75.6
86Ab9763	74.2	110	7.5	48.6	74.6
86Ab9907	75.0	110	9.3	46.6	71.9
Boone²	100.0	110	17.2	43.0	76.5
LSD 0.05	16.4	1.2	21.7	3.5	23.0
<i>Six-rowed</i>					
Morex	80.0	108	10.0	43.8	56.2
Russll	70.0	108	24.0	41.8	43.1
85SR431	66.7	108	15.5	39.7	63.0
85SR598	82.0	108	9.7	43.8	69.9
Plaisant²	98.0	109	4.3	40.4	88.1
LSD 0.05	15.7	1.2	24.5	3.5	16.0

¹These data are from the 1994-95 season only.

²Winter barley variety.

Table 3. Malting quality data ¹ for selected spring varieties and selections barley grown in trials in North Carolina and Idaho.

Entry	Plump Barley (%)	Malt Extract (%)	F/C Diff. (%)	Barley Protein (%)	Ratio Wort N / Malt N (%)	Dia-static power (Deg)	Alpha Amylase (20° Units)	Beta-Glucan (%)
North Carolina 1993-94 & 1994-95								
<i>Two-rowed</i>								
Crystal	89	79.8	1.2	12.8	40.2	111	49.7	667
Klaces	86	80.5	1.8	11.8	42.3	93	47.0	889
86Ab1527	92	80.2	2.0	12.3	40.5	109	44.7	830
86Ab2317	88	81.1	1.4	12.4	44.2	111	54.3	583
86Ab2626	88	80.9	1.6	12.0	43.2	110	50.5	601
87Ab9907	87	81.2	1.4	12.1	43.9	116	54.2	496
87Ab9763 ²	95	81.0	1.6	12.4	39.8	95	46.6	523
Idaho 1994 & 1995								
Crystal	85	79.0	2.2	13.4	33.6	128	45.0	592
Klaces	79	79.3	2.6	12.0	34.5	98	40.2	764
86Ab1527	94	79.5	2.7	12.5	31.6	101	34.3	276
86Ab2317	91	78.9	1.9	12.7	32.7	141	43.4	319
86Ab2626	84	80.7	1.6	12.	39.2	132	44.7	227
87Ab9763	85	79.7	1.9	12.3	35.4	126	45.4	421
North Carolina 1993-94 & 1994-95								
<i>Six-rowed</i>								
Morex	81	79.0	1.9	12.3	41.2	124	48.2	1159
Russell	74	79.5	1.8	10.9	44.7	116	41.8	538
85SR431	72	78.2	2.3	11.7	40.6	138	41.7	1020
85SR598 ²	78	79.9	1.3	9.8	43.9	116	41.3	906
Plaisant ²	58	75.9	3.2	10.4	34.3	79	31.7	987
Idaho 1993 & 1994								
Morex	93	79.0	2.5	12.5	30.8	105	34.1	
Russell	91	79.4	3.0	10.4	42.2	95	31.8	
85SR431	90	77.8	3.5	11.7	36.6	108	31.0	
85SR598	92	79.3	4.3	9.5	40.8	82	27.5	

¹Malting quality data courtesy of USDA-ARS Cereal Crops Research Unit, Madison, Wisconsin. Agtron (0-100) values averaged 40 and 68, respectively, for North Carolina and Idaho.

²These data are from the 1994-95 season only.

THE PRODUCTIVE TRAITS AND GRAIN QUALITY OF WINTER AND SPRING MALTING BARLEY

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INTRODUCTION: Barley represent a very important agronomic species. This species of plants are grown all over the world on 80 million hectare (Trofimovska, 1972) and in SR Yugoslavia (Serbia and Montenegro) this species grown on 100.000 hectares. Today, in Yugoslavia for three time and more grain yield of barley was increased than in period earlier the 1940 year. This increasing of yield based on new barley high yielding malting and forage barley cultivars. New barley cultivars are characterize, short stem, large grain, resistance to diseases, resistance to attrition, lodging resistance, frost and drought resistance. The genetic potential for grain yield at new barley cultivars is above 10,5 t/ha and for grain extract content in average 78,3% to 81,1% and more. New cultivars are very important for breeding process, and agricultural production as well as for science.

MATERIAL AND METHOD: Grain yield was measured in cultivar microtrials designed in a randomized block in experimental plot 5 m² with five repetition (Tavcar, 1952).

Classification of seed size were made on battery. The first class represent seed large 2,5 mm and up. Grain protein content was determined by Kjeldahl method (nitrogen content x 6,25 in dry seed with 10% of water). Grain extract content was determined forward Bishop formulae: E=A- 4,7 +0,16. where E= extract of grain; A= constant; G= mass 1000 kernels according to Malcev (1967), Kosha (1974).

RESULT AND DISCUSSION: Grain yield and content of size seed first class were presented in Table 1. Average grain yield at two row winter malting barley varied from 6279 kg/ha at the NS-331 to 7003 kg/ha at the OSK.5.194/10-85 line. In spring malting barley line and cultivars the grain yield varied from 5027 kg/ha at the Jastrebac cultivar to 5310 kg/ha in NS-404 cultivar.

The average grain yield of winter barley line and cultivar is 6727 kg/ha and in malting barley line and cultivar 5140 kg/ha. In winter barley line and cultivar average impact of first class of seed is varied from 94.04% at the KG-8/4 to 97.95% at the OSK.5.194/10-85 line. I average for all winter barley cultivars the average value of impact first class size seed was 96.44% and for spring barley cultivars was 89.75% (Table 1).

The line and cultivars of two row winter malting barley showed for 1587 kg/ha higher grain yield and higher impact of seed first class for 6.69% than spring malting barley line and cultivars. This results are in agreement with reports (Maksimovic et al., 1994).

The variation of grain crude protein content in winter barley cultivar and lines, in average, from 10.24% at the Jagodinac cultivar to 11.11% at the OSK.5.194/10-85 line were found. In spring malting barley line and cultivars, variation of crude protein content was from 11.18% at the NS-404 cultivar to 11.99% at the OSK.7.103/11-88 line.

The average value for all line and cultivars in all year of investigation, crude protein content of winter barley was 10.67 and spring barley 11.65 (Table 2). This indicating that

in average crude protein content was lower for 0.98% than in spring barley cultivars. The quality of grain is very important for production malt and beer (Maksimovic et al., 1995; Kodanev, 1964; Trofimovska, 1972; Malcev 1976; Kosha, 1974, Ulonska et al., 1973).

Variation of content of extract of grain in winter malting barley line and cultivars, in average, from 80.28 at the KG-8/4 line to 81.08% at NS-293 cultivar were found. In two row spring barley line and cultivars, extact of grain is varied from 78.32% at the OSK.7.103/11-88 line to 75.52% at the NS-404 cultivar. Its indicating than in malting barley, in average, of extract was higher for 1.73% than extract content of grain in spring barley cultivar and lines (Table 3).

The results indicating that winter barley and line cultivars in average had higher grain yield, higher impact of seed of first class, higher content of extract of grain and lower crude protein content than in spring barley line and cultivars.

Table 1. Grain yield and content of first class size seed of winter and spring malting barley line and cultivars in microtrials in Kragujevac

Cultivar	Average grain yield 1990-1992	Content of first class size grain		
		1991	1992	Average
I Winter Barley				
1.Kg. 8/4 6	6.831	91.57	96.50	94.04
2.Jagodina6c	6.832	94.74	96.48	95.61
3.OSK.56.194/10-85	7.003	97.98	97.93	97.95
4.NS.2693	6.568	96.66	97.93	97.29
5.NS6.331	6.279	96.68	98.05	97.37
6.Z6A.88	6.815	95.61	97.15	96.38
Average	6.727	95.54	97.34	96.44
II . Spring barley				
1.Jastrebac	5.027	90.94	93.54	92.24
2.Kg. 2/10	5.140	87.68	91.86	89.77
3.Za 9/89	5.084	91.21	94.14	92.67
4.NS-404	5.310	91.71	89.85	90.78
5.Jaran	5.213	80.59	95.69	88.14
6.OSK.7.103/11-88	5.027	77.74	92.08	84.91
Average	5.140	86.65	92.86	89.75

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Table 2. Grain protein content in winter and spring barley line and cultivars in microtrials in Kragujevac

Cultivar	Grain protein content (%)		
	1991	1992	Average
I .Winter Barley			
1.KG. 8/4	10.78	10.50	10.64
2.Jagodinac	10.27	10.20	10.24
3.OSK.5.194/10-85	11.39	10.83	11.11
4.NS.293	10.39	10.18	10.28
5.NS.331	11.19	10.68	10.93
6.ZA.88	10.88	10.75	10.82
Average	10.82	10.52	10.67
II Spring barley			
1.Jastrebac	11.75	11.18	11.46
2.Kg. 2/10	11.96	11.26	11.61
3.Za 9/89	11.61	11.99	11.80
4.NS-404	11.57	10.80	11.8
5.Jaran	12.25	11.50	11.87
6.OSK.7.103/11-88	12.44	11.53	11.99
Average	11.93	11.38	11.65

Table 3. Content of extract in grain of winter and spring barley line and cultivars in microtrial in Kragujevac

Cultivar	Content of extract in grain (%)		
	1991	1992	Average
I. Winter barley			
1.Kg. 8/4	80.01	80.56	80.28
2.Jagodinac	80.89	80.67	80.78
3.OSK.5.194/10-85	80.41	80.51	80.46
4.NS.293	80.98	81.18	81.08
5.NS.331	80.13	80.75	80.44
66.ZA.88	80.62	81.03	80.82
Average	80.51	80.78	80.64
II. Spring barley			
1.Jastrebac	78.17	79.67	78.92
2.Kg. 2/10	77.94	79.73	78.84
3.Za 9/89	78.51	79.50	79.01
4.NS-404	78.63	80.42	79.52
5.Jaran	77.88	79.86	78.87
6.OSK.7.103/11-88	76.97	79.63	78.30
Average	78.02	79.80	78.91

BARLEY BREEDING FOR MALTING QUALITY IN CENTRAL CHERNOZEM PROVINCE OF RUSSIA. V.D. Navolotsky, R.N. Kalendar¹, V.P. Netsvetaev, Belselect Co., Seed Station, Settl. Oktyabrsky, Belgorod Reg., 309090, Russia; ¹Plant Breeding and Genetics Institute, Odessa, 270036, Ukraine.

Introduction. Central Chernozem Province (CCP) is the most important in Russia for malting barley production. Total sowing area of barley in this province is about 1.5 mill. ha. and it is possible to grow grain of the barley with a good malting quality almost every season except in the most droughtly south-eastern part of Voronezh region. Precipitation in the CCP averages 500-600 mm per year, but variability among seasons is very high. The soil is typical chernozem (black soil), about 1-1.5 m deep, with 4-5% humus. The most widespread malting varieties are Odessky 115, Odessky 100, Zazersky 85, Dvoran and Karina. All cultivars have good productivity and adaptation, but most of them do not have enough resistance to lodging, drought or diseases, especially to loose smut, covered smut, Rhyncosporium and mildew.

Materials and Methods. 24 varieties of spring barley were investigated in 1994-95. 94 cultivars were investigated for some malting traits in connection with biochemical and molecular markers. Statistical analysis was done between biochemical or molecular markers and characters which determine malting quality. Biochemical markers are Amy 1, 2 (α -amylase) Bmy 1 (β -amylase), Est 1, 2, 4, 5, 12 (Esterases). The P6, P39, P56 primers were used for identification of molecular markers. So, primer P6 gave products of polymerization with molecular weights 810, 850, and 915 b; P39 with 890, 900, 1500, 2300, and 2350b; P56 with 1100b.

Results and Discussion. Mean yield in 1994 was 7.29 ± 0.51 t/ha, but only 3.24 ± 0.37 t/ha in 1995. Yields of varieties in 1994 and 1995 were not correlated ($r = 0.20 \pm 0.26$). Climatic conditions in 1994 were very favourable, but 1995 was very droughty. The correlation between yield and extract content was not significant ($r = -0.27 \pm 0.21$), the correlation between protein content and extract content was negative and significant ($r = 0.62 \pm 0.13$). The malting quality of malting barley in the Belgorod region will be good only in favourable growing seasons.

All molecular markers were associated with agronomic traits and with malting quality traits (Table 2). Tables 1 and 2 show that molecular biochemical markers may be used to indicate genotypes with higher malting quality. Extract content in grain of malting varieties was 77-78% and in grain of fodder cultivars 74-76%. Climatic conditions in the Belgorod region are more favourable for selecting varieties with good malting quality than in Odessa. Breeding strategy of malting barley should be focused on creating varieties with a good lodging resistance, resistance to the main diseases and adaptation to variable climatic conditions.

Table 1. Relationship between molecular markers and some agronomic and malting quality traits (24 var., 4 rep. x 25 m², 1994; Belgorod).

Character	Marker	Absence (0) or presence (1) of product	Means of alternating groups	Standard errors	No. of varieties	t value
1000 G.W., (g)	P56#1100	0	46.5	0.86	11	
		1	49.5	0.86	13	2.45*
Hull content %	P6#810	0	8.91	0.13	16	
		1	8.32	0.15	8	2.84**
	P39#1500	0	8.59	0.09	21	
		1	9.58	0.25	3	3.54**
Extract (%)	P39#1500	0	77.3	0.20	21	
		1	76.1	0.33	3	2.29*
Sieving 2.8 (%)	P56#1100	0	1.82	0.44	11	
		1	3.54	0.45	13	2.70*
	P6#810	0	49.4	3.42	16	
		1	64.0	3.60	8	2.66*
2.2	P6#810	0	35.9	2.86	16	
		1	26.1	3.39	8	2.08*
	P56#1100	0	38.3	3.39	11	
		1	27.8	2.76	13	2.44*
Susceptible to Eury- gaster integriceps Put. (%)	P39#2300	0	69.3	4.58	15	
		1	87.4	5.08	9	2.55*

* P < 0.05, ** P < 0.01

Table 2. Relationship between some molecular and biochemical markers and some malting quality (94 traits, 94 varieties, Odessa, 1994).

Characters	Marker	Allele	Means of alternat. groups	Standard error	No. of varieties	t
Extract content, %	Amy 2	1	75.54	0.09	81	
		2	75.11	0.31	10	2.15*
	P39#1500	0	75.80	0.09	79	
		1	75.11	0.24	15	2.85**
	P39#900	1	75.39	1.17	38	
		1	75.89	0.09	56	2.74**
	P39#890	0	75.78	0.09	78	
		1	75.23	0.22	16	2.30*
	Amy 2	1	15.36	0.12	81	
		2	16.89	0.41	10	4.13***
Protein content, %	Sod S	1	15.32	0.11	80	
		2	16.86	0.59	9	3.90***
	P39#1500	0	15.38	0.12	79	
		1	16.15	0.43	15	2.31*
	P39#2300	0	15.71	0.16	59	
		1	15.15	0.17	35	2.21*
	Est 12 ^a	1	9.08	0.06	90	
		2	10.50	0.45	3	3.84**
α -amylase activity con. un.	Bmy 1	1	180.4	8.07	37	
		2	204.1	6.94	50	2.22**
	Sod S	1	183.8	4.80	80	
		2	223.9	26.50	9	2.40*
	P6#810	0	197.7	6.06	71	
		1	171.4	8.54	23	2.24*
	P6#915	0	179.2	6.57	51	
		1	205.6	7.65	43	2.63*
	P39#890	0	197.6	5.43	78	
		1	160.5	12.40	16	2.80**
Malting index	Est 12 ^a	1	60.9	0.77	90	
		2	49.0	3.05	3	2.78**

*P<0.05; **P<0.01; ***P<0.001; ^a - associated with Vv

Influences of the starchy endosperm on α -amylase gene expression. R.W. Skadsen,
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Introduction. After germination in cereal seeds, the activities of α -amylases and many other hydrolytic enzymes greatly increase in the starchy endosperm, resulting in the mobilization of starch and other reserves to the developing seedling. The study of this phenomenon was simplified by the isolated aleurone system, in which aleurones from deembryonated half-seeds are treated in solution with gibberellin (GA)(1). This causes the synthesis of α -amylases and their secretion into the incubation medium, which is thought to mimic the process as it occurs in germinating seeds.

α -Amylase activity in barley results from two isozymes, the high-pI and low-pI α -amylases. In seedling kernels, the activity of the high-pI isozymes is appreciably greater than that of the low-pI isozymes, accounting for 90 to 97.9% of the total α -amylase activity in various cultivars of different ages and under a variety of conditions (2-9). Isozyme activities are quite different in the isolated aleurone system, where the low-pI isozyme and its mRNA either predominate or are equal to the high-pI (10-17). These observations suggest that factors governing α -amylase expression differ significantly between the two systems. One obvious difference is the presence or absence of the starchy endosperm. This study presents observations which suggest that the starchy endosperm modulates α -amylase expression.

Materials and Methods. Intact seedlings, half-seeds and isolated aleurones were grown and prepared as described (4). Etiolated seedlings were grown for 1 to 7 days (from start of imbibition) at 16°C. Aleurones were removed, frozen in liquid nitrogen and stored at -80°C. For kernel (aleurone plus starchy endosperm) samples, scutella, roots and shoots were removed before freezing. For isolated aleurone studies, aleurones were removed from the starchy endosperm of 4 or 5-day-old deembryonated half-seeds and rinsed free of starch with succinate/Ca buffer. GA treatments were conducted on aleurones in petri dishes containing succinate/Ca buffer with penicillin, gentamycin and 10⁻⁶ M GA₃ as described (4). Dishes were shaken at 21°C for varying periods. To examine whether early signals influence GA-responsiveness, deembryonated half-seeds were prepared from dry seeds and from untreated germinating seedlings of various ages. These were treated with GA in the same way as the isolated aleurones. Isolated aleurone treatments were also conducted with GA-supplemented media containing endosperm homogenates. Endosperm were homogenized in succinate/CaCl₂ buffer (40 endosperms per 10 ml). Twenty aleurones were added to 10 ml of homogenate in 5-cm petri dishes. GA treatments and sample preparations were as described above. Treatments were conducted for up to 36 h.

Tissues were prepared for α -amylase enzyme assays and isoelectric focusing (IEF) as described (4). For activity gels, heat-treated homogenate was applied to IEF gels of ampholyte pH range 4.0 to 6.5. After IEF, α -amylase activity was detected by impregnating the gels with Lintner starch and staining in iodine (3). RNA was prepared from aleurones and kernels and analyzed on northern blots as previously described (4). Filters were hybridized and washed under high stringency (62.5°C). High- and low-pI α -amylase mRNAs were detected with the high-pI cDNA clone pM/C (18) and low-pI

cDNA clone E (19). Autoradiographs were scanned densitometrically.

Results and Discussion. In intact seedlings, high-pI isozyme activity increased strongly through at least day 6, while its mRNA increased strongly to peak levels between days 3 and 5 and remained at high levels through day 7. The low-pI isozyme and mRNA became detectable by day 3 and increased steadily through day 7. In the Himalaya cv., the ratio of high- to low-pI mRNA was 3.5 at day 5 and 1.8 by day 7. This ratio was much higher in Morex and Steptoe cvs. The high ratio of high-pI to low-pI α -amylase in intact seedlings may result from either stimuli from the embryonic axis or from factors present in the endosperm or from an interaction of both. These alternatives were tested by preparing deembryonated half-seeds from 0 to 72 h old intact seedlings and then incubating these with and without GA. After 24 h, high-pI mRNA was always present at greater levels than low-pI mRNA. Because this pattern was already established in 0 h half-seeds, where no preconditioning signal transduction from the embryo occurred, it was concluded high-pI predominance resulted at least partly from factors associated with the starchy endosperm. In another study, deembryonated half-seeds were left on moist germination paper and analyzed on successive days. Those receiving no GA produced gradually increasing levels of both high- and low-pI isozymes in roughly equal amounts, and neither isozyme was secreted into the endosperm. Half-seeds imbibed in 10^{-6} M GA produced four times as much total activity, strongly favored high-pI expression over the six-day period, and secreted both isozymes. Thus, an endosperm factor may augment the effects of GA (or vice versa).

To detect possible signals associated with the endosperm, aleurones were prepared from deembryonated Himalaya half-seeds and incubated with GA and a thick homogenate of the starchy endosperms from the same half-seeds. The homogenate caused a prolonged increase in high-pI mRNA levels, which reached a peak at 30 h and then declined. Without the endosperm homogenate, high-pI mRNA reached peak levels by 12 h. Low-pI mRNA increased steadily for 36 h and was at similar levels in both systems. Experiments were also conducted with isolated aleurones from Morex, which were incubated either with GA alone or with GA plus endosperm homogenates from 5-day-old deembryonated half-seeds of Steptoe or Morex. As in Himalaya aleurones, endosperm homogenates reversed the high-pI to low-pI mRNA ratio of isolated aleurones, causing high-pI mRNA to predominate. Possible osmotic effects from the homogenate were tested by incubating aleurones in solutions of 7.5% and 15% mannitol or 10% PEG. These produced no increase in relative levels of high-pI mRNA.

These findings strongly suggest that the starchy endosperm modulates the expression of α -amylase genes in barley. Incubation of isolated aleurones with endosperm homogenates caused a shift toward some facets of high-pI expression which are seen in the intact seedling system. These included elevating the high-pI/low-pI mRNA ratio and prolonging the period of high-pI mRNA accumulation. In addition, the endosperm homogenate increased the overall level of high-pI enzyme. To date, efforts at separating an active principle from the crude endosperm homogenate have been unsuccessful.

The resolution of the signalling question is important in determining whether α -amylases and other hydrolytic enzyme genes are controlled 1) autonomously by innate temporal expression programs or 2) by modulating factors which help the genes to

monitor hydrolysis and adjust their activities accordingly. In rice cells, some α -amylase genes are induced by sucrose starvation, while others are constitutively expressed (20). The "carbon metabolite signal hypothesis" proposes that α -amylase genes in the aleurone are regulated by GA levels, which in turn are controlled by the scutellum according to the flux of sucrose resulting from starch hydrolysis (21). The possibility of direct effects of the starchy endosperm upon gene expression in the aleurone are not included in this scheme and have not previously been noted elsewhere. It is possible that the active factor controlling this phenomenon is part of a more complex set of hormones and/or metabolites which may allow feedback signalling between the aleurone and endosperm.

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Comparison of progress in breeding winter and spring barley for malting quality in the Czech Republic. J. SPUNAR, M. SPUNAR, Agricultural Research Institute, Ltd., Havlickova 2787, 767 41 Kromeriz, Czech Republic

Introduction. Central Europe, particularly the Czech Republic is considered as the country producing spring barley of top malting quality. Malt is a very important export commodity. Spring barley for top malting parameters has been bred in this area for more than 120 years.

Winter barley has been considered as feed barley, particularly the 6-row one. In the last 10 years dramatic progress has been reached in the breeding of 2-row winter barley for malting quality. With respect to the fact that 2-row winter barley provides yield comparable with 6-row barley and 10-15% higher than spring barley, the utilisation of winter barley for malting purposes starts to be intensively discussed.

Materials and Methods. Winter barley was cultivated in the period 1993-1995 after forecrop winter rape. The period of sowing was optimal with respect to soil and climatic conditions, September 25.

Spring barley was cultivated after forecrop sugar beet. Sowing was carried out with respect to climatic and soil conditions in the spring, in March.

Nitrogen fertilization was carried out at the limited amount of 20-30 kg/ha always before sowing. Common herbicides were used in accordance with recommendation of producers. No fungicide treatment was carried out.

Varieties of winter and spring barley were used as follows:

6-row winter barley - Okal (CZ) and Kromoz (CZ)

2-row winter barley - low malting quality, Marinka (NL), Monaco (F), Marna (F)

2-row winter barley - high malting quality KM 774 (CZ) - new line of the authors

Tiffany (D) - tested in official state trials

2-row spring barley - top malting quality Akcent (CZ), Rubin (CZ)

All varieties without indication are in the List of Released Varieties in the Czech Republic.

Results and Discussion. Six-row winter barley was superior in yield and 2-row winter barley in TGW /Tab.1/. As for malting quality, extract content is considered as the most important parameter. Tab. 1 shows that 6-row winter barley and 2-row winter barley with low malting quality did not reach the minimum level 80%. This level is considered minimal by Czech malting industry. On the contrary, 2-row winter barleys KM 774, Tiffany reached a higher level than top malting quality spring barleys Akcent and Rubin.

As well as other parameters of malting quality were comparable or better than in spring barley.

According to Schildbach (1994) in the central and western areas of Europe, including the British Isles, there are the countries with world's most extensive malting barley production. Furthermore, this region has the highest malt requirements, the largest malting capacity and the greatest malt export volume.

This region lies in the favourable climatic conditions which enable the simultaneous growth of winter barleys in autumn sowing and spring barley in spring sowing.

Sage (1994) informed that in England it was much easier to combine the higher yields of winter barleys with the good malting qualities of spring barleys since the crosses do not need to have the winter-hardy characteristics which are essential for continental mainland.

Taking Germany, a neighbouring country of the Czech Republic, as an example, it can be shown (Schildbach and Burbridge, 1993) that the barley yields have risen over last 20 years from 4.2 to 6.1 t/ha whilst the spring barley yields have only improved from 3.4 to 4.3 t/ha. Thanks partly to the longer vegetation period, the yield advantage of winter to spring barleys has risen from 0.74 to 1.78 t/ha. In relative comparison it represents yield advantage 20-30%.

Because of the economic advantages the cultivation acreage of winter barleys in the three most important malting barley producing countries, Great Britain, France, Germany, has increased from 1.4 to 4.6 million ha. Also due to the higher risk of quality loss, the spring barleys have suffered a rapid reduction in acreage from 6.2 to 2.5 million ha.

Back et al. (1995) have confirmed that varieties like Angora recorded drastical improvement of malting quality parameters and several other lines provide further perspectives. Fuchs (1993) characterised very positively in economic and malting parameters variety of Barlena. Narziss (1993) confirmed improvement of beta-glucan content and filterability of winter barley.

All these results are in accordance with the results in the Czech Republic.

Conclusion

1. Varieties of winter barley both 6-row and 2-row released in the Czech Republic till 1995 can be considered unsuitable for malting purposes.
2. Variety Tiffany, KM 774 manifested that they have comparable or better parameters of malting quality than spring barley. As well as yield and TGW reached a very high level.
3. In spite of very positive results winterhardiness must be carefully observed and verified as the Czech Republic is situated on the border of continental and oceanic climate.

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Tab.1 Comparison of yielding and malting parameters of winter and spring barley
Krömeriz, performance trials, 1994-95

Variety	Yield t/ha	TGW. g	Protein cont. %	Extr. cont. %	Relative extrakt %	Degree of att. %	Diastatic power	Kolbach number W.K.	Friabi- lity %
six-row winter barley									
Okal	7,81	41,1	10,3	78,2	29,3	81,9	280	33,8	65
Kromoz	7,93	44,3	9,8	79,1	32,2	81,7	310	36,8	71
two-row winter barley of low malting quality									
Marinka	6,98	47,7	10,2	80,6	42,5	80,7	180	45,2	74
Monaco	6,78	53,8	10,6	79,3	36,8	81,5	310	37,5	73
Marna	6,25	55,9	11,3	78,2	33,0	80,3	380	33,1	50
two-row winter barley of high malting quality									
Tiffany	7,17	54,9	9,4	83,1	42,2	83,3	330	49,7	89
KM 774	7,46	54,4	10,8	80,9	43,5	82,5	225	48,6	83
two-row winter barley of top malting quality									
Rubin	6,98	42,1	10,1	80,8	43,9	80,4	255	47,7	90
Akcent	7,84	42,8	11,4	80,8	48,5	80,1	325	45,5	87

Breeding Barley for Malt Whisky Distilling. J.S. SWANSTON and W.T.B. THOMAS, Scottish Crop Research Institute, Mylnefield, Invergowrie, Dundee DD2 5DA, UK.

Introduction. Scotland is unique amongst the main barley growing areas of western Europe in that around 60% of the spring crop is malted, but only half the malt is used in brewing. The distillation of malt whisky, for sale or blending, consumes approx. 400,000 tonnes of malting barley per annum. In distilling, spirit yield, i.e. the quantity of alcohol obtained from a given amount of malted barley, should be maximised. It is determined by 2 factors. As in brewing, a high level of hot water extract (HWE) is essential, but the extract must also contain a high proportion of fermentable sugars. Fermentable extract is the product of HWE and fermentability and, from this, spirit yield can be predicted.

The Scottish Crop Research Institute (SCRI) has developed novel breeding methodology (Swanston *et al.*, 1987) and techniques for rapid assessment of malting quality, either through predictive tests (Allison *et al.*, 1979) or by scaling down malting and malt assessment (Swanston, 1988). Preliminary attempts to increase the throughput of fermentability determinations are reported here.

In addition, SCRI has utilised F1 doubled haploids (DH) to locate quantitative trait loci (QTL's) for quality characters (Thomas *et al.*, 1996). Such approaches can be applied to factors specific to distilling and offer both a potential breeding system and the opportunity to develop marker assisted selection. However, close genetic linkages will not be broken during the production of F1 DH lines and it is necessary to ascertain whether this is an appropriate strategy when selecting for several factors. Ethyl carbamate is an undesirable trace component of distilled beverages and cyanogenic glycosides, produced in the acrospires of malting barley, are an important precursor. Some genotypes, however, produce very low levels and these can be selected by a rapid screening test (Cook and Oliver, 1991). Here the potential to select, simultaneously for high HWE and low levels of cyanogenic glycoside is considered and a selection scheme, integrating field and laboratory methods, is proposed.

Materials and Methods. Samples of a standard malt supplied by the Institute of Brewing (IOB) were extracted according to the IOB recommended method (1977), with the exception that 20g of grist was used and the final volume was 206ml. After determination of original gravity, 100ml samples of wort were fermented either with 0.5g yeast, of the strain recommended by the IOB, for 48 hrs, or with 6g yeast for 20hrs. Samples were shaken, at slow speed, throughout at 25°C. At completion of fermentation, samples were filtered, with final gravities determined. Fermentabilities were calculated and compared with data provided for the malt standard.

Spirit yields were predicted after extraction and fermentation of 29 malt samples. These were derived from nine cultivars, grown in trial at SCRI and at a site in Banffshire in 1995 and also from 2 cultivars, Triumph and Golden Promise whose fermentable extracts had been adjusted by either reducing the ratio of grist to water, or by adding small quantities of unmalted grain. Five such malt samples were produced for each cultivar. In addition, the IOB standard malt was included as a control.

The presence of cyanogenic glycoside was determined, by a rapid method (Cook and Oliver, 1991) on 160 inbred lines from a cross between the cultivar Derkado and an SCRI breeders' line, grown in a trial of 2 replications at SCRI in 1994. Grain was also assessed for milling energy, which measures the resistance of the grain structure to mechanical disruption, and is negatively correlated with ease of modification of both the cell wall and protein matrix (Camm *et al.*, 1990). It will thus give a useful indication of the accessibility of the starch granules to enzymes during malting. In addition, samples were malted and HWE was determined by a small-scale method (Swanston, 1988).

Results and Discussion. Comparison of the fermentability results (Table 1) with the standard data provided, suggested that accurate and repeatable data could be obtained with 100ml samples. Reducing the fermentation time, by adding more yeast, would be a major advantage in increasing sample throughput and, following promising initial results here, experimentation will continue.

TABLE 1. Comparison of fermentability results obtained by 3 techniques.

Method	IOB Recommended	100ml (48hrs)	100ml (20hrs)
Mean Fermentability (%)	85.74	86.38	85.77
Standard Error		0.306	0.524

In a breeding system there is initial field selection, on single plant or ear rows, for agronomic and disease resistance characters. A first yield trial, on one site, will be followed by at least two years of trialling at a range of sites, before submission of the most promising genotypes into the National testing procedure. Selection of barleys for distilling quality will also be in 3 stages. As spirit yield is dependant on grain with a high proportion of readily accessible starch, initial selection will be for large grains, which should have higher starch content, and low milling energy. Grain from the first yield trial will be malted and HWE will be assessed on 2g malt samples (Swanston, 1988), permitting rapid throughput and effective screening.

There was good agreement (Fig. 1) between predicted spirit yield and HWE, as fermentability only indicates the percentage of soluble material which can be utilised by the yeast and high fermentable extracts are not, therefore, possible without high HWE. At present, fermentability will not be assessed until later generations where greater precision is achievable by extracting 20g of grist. However, detection of QTL's for fermentability may permit future development of marker assisted selection at earlier stages of the programme.

The 160 DH lines were segregated into normal and very low producers of cyanogenic glycoside. The low producing lines had a lower mean milling energy (Table 2), but there was no difference between means for extract. It therefore was possible, in F1 DH lines, to achieve the desired combination of low cyanogenic glycoside production and high HWE.

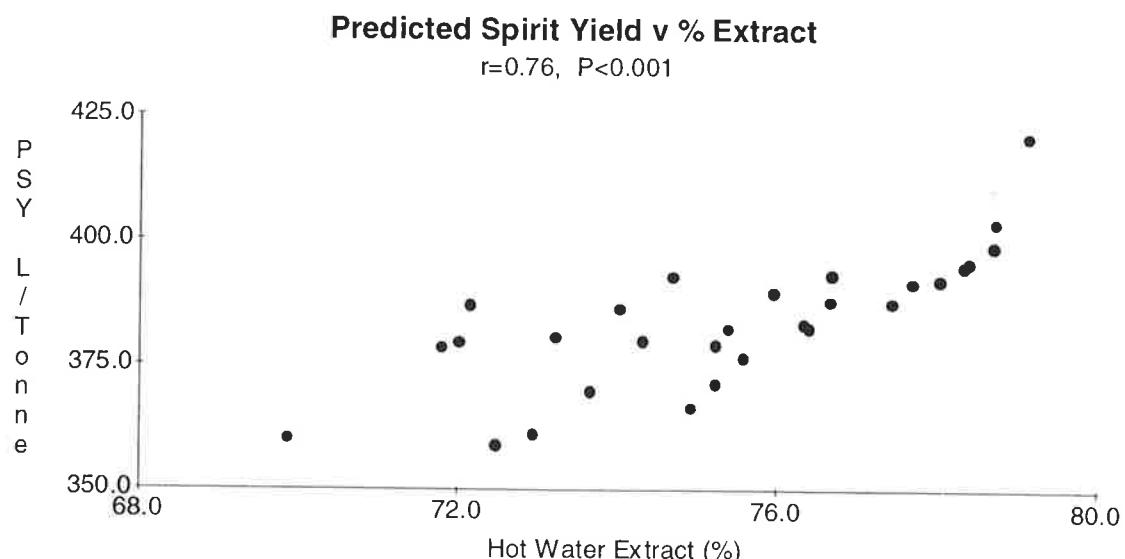


FIGURE 1. Predicted spirit yield plotted against hot water extract for 29 malt samples.

TABLE 2. Comparison of low and normal cyanogenic glycoside producing inbred lines for mean levels of grain milling energy and malt extract.

	Milling Energy (Joules)	Hot Water Extract (%)	
Low	704.3**	76.3	(**- significant at the 1% level)
Normal	734.8	76.5	

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Mechanism and inheritance of hull-cracked grains in barley. K. TAKEDA, Research Institute for Bioresources, Okayama University, Chuo 2-20-1, Kurashiki 710, Japan.

Introduction.

Recently hull-cracked grains which cause low germinability and low malt extract have been observed in malting barley varieties mainly in the western part of Japan (Hamachi et al. 1989). Hull-cracking occurs on either side of the grains in two-rowed and covered varieties but it is very rare in six-rowed varieties and wild species. The author has studied the varietal variation, mechanism and inheritance of this new marformed grain character of malting barley, and will present the summary of the study.

Materials and Methods.

A. Varietal variation: 615 two-rowed and covered barley varieties including 194 local and 421 improved ones were grown in the field. Five spikes each from three plants of the varieties were sampled and the percentage of hull-cracked grains was recorded (Kanatani et al. 1996).

B. Mechanism: Nine two-rowed and covered varieties with various percentage of hull-cracked grains were grown in eight different ripening conditions. Percentage of hull-cracked grains and 1,000-grain weight were recorded (Kanatani et al. 1996).

C. Inheritance. C-1. Diallel analysis: A reciprocal diallel set among eight two-rowed and covered varieties with various percentage of hull-cracked grains was grown in five different ripening conditions with two replications. Percentage of hull-cracked grains was recorded and was transformed into degree of angle for the statistical analysis (Takeda and Kanatani 1995).

C-2. F₂ segregation: The F₂ generation of a half set of the diallel crosses mentioned earlier was grown in the field for investigating the segregation mode of the percentage of hull-cracked grains. Each of 28 F₂ populations consisted of 100 to 150 plants (Wu et al. 1994).

C-3. Selection experiment: About 200 plants of four F₂ populations were examined for the percentage of hull-cracked grains. Then the top and bottom 5% each of the populations were selected for the percentage of hull-cracked grains and for 1,000-grain weight, respectively, and the F₃ progenies consisting of 15 plants each were raised to

examine the selection response and correlated response (Wu et al. 1995).

C-4. Family analysis: Nittakei 8 and Nittakei 22, whose percentage of hull-cracked grains were particularly high, and their pedigree were grown in the field to check the percentage of hull-cracked grains (Kanatani et al. 1996).

Results and Discussion.

A. Varietal variation: Hull-cracked grain percentage showed a markedly skewed frequency distribution pattern; 513 varieties (83% of the total) showed less than 5% hull-cracked grains, and the maximum percentage was as high as 61%. Improved varieties developed hull-cracked grains more frequently than local varieties, indicating varietal improvement indirectly caused the hull-cracked grains.

B. Mechanism: Variances due to varieties, environment and their interaction were all statistically significant. Some of the varieties developed almost no hull-cracked grains throughout the environmental conditions examined, while others sharply responded to the environmental conditions. The environmental correlation coefficient between 1,000-grain weight and percentage of hull-cracked grains was as high as 0.918, indicating that hull-cracked grains had developed under favorable ripening conditions. Primary factor of hull-cracking seemed to be the imbalance between size of hull and ripening of grain. To increase malt extract, selection for lower hull percentage has been practiced in malting barley. This has been attained by thinning hull and/or improving grain plumpness. Such breeding may be the cause of higher percentage of hull-cracked grains in the improved malting barley varieties.

C. Inheritance. C-1. Diallel analysis: The percentage of hull-cracked grains was predominantly controlled by additive genes and the dominance effect was also significant. Low percentage of hull-cracked grains was partially dominant over the high percentage. Average dominance was ca. 0.5. Epistasis among the genes was not significant. Maternal effect was absent. Heritability was about 0.9 and 0.8 in a broad and narrow sense, respectively. The percentage of hull-cracked grains was markedly affected by the different ripening conditions, but the character expression for the trait was parallel among the ripening conditions.

C-2. F_2 segregation: The percentage of hull-cracked grains showed continuous and transgressive segregations in 28 F_2 populations derived from half diallel crosses among

eight parents. Heritability of the trait in a broad sense was 0.43 to 0.80 in the 28 F_2 populations.

C-3. Selection experiment: Broad sense heritability of the percentage of hull-cracked grains estimated in the F_2 populations was 0.5 to 0.6. The realized heritability estimated from the selection response was 0.4 to 0.6. Correlation and regression coefficients between F_2 and F_3 were about 0.5. Genetic correlation coefficient between percentage of hull-cracked grains and 1,000-grain weight estimated from the correlated response was -0.1 to -0.3. Because of the high heritability of the percentage of hull-cracked grains, selection for decreasing the percentage of hull-cracked grains may be easy, and because the genetic correlation between percentage of hull-cracked grains and 1,000-grain weight is low, selection for the percentage of hull-cracked grains may induce little or no change in the 1,000-grain weight.

C-4. Family analysis: Nittakei 8, whose percentage of hull-cracked grains is 29%, was derived from a three way cross among low hull-cracking Haruna Nijo (1%)/Amsel (1%)/Seijo 17 (1%). Nittakei 22 (26%) was also derived from a three way cross among Haruna Nijo (1%)/Seijo 8 (5%)/Cambrinus (4%). These indicate that percentage of hull-cracked grains are controlled not by simple major gene(s), but by the accumulation and interaction of multiple genes controlling its component characters.

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ENVIRONMENTAL EFFECTS ON MALTING QUALITY OF BARLEY IN INDIA

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INTRODUCTION: Barley is mainly utilised for food, feed and fodder in India and about 12-15% of the barley also consumed for malting (Jindal, 1994). Mainly the six-row spring barley is cultivated, which is more suitable as feed barley. Recently a two row malting barley culture 'ALFA 93' has been recommended for commercial cultivation (Verma and Tandon, 1993). Though barley is cultivated in diverse agroclimatic zones, but mainly barley from Haryana, Northern Rajasthan, Punjab and Western U.P. is preferred for malting (Bhatnagar, 1994). There is no information available on proper production technology, suitable environments/cultivars for malt barley. The present study was undertaken to generate preliminary information on varieties and suitable environments for malting barley cultivation.

MATERIALS AND METHODS: Eleven genotypes (six strains of two-row and five strains of six-row type) of barley were cultivated at four locations during winters of 1993-94 and 1994-95. The grain samples from each location were analysed for various malting quality parameters.

RESULTS: The results have been summarised in table 1 and table 2 for varietal performance and environment/location effects, respectively. The following observations can be made from these results.

- The genotypes BCU 73 and DWR 2 in two row barley and RD 2503 in six row type are suitable for malting, as indicated by their husk(%), Protein(%), TCW, Thin grain(%), Diastatic power (D.P.) and hot water extract (HWE). PL 419 is also desirable for malting except slightly lower HWE recovery.
- Amongst the genotypes studied, there was not much difference between the two row and six row barley for most of the parameters, except husk content, T.C.W. (grain and malt). The advantage of two row barley for various parameters like low protein, higher malt extract, more proportion of bold grains etc., could not be established under Indian conditions. However for husk content, T.C.W. of barley grain as well as of malt, and upto some extent the proportion of thin grains (<2.2mm), the two-row barley definitely had an edge over the six-row type.
- The effect of environment on malting quality (table 2) is quite evident, that Hisar has the much needed ideal environment for the production of malt barley. Karnal and Durgapura are also equally suitable with all desirable quality traits except slightly low HWE.

Kanpur location seems to be unsuitable for the cultivation of malting barley with undesirable levels for husk content, T.C.W. (grain), proportion of thin and bold grains, T.C.W. (malt) and H.W.E. All these factors put together makes the barley cultivated there, unsuitable for malting and more suitable as feed barley.

DISCUSSION: The spring type, early maturing six row barley with high grain yields is cultivated in India for feed, fodder & human consumption, and the interest in malting type barley

(that too in two-row type) is very recent in origin. The material under study includes the exotic two-row barley genotypes, (most of them are late maturing and semi winter type in growth habit) and indigenously bred six-row improved genotypes. The comparision of two and six row barley seems to be affected by these factors. The early maturing two-row genotypes BCU 73 and DWR 2 performed satisfactorily, while others like Clipper, Alfa 93, DWR 1 etc. with late maturity could not express fully at locations like Kanpur and Durgapura, resulting in no specific advantage for malting qualities and yield in two row barley. The poor performance of Clipper and Alfa 93 for characters like T.C.W. (grain), proportion of thin and bold grains, T.C.W. (malt), put the rest of two row type at par with six-row barley. However, varieties like RD 2503 and PL 419 in six-row type are comparable to any of the two row barley for malting qualities. Therefore, It is necessary to develop the two-row barley with spring growth habit and local adaptation, to make its cultivation competitive with six-row type for grain yield with advantage in malting qualities for which enogh genetic variability is available (Verma and Nagarajan, 1994).

The environment seems to play an over riding influence on the malting quality of barley as has been observed and our results indicate that there are locations in India such as southern Haryana (Hisar) where high quality malt barley can be cultivated. The environmental conditions at Kanpur, affected adversely the malting quality of the grain.

ACKNOWLEDGEMENTS

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Table 1: Performance of barley genotypes for malting quality parameters at 4 locations during 1993-94 and 1994-95.

Genotypes	BARLEY						MALT				
	Husk (%)	TCW (g)	Protein (%)	Thin grains (<2.2 mm) (%)	Bold grains (>2.5 mm) (%)		G.C. (%)	T.C.W. (%)	D.P. (^L)	Malt yield (%)	H.W.E. (%) (d.b.)
2-ROW BCU 8	10.9	46.6	10.5	2.28	86.5		99.2	41.5	106	86.1	74.5
BCU 73	11.1	59.3	9.9	1.82	90.2		98.4	52.0	107	85.5	75.5
DWR 1	11.1	43.0	9.8	3.99	78.9		98.4	38.0	104	85.2	75.3
DWR 2	10.9	57.7	9.6	2.01	88.6		98.0	51.2	102	85.0	75.7
CLIPPER	12.5	40.9	10.2	7.48	70.0		99.1	36.3	103	85.4	75.0
ALFA 93	10.2	40.0	10.3	3.99	80.9		98.5	35.0	100	85.1	75.3
Mean	11.1	47.9	10.0	2.80	82.5		98.5	42.5	104	85.4	75.2
6-ROW RD 2503	11.7	46.2	9.1	1.93	89.4		97.9	40.5	110	85.8	76.0
RD 2511	13.6	41.2	8.7	6.50	72.3		98.2	36.4	106	85.9	74.5
K 508	14.0	39.1	9.3	5.48	68.3		98.8	34.6	103	86.3	74.9
PL 419	11.9	49.0	9.5	1.59	93.8		96.7	42.6	100	85.3	74.5
DL 88	14.7	46.8	8.8	2.80	87.1		99.1	40.9	95	85.9	74.4
Mean	13.2	44.5	9.1	3.20	82.2		98.1	38.9	103	85.9	74.9

Table 2: Effect of environment on malting quality.

Parameters	Locations			
	Karnal 29°N 77°E	Hisar 29°N 75°E	Durgapura 27°N 76°E	Kanpur 27°N 81°E
Barley				
Husk (%)	11.4	11.7	11.1	14.3
T.C.W. (g)	46.6	46.6	47.0	39.3
Protein(%)	9.6	8.0	10.0	10.8
Bold grain(%) (>2.5 mm)	84.4	91.9	87.9	70.7
Thin grains(%) (<2.2 mm)	3.3	1.48	2.6	7.81
G.C. (%)	98.7	97.8	99.1	98.7
Malt				
T.C.W.(g)	41.0	42.1	44.6	34.8
D.P.(L)	108	88.0	108	112
Malt yield(%)	85.2	86.3	86.0	84.9
H.W.E.%(d.b.)	75.9	77.0	75.0	72.6

-- Undesirable values are shown in bold figures.

-- Results pooled over two years on 11 genotypes.

Section IV: Nutritional Quality 1 – Food and Industrial

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Inheritance of oil content and composition in oat (*Avena sativa*). C.I.
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Introduction

The genetic control of oil content and composition has been elucidated for a variety of crops. Several previous studies have shown that total oil content is a highly heritable trait that is polygenetically inherited and whose gene action is largely additive (Kalbasi-Ashtari and Hammond, 1977; Thro and Frey, 1985). This makes oil content a good candidate for improvement by breeders.

The inheritance of total oil content is a function of the genetic control over the accumulation of constituent fatty acids. The fatty acid profile of the oat oil may be as important as the total oil content to end users because the composition of the oil is important in determining its stability and nutritive value. The objective of this study was to attempt to correlate differences in oil content between the oat cultivars A.C. Marie and Cascade to differences in the relative amounts of specific fatty acids.

An increase in understanding of the basis of variation for oil content is a necessary step towards the identification of molecular markers for the genes involved.

Materials and Method

An F₈ population consisting of 223 bulked lines from a cross between the high oil cultivar A.C. Marie and the low oil cultivar Cascade was grown in three reps at each of three sites in Manitoba in 1995.

To determine the total oil content of these lines, approximately 20 g of whole oats were ground to a coarse flour texture. A 4 g sample was placed in a Labconoco lipid extractor where it was processed for 7 hours using petroleum ether (35 to 60°C bp) as a solvent. The solvent was evaporated off and the resulting oil weighed to determine total oil content.

The fatty acid composition was determined by converting a 50 µl sample to fatty acid methyl esters using 2 ml of a 2 percent concentrated sulfuric acid in methanol (v:v) solution as a catalyst. This was allowed to react at room temperature for several hours. The resulting methyl esters were analysed in a Hewlett Packard 5890 gas chromatograph using a Supelco Wax 10 column (15 m x 0.32 mm). The resulting peaks were identified by comparison to known standards and the relative amounts of individual fatty acids were determined by comparing peak areas.

Results and Discussion

The total oil content and oil composition for the 223 F₈ lines from the A.C. Marie x Cascade cross were determined as described above and are shown in Figure 1. The average values for the parents are shown in Table 1. A wide range of values for overall oil and individual fatty acid content were found which indicates a high degree of variability between the F₈ lines. The distribution found on each histogram is roughly normal and thus indicate that the inheritance of each individual fatty acid is polygenetically controlled.

The histograms for total oil, palmitic acid, oleic acid and linoleic acid all exhibit transgressive segregation. This would appear to indicate that the parents contain different loci that contribute to the increased accumulation of individual fatty acids.

Regression analysis of the individual fatty acids showed no significant correlation between them. There was a strong correlation between the total oil level and specific fatty acids although intuitively it is expected that the amount of a specific fatty acid will increase as the overall oil content increases.

Alternatively, the apparent transgressive segregation may be induced by environmental effects. None of the transgressive segregants exhibit values outside of a standard error of one of the parental means.

It certainly appears that oil content is polygenetically inherited in this cross. The next step in this project will be to locate genetic markers for these loci using associated fragment length polymorphisms (AFLP*) to create a map of the cross and then correlate the phenotypic data to the genotypic data to determine if any of the AFLP sites are linked to the traits of interest.

* Trademark of Keygene Inc.

Table 1. Total oil content and specific fatty acid content means, in mg, for the parental cultivars A.C. Marie and Cascade. Means are plus or minus the standard deviation indicated.

Cultivar	Total oil	Palmitic Acid	Oleic Acid	Linoleic Acid
A.C. Marie	282.4 ±10.3	54.5 ±5.8	98 ±5.8	112.3 ±5.5
Cascade	130.6 ±16	25.5 ±4.1	36.4 ±6.7	56.7 ±8.1

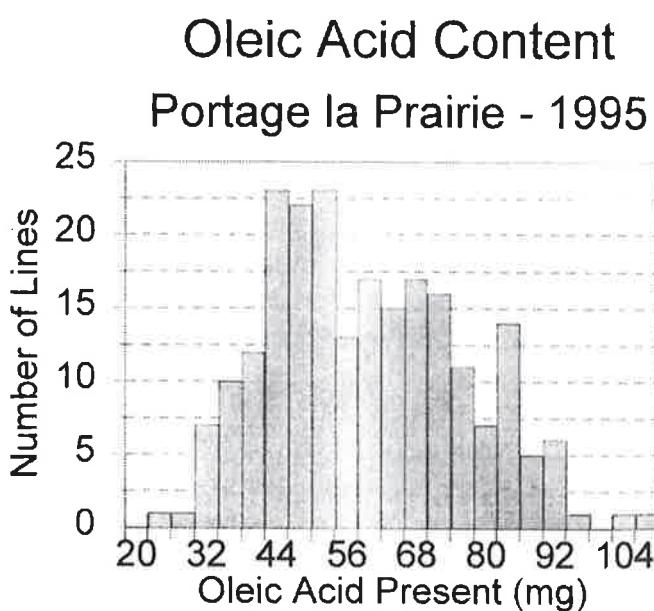
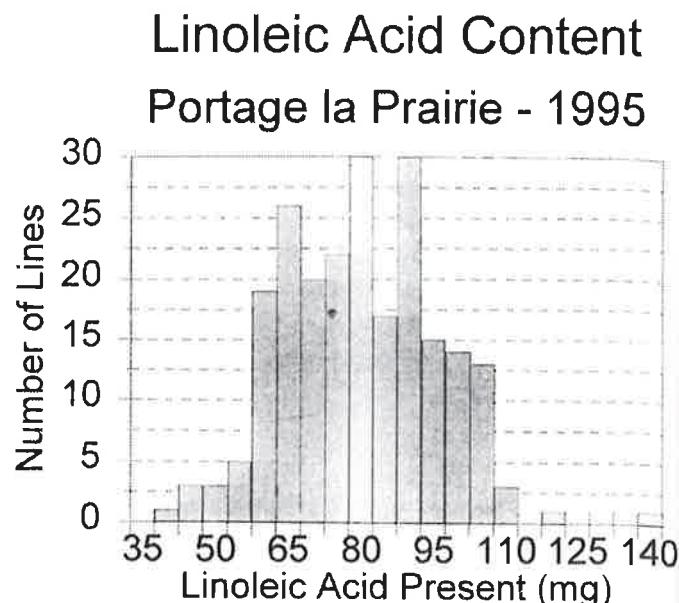
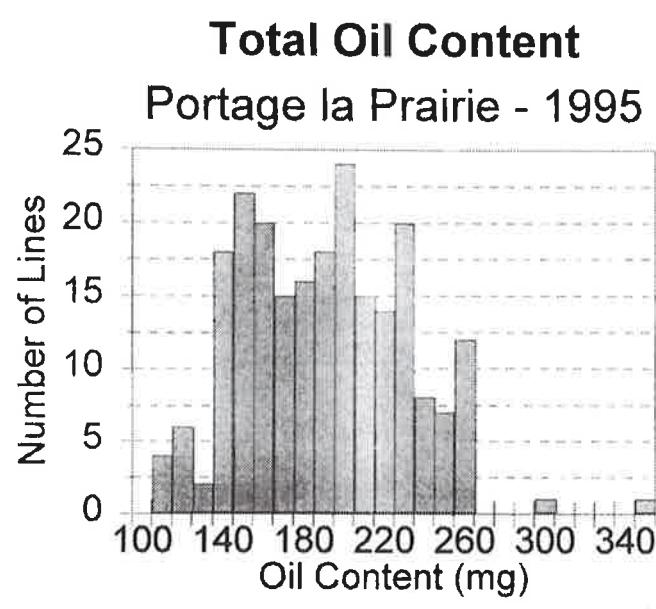


Figure 1. Total oil and specific fatty acid content, in mg, of 223 F₈ lines of the A.C. Marie x Cascade cross.

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Milling of hull-less barley for the production of bran and flour.

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Introduction. Traditionally, hulled or hull-less barley (HB) has not been roller-milled, like wheat and oats, to produce bran and flour. Rarely have true barley bran and flour been produced and investigated. Barley reacts differently than wheat to conventional roller-milling. The bran is brittle and shatters, regardless of tempering conditions, unlike wheat bran which separates as large stable flakes. Because of brittleness of barley bran, roller-milled HB flour has higher ash content and is sometimes darker than wheat flour milled under identical conditions.

However, HB can be dry-milled or milled after tempering with equipment routinely used for wheat milling (McGuire 1987; Bhatty 1987, 1993). Milling yields of 16 diverse genotypes of barley, tempered to 13% moisture and milled in a Buhler mill, varied from 51 to 72%; one sample of barley milled in a Miag-Multomat Mill yielded 63% flour, 23% shorts and 13% bran (McGuire 1979). Milling of hulled and hull-less barleys under different tempering conditions in an Allis-Chalmers experimental mill gave flour yields of about 72%; the bran and shorts, which were not separated, yielded the balance 28% of the recovered product (Bhatty 1986, 1987). It is thus clear that on roller-milling of barley, bran does not separate from the short fraction; part of it also appears in the flour fraction.

Several cultivars of HB have been registered in Canada, including those containing low amylose or waxy starch. The waxy cultivars yield bran and flour fractions having unique starch properties. The objective of the present study was to determine the milling behavior of regular and waxy starch barleys and the physico-chemical properties of the roller-milled bran and flour fractions.

Materials and Methods. Two Canadian registered cultivars of hull-less barley CDC Candle and CDC Dawn, and one registered cultivar of soft white wheat AC Reed were used in the milling study. CDC Candle was a waxy starch and CDC Dawn a regular starch HB. The wheat cultivar was included for comparison.

Milling. One kg samples of HB and wheat were milled dry (as-is moisture), after drying (only HB) or tempering in a Buhler mill to obtain in each case eight fractions; three break flour, three reduction flour, bran and shorts. The break and reduction fractions were combined to obtain total flour and the bran and shorts combined to obtain the bran, and expressed as percent of the recovered product.

Analyses. Grain hardness was determined with the Brabender microhardness tester; color with the Hunterlab ColorQuest spectrophotometer; starch damage, protein, starch, β -glucan, ash, water-holding capacity and farinograph absorption using the AACC official procedures (1995).

Results and Discussion. The two HB cultivars were softer than the AC Reed

wheat and had grind times of 68 to 98 sec compared to 47 sec for wheat; the longer grind time indicates softer grain. The two HB contained 13 to 16% protein ($N \times 6.25$), 62 to 75% starch and 5 to 7% β -glucan; the higher value of β -glucan was for the waxy cultivar, CDC Candle. AC Reed wheat contained < 1% β -glucan.

Milling yields of total flour varied considerably between the two HB, and were related to grain hardness which was varied by adjusting its moisture content. Data in Table 1 show grain moisture, hardness, total flour and bran yields in CDC Dawn and CDC Candle. At every moisture level, the total flour was higher and the bran lower in the waxy cultivar, CDC Candle than in CDC Dawn. In CDC Dawn the as-is grain moisture (dry milling) was 9%, and at this moisture total flour yield was 60% and the bran yield 40%. In CDC Candle, the total flour and bran yields at the as-is moisture of 8% were 38% and 62%, respectively. Increasing the grain moisture from 9% to 16% by tempering decreased the flour yield from 60% to 47% in CDC Dawn, but only from 38% to 34% in CDC Candle. Conversely, total flour yields were increased in both the cultivars by drying the grain to 5% moisture. The increase in flour yield was higher in regular starch (CDC Dawn) than in waxy starch HB. The changes in flour yield on adjusting the grain moisture levels were largely due to changes in the reduction flour fraction. The HB became hard on drying and soft on tempering. Thus, within a HB cultivar, flour yield was greatly influenced by grain moisture, which in turn affected grain hardness.

The composition of HB flour was compared with that of AC Reed wheat flour milled under identical conditions. HB flour was nearly as white as wheat flour, had higher ash and β -glucan, particularly CDC Candle flour. Starch damage, although higher than in wheat flour, was low. The barley flour had higher water, mixograph and farinograph absorptions, particularly CDC Candle flour due to its higher β -glucan content.

Conclusions. Although whole grain products such as flakes, grits and pearls may be commercially produced and used in food products, roller-milled barley products such as bran and flour have the most potential in food applications. Hull-less barley may be dry milled (as-is moisture) using conventional equipment used for wheat milling. The bran may be used in ready-to-eat cereals, high fibre bakery products such as cookies and muffins. Barley flour may be blended with wheat flour for making pastry, cookies, muffins, cakes and flatbreads, or used as food thickener. Data are reported here which show that regular starch HB (CDC Dawn) milled quite differently than the waxy starch HB (CDC Candle). Yields of flour (break and reduction) and bran (shorts and bran) largely depended on grain moisture (5 to 16%) which affected grain hardness. The HB flour from the two cultivars was white, had higher ash, β -glucan, starch damage, mixograph and farinograph absorptions than the AC Reed wheat flour milled under identical conditions.

Table 1. Milling Yields of CDC Dawn and CDC Candle Cultivars of Hull-less Barleys at Various Moisture Levels

Cultivar	Moisture, %	Hardness, sec	Flour yield, %	Bran yield, %
CDC Dawn	5	24.5	85.3	14.7
	7	28.0	77.2	22.8
	9*	67.5	59.6	40.4
	12	126.5	56.7	43.3
	14	240.5	49.1	50.9
	16	413.0	47.4	52.6
CDC Candle	5	43.0	65.7	34.3
	7	47.0	51.4	48.6
	8*	98.0	38.4	61.6
	12	174.5	33.7	66.3
	14	389.5	32.7	67.3
	16	404.5	34.1	65.9

* Milled as-is

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Image analysis for assessing effects of seeding rate and disease control on oat grain quality. G.M. BRIDGER, C. CHUNGU and D.E. MATHER, Department of Plant Science, McGill University, 21111 Lakeshore, Ste-Anne-de-Bellevue, Québec H9X 3V9, Canada.

Introduction: Plump, uniformly sized oat grain is preferred for milling. Conventional indicators of grain quality include test weight, groat percentage, and average grain weight. These are known to differ among oat cultivars, and to be affected by environmental conditions during oat production. Symons and Fulcher (1988) have demonstrated that image analysis can be used to assess within-sample variation for grain size attributes, including the area, perimeter, length, and width of individual grains. Here, we used image analysis to assess the effects of two management factors (seeding rate and fungicidal control of crown rust) on three oat genotypes.

Materials and Methods: Field experiments: Experiments were conducted at Ste-Anne-de-Bellevue, Québec and Winchester, Ontario in 1994 and 1995. Experiments were arranged as split-plot factorials with four blocks. Genotypes (the cultivars Newman and Ultima in 1994; Newman, Ultima and the breeding line OA961-2 in 1995) formed the main-plot units. A factorial arrangement of seeding rates (250, 350, and 450 seeds m⁻²) and fungicide treatments (0 or 125 g a.i. ha⁻¹ propiconazole) was randomized to subplots. Ultima is susceptible to crown rust (*Puccinia coronata* Cda. f. sp. *avenae* Eriks.). Newman was resistant when it was released but its resistance is not effective against all currently predominant races of *P. coronata*. OA961-2 is resistant to predominant races of *P. coronata*. Propiconazole was applied with a backpack sprayer prior to the onset of crown rust symptoms. Each subplot consisted of a 4.32-m² 4-row plot at Winchester, and a 3.42-m² 6-row plot at Ste-Anne-de-Bellevue. Plot ends were trimmed prior to harvesting. At Ste-Anne-de-Bellevue, the 4 centre rows of each plot were harvested by plot combine. At Winchester, the 2 centre rows of each plot were hand harvested, then threshed. Grain yield was adjusted to 0% moisture and converted to kg ha⁻¹. Groat percentage was determined by passing a clean 50-g sample once through a Quaker Oats laboratory-sized impact dehuller. Test weight was determined as the weight of 100 mL of grain converted to kg hL⁻¹.

Image analysis: Two samples of grain were examined from each subplot. Each sample consisted of about 100 grains placed flat onto the sticky surface of a 12 cm × 12 cm sheet of clear tack paper, such that grains were not in contact with one another. Samples were placed on a light-stand with substage illumination. A camera mounted to a light stand and connected to a Leco 2001 image analyzer (Leco Instrument Ltd, Mississauga, Ontario) was used to capture the silhouetted images. Spatial resolution was 0.29 mm/pixel. Each grain was automatically identified and measured for area, perimeter, length, width, roundness ($4\pi \times \text{area}/\text{perimeter}^2$), and aspect ratio. Imaging data from the 2 samples per subplot were combined, then means and standard deviations were calculated and subjected to analysis of variance. Simple correlation coefficients of imaging variables with groat percentage and test weight were calculated.

Results and Discussion: In 1994, post-anthesis crown rust symptoms were severe on unprotected subplots at both sites. Fungicide application was effective in suppressing crown rust, and it resulted in higher grain yields, test weights and groat percentages. At Ste-Anne-de-Bellevue (Table 1), fungicide application resulted in increased grain area and width, and reduced variability of width (more uniform grain size). At Winchester (data not shown), there were no significant changes in grain morphology in response to the fungicide application, but increasing the seeding rate increased test weight and grain roundness, and reduced grain length and aspect ratio.

In 1995, the development of crown rust was delayed by a 5-week drought from the end of tillering until flowering. Crown rust symptoms appeared on Newman and Ultima at the mid-milk stage. Symptoms were light to moderate, and sporadic, at Winchester and light at Ste-Anne-de-Bellevue. At Winchester, grain yield, test weight and groat percentage were not affected by seeding rate but were increased by fungicide application. Improved grain quality was associated with a reduction in grain perimeter and length, and an increase in roundness. At Ste-Anne-de-Bellevue (Table 1), application of fungicide affected grain characteristics of Newman, increasing grain size and reducing grain size variation. Increasing the seeding rate had similar effects.

Table 1. Means (and *mean standard deviations*) of 4 image analysis variables and means of grain yield, test weight and groat percentage of oat grain grown with and without application of propiconazole fungicide at Ste-Anne-de-Bellevue in 1994 (averaged across the cultivars Newman and Ultima) and in 1995.

Fungicide application:	1994		1995					
			Newman		OA961-2		Ultima	
	No	Yes	No	Yes	No	Yes	No	Yes
Grain area (mm ²)	23.1 4.7	23.8 4.7	13.9 4.6	15.1 3.8	16.0 5.0	16.1 5.0	12.5 4.0	13.0 4.0
Grain length (mm)	10.2 1.1	10.3 1.1	9.5 1.6	9.9 1.3	9.9 1.7	9.9 1.6	9.0 1.4	9.1 1.4
Grain width (mm)	3.21 0.40	3.26 0.38	2.46 0.47	2.54 0.43	2.68 0.50	2.67 0.49	2.34 0.46	2.41 0.45
Grain aspect ratio	3.20 0.31	3.18 0.31	3.91 0.57	3.96 0.58	3.74 0.55	3.74 0.54	3.89 0.61	3.89 0.59
Grain yield (kg ha ⁻¹)	1943	2399	2192	2165	2057	2015	2092	2203
Test weight (kg hL ⁻¹)	46.3	52.3	52.0	52.2	56.0	56.4	49.8	50.3
Groat percentage	75.0	77.5	75.1	74.9	76.2	76.4	73.3	73.6

Means in **bold font** indicate a significant effect of fungicide application ($P < 0.05$).

Grain quality differences among cultivars were consistent among environments. Test weight and groat percentage were highest for OA961-2, followed by Newman, then Ultima, except at Winchester in 1994, where there were no cultivar differences. Image analysis consistently showed that OA961-2 grain was larger and had a lower aspect ratio than Newman grain. Grains of Newman were larger than those of Ultima, but grains of Ultima were more round and had a lower aspect ratio than those of Newman.

At Ste-Anne-de-Bellevue, grain area, length and width, and the standard deviation of aspect ratio were strongly related to test weight and groat percentage (Table 2). The only correlation that was significant in all environments was a negative correlation between the standard deviation of aspect ratio and groat percentage.

Table 2. Correlation coefficients of treatment means for 4 image analysis variables and their *standard deviations* with treatment means for grain yield, test weight and groat percentage of oat grain grown in a cultivar-fungicide-seeding rate experiment at Ste-Anne-de-Bellevue in 1994 and 1995.

	Grain area		Grain length		Grain width		Aspect ratio	
	1994	1995	1994	1995	1994	1995	1994	1995
Test weight	0.86 0.62	0.86 0.53	0.82 0.74	0.70 0.36	0.85 -0.56	0.88 0.35	-0.57 -0.76	-0.74 -0.76
Groat percentage	0.87 0.62	0.84 0.52	0.83 0.74	0.73 0.37	0.87 -0.58	0.85 0.29	-0.57 -0.77	-0.65 -0.74

Correlation coefficients presented in bold font are significant at P < 0.05.

Conclusions: Image analysis consistently detected differences among cultivars over a range of environments. In a year of severe crown rust, management-induced grain quality differences were detected by test weight and groat yield, but these were not consistently associated with changes in grain morphology. In a droughty year with light to moderate crown rust, management-induced quality differences were detected by image analysis, but these were not consistently associated with changes in test weight or groat percentage. Based on these results, image analysis does not seem useful as a predictor of conventional measures of grain quality, but it provides complementary information, allowing the detection of minor but potentially important differences in grain morphology and uniformity. It can also be used to reliably assess grain quality differences among cultivars.

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Tocochromanols: tocotrienols and tocopherols from *Hordeum vulgare* L. grains. M.L. COLOMBO¹, A. CORSINI¹, A. MOSSA¹, L. CATTIVELLI², A.M. STANCA², and R. PAOLETTI¹, ¹Institute Pharmacological Science, University of Milan, I-20133, Via Balzaretti, 9, Milano, Italy; ²Experimental Institute for Cereal Research, Section of Fiorenzuola d'Arda, I-29017, Via S.Protaso, 302, Fiorenzuola d'Arda (PC), Italy

Introduction. Barley grains and palm oil are the richest natural source of vitamin E (tocochromanols = tocopherols and tocotrienols). The potential activity of such compounds is of great interest and the biological role of these derivatives in human nutrition and metabolism has been extensively studied (Schuep and Rettenmaier, 1994). There is a lower tendency to develop atherosclerosis, among peoples whose diet is rich in cereal grains (Quershi et al., 1986). Oily barley extracts have been discovered to have an interesting antioxidant activity, to lower serum lipids and cholesterol levels (Newman and Newman, 1992). The inhibition of cholesterol biosynthesis is a good approach to the reduction of Low Density Lipoprotein (LDL), a key risk factor in coronary heart disease and thrombosis. The tocotrienols are structurally related to the lipid-soluble antioxidant vitamin E (tocopherols) and are the intermediates in the biosynthesis of a-tocopherol. It would appear that methylation in the number and position on the chromanol ring occurs before saturation of the side chain (Pennock et al., 1964). Tocotrienols decrease the activity of 3-hydroxy-3-methyl glutaril CoA reductase (HMGR), the enzyme rate-limiting in the biosynthesis of cholesterol, with a mechanism controlling the HMGR degradation (Khor et al., 1995). Tocotrienols show different hypocholesterolemic activity, highly active are those lacking 5-methyl substitution. The chirality at C-2 appears to play a minor role for inhibitory activity (Pearce et al., 1994). Although tocotrienols are known to possess a lower biological vitamin E activity than tocopherols, recent findings suggest that a-tocotrienol is a better antioxidant than a-tocopherol. The tocopherols were nearly always considered for their antioxidant activity. The antioxidant effect of g- and d-tocopherols was in general much higher than that of a- and b- tocopherols. In comparison with fully methylated tocochromanols, the dimethyltocols show 40 % of potency and monomethyltocols only 7.5% or minus. These data may be approximate, since the values vary appreciably with the method of assay used. Although the antioxidant function of tocopherols is well known, its mechanism of action is not fully elucidated (Paz Sanchez-Migallon et al., 1996). Recently has been reported a different biological activity of d-a- and d-b-tocopherol on vascular smooth muscle cell, in spite of the strong similarity between the two molecules. d-a-tocopherol inhibits cell proliferation, whereas d-b-tocopherol prevents the inhibition of cell growth caused by d-a-tocopherol (Tasinato et al., 1995). Because humans and most animals are unable to synthesize vitamin E, they have to rely an other source to obtain it. Supplementation of foods is then an alternative to provide an adequate vitamin E supply. In light of these findings, tocochromanols are a major biological antioxidant and cytostatic compounds with significant changes in cell differentiation, alteration of cell membranes and increased autophagic activity (Lupulescu, 1993). In order to obtain the tocochromanol fraction, a lipophilic extraction is required. Several techniques have been developed to obtain tocotrienol fraction from vegetable material using traditional solvent extraction methods (Qureshi et al., 1986; Peterson and Qureshi, 1993). The developed extraction method provided the use of supercritical CO₂, leading to high purity and productivity tocotrienol concentrates from *Hordeum vulgare* L.

cultivars (Colombo et al. 1995). There is much interest in separating naturally - occurring compounds for human nutrition and health using supercritical CO₂, considering that there aren't traces of organic solvents. Carbon dioxide is a useful supercritical solvent because it is non-toxic, inexpensive, non-flammable and environmentally acceptable; and it has a great potential for the extraction of hydrophobic compounds biologically related to isopentenyl PP having an isoprenoid moiety. The recovered lipophilic fraction was examined in its components by means of MS spectrometry and the activity of the different fraction was tested *in vitro* on rat aortic myocytes.

Materials and Methods. The barley caryopses were obtained from replicated yield trials (Experimental Institute for Cereal Research, Fiorenzuola d'Arda, PC, Italy) from following barley genotypes : Proctor, Nudinka, Fior 1879, Asso, Trebbia and Gitane. To identify the tocopherols and tocotrienols free and/or in the esterified form (Van Niekerk, 1973), the analyses were carried out, according to literature data (Govind Rao and Perkins, 1972), either on the total lipidic fraction or on the unsaponifiable fraction. The analyses of the extracts were carried out on an analytical (Si 60) straight phase HPLC equipped with a Beckmann multichannel UV system (190 - 600 nm) coupled with a Perkin Elmer fluorescence spectrophotometer. The HPLC analyses were also performed on an analytical reverse phase (RP 18) and monitored using an ESA electrochemical coulometer detector. The components of the HPLC chromatograms were separated by semipreparative column (250 x 10 mm) HPLC on straigh phase (Si 60) and their structure analyzed and compared by different MS spectrometry tecnicas : Electrospray, Field Desorption, FAB, Chemical Ionization.

Results. The extraction method employed in this work (supercritical CO₂ process) yields an high selectively tocochromanols fraction without preliminary treatment of the samples, which can be necessary in other methods. The determination of vitamin E active components does not require a saponification step. The lipophilic extract was monitored by UV Photo Diode Array and fluorescence HPLC detectors and by UV multichannel and electrochemical coulometric HPLC detectors. The fluorescence and the electrochemical detectors are highly selective, and the unspecific peaks are eliminated. The coulometric efficiency allows a complete voltammetric resolution of analytes as a function of their reaction potential. In the same straight phase by means of a semipreparative HPLC system in isocratic conditions, the separation of seven / eight tocochromanols from six genotypes was achieved. The identity of the compounds was studied by means of mass spectrometry. The MS analyses revealed the presence of tocotrienols, tocopherols and other related compounds with higher molecular weight. In order to better understand the nature of the compounds which occur in the tocochromanols purified peaks with a molecular weight higher than vitamin E constituents, the results of MS analyses obtained with different tecnicas were compared. As first result of our work we can suppose that in barley grains occurs the formation of tocochromanols and of their dimers which are able to inhibit cell proliferation of rat aortic myocytes.

Conclusions. Barley contains almost all the known tocopherols and tocotrienols. There is much interest in separating natural compounds for human nutrition and health using solvent - free techniques, such as supercritical CO₂. In the present study we anlyzed the

tocochromanol fraction obtained from *Hordeum vulgare* L. cultivars genetically characterized. The tocopherol mixture, monitored by means of HPLC fluorescence and HPLC electrochemical detectors, was separated in its components. The MS data of the purified compounds revealed the presence either of tocopherols and tocotrienols as of other compounds with a higher molecular weight.

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Polymorphisms within waxy gene in indigenous barley cultivars revealed by the polymerase chain reaction. Eiji Domon, Barley breeding laboratory, Shikoku National Agricultural Experiment Station, 1-3-1 Sen'yu-cho, Zentsuji, Kagawa 765, Japan

Introduction. The starch component in endosperm is important in grain quality of barley. The barley waxy gene (*Wx*) is known to code for a granule-bound starch synthase (GBSS), which catalyzes amylose biosynthesis in endosperm of developing kernels and in pollen grain. Waxy mutants have glutinous endosperm composed of lower amylose starch. Recently, Ishikawa *et. al.* (1994) surveyed amylose content of endosperm starch in indigenous waxy barley cultivars and reported that all cultivars studied showed lower amylose content ranging from 2.4 % to 10 % compared with non-waxy cultivars of around 25 %. They also conducted SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and revealed that *Wx*-protein was considerably reduced in indigenous waxy cultivars. It is suggested that *wx* allele from indigenous cultivars may conserve intact waxy structural gene which maintains GBSS activity but its expression may partially be suppressed by structural alteration or a point mutation in the regulatory region.

In the present study we investigated 5' leader sequence of waxy gene from cultivated and wild barley with the polymerase chain reaction (PCR) technique. The PCR products from indigenous waxy cultivars and some non-waxy cultivars showed length polymorphism indicating some structural alteration between the two primer sequences. DNA sequencing of cloned PCR products revealed that, in the *wx* allele, there were a deletion including whole exon I and an insertion located in the intron 1. The inserted sequence was completely conserved between novel *Wx* allele and *wx* allele.

Materials and Methods. Two sets of *Wx/wx* near-isogenic lines were used, one set is Shonupana and Washonupana, another is Oderbrucker and Waxy Oderbrucker. 63 accessions of cultivated barley (*Hordeum vulgare* L.) and 3 accessions of wild barley (*Hordeum vulgare* ssp. *spontaneum*) were used for investigating genetic diversity in waxy gene.

The PCR was processed at 94 °C for 4 min, at 52 °C for 2 min, and at 72 °C for 2 min for 1 cycle, followed by 34 cycles at 94 °C for 1 min, at 52 °C for 2 min, and at 72 °C for 2 min for 1 cycle, with a final extension step of 72 °C for 2 min. The primers designated p-197 (5'-CAA ACA GAC GAC AAG CGG AG AA-3') and p+606 (5'-TAG AAA AAG AAA AAC ATC AAG CA-3') were used.

The amplified DNA fragments were separated by electrophoresis, cloned and sequenced with Taq DyeDeoxy Terminator Cycle Sequencing Kit on ABI PRISM 377 DNA Sequencing system (Perkin Elmer).

Results and Discussion. The two primers used for PCR were based on a barley waxy gene already reported as X07391 and the target sequence was 803 bp long, spanning from 197 bp upstream of transcription starting point (position +1) to 606 bp downstream within the intron 1. When two primers, P-197 and P+606, were used with genomic DNA from *Wx/wx* near-isogenic lines, the PCR products of about 800 bp and 600 bp were amplified. A DNA fragment of predicted size (around 800 bp) was present in the two *Wx* near-isogenic lines, while 600 bp fragment was amplified instead of 800 bp fragment in the two *wx* near-isogenic lines. This result indicates that these 800 bp and 600 bp fragments are both specific for the waxy loci and clearly correspond with *Wx* and *wx* alleles respectively. A deletion mutation was

strongly suggested to have occurred in this region of *wx* allele.

Indigenous *wx* cultivars have much lower amount of *Wx*-protein with the same molecular weight of 60 kD than *Wx* strains (Ishikawa et. al. 1994). Therefore, the *wx* allele from these cultivars should still have conserved intact GBSS with amylose synthase activity. On the other hand, Rosichan et. al. (1979) construct a fine genetic map of waxy locus using an indigenous and some waxy mutants induced by sodium azide and they indicated that some sort of mutation lies in the terminal region of *wx* allele from indigenous waxy cultivar.

Screening 63 cultivated and 3 wild accessions, 3 types of alleles were found. In all indigenous waxy cultivars, 600 bp fragment were amplified and 800 bp and 1000 bp fragments from non-waxy cultivars. All two-rowed type showed 800 bp fragment and the 1000 bp fragment was observed in six-rowed type. Apparently, an insertion mutation should have taken place in the target sequence of 1000 bp fragment from these *Wx* cultivars.

The DNA sequence of these PCR products are aligned in Fig.1. The deletion in *wx* allele was 403bp, spanning from position -149 bp to position +254. This result shows that the whole exon 1 of the *Wx* allele was located within the deleted region and that the loss of original transcription starting point in *wx* allele probably cause the reduction of the *Wx*-protein in indigenous waxy cultivars.

The insertion found in novel *Wx* allele and *wx* allele was located within the intron I. The underlined sequence of AAGAAA of X07391 and the PCR product from Shonupana was changed in these alleles, instead of 2bp sequence GA, the 193 bp sequence with three initiation codon was inserted (Fig. 1). In addition, another 5 bp sequence TTGGA was inserted at 30 bp upstream of *Nar*I site in the intron I, which was not found in this part of X07391.

These structural alterations suggest that the *wx* allele was originated from the *Wx* allele with two successive insertion/deletion events. First, the insertion mutation in the intron I was happened, and then the deletion mutation changed the phenotype of the endosperm.

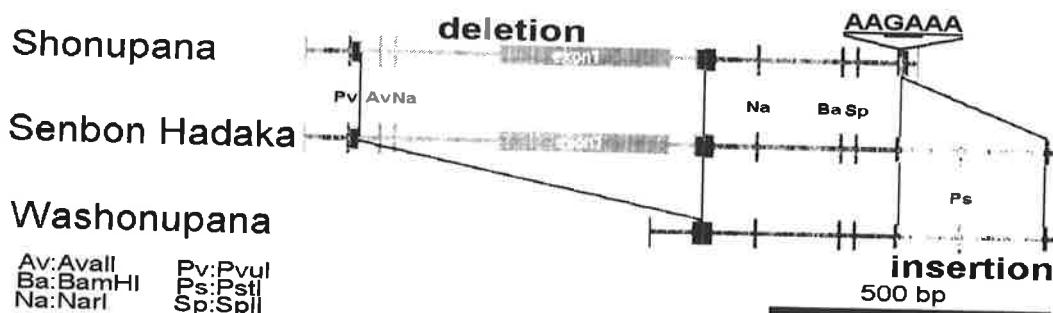


Fig. 1 Schematic representation of the PCR products from cv. Shonupana (top), cv. Senbon Hadaka (center) and Washonupana (bottom). Upper two cultivars are non-waxy type and another one is waxy type.

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Evaluation of Covered, Hulless and Waxy Barleys for Food Quality. M.J. EDNEY, Grain Research Laboratory, Canadian Grain Commission, 1404-303 Main Street, Winnipeg, Manitoba R3C 0G8 and B.G. ROSSNAGEL, Crop Development Centre, University of Saskatchewan, 51 Campus Drive, Saskatoon, Saskatchewan S7N 5A8

Introduction. The quality of North American barley does not always meet the special requirements of the food processing industries of south-east Asia. The major uses of North American barley in this market are as rice extenders or in miso soup (Bhatty 1993). Hulless barley, by tradition, is used in miso soup, while covered barley has been the preferred barley type for rice extenders, although waxy barley is now becoming the barley type of choice.

Two of the major quality considerations for both uses are kernel texture and colour of the pearled barley (Pomeranz 1974, Hulse 1995). Barley for miso soup, requires a uniform texture to insure that all the barley kernels absorb liquid at a constant rate during miso aging. A mixture of steely (vitreous) and non-steely kernels is particularly undesirable because of differences in water absorbency.

Barley rice extenders, which are presently used to enhance the nutritional quality of rice, must resemble rice in the final product. The processed barley, which involves pearling followed by rolling or splitting of the kernels, therefore, must be white. Poor barley texture can lead to breakage or powdering during processing.

The present study looked at how barley type, growth location, plumpness, protein and moisture affected the quality of barley for food. Numbers of steely and broken kernels in pearled samples were used as indicators of barley quality. Lower numbers indicated better quality. The colour of the pearled product was also measured.

Materials and Methods. Three types of barley were tested for food quality; covered, hulless (normal starch) and waxy. The cultivars and their types are listed in Table 1. All the cultivars were grown at 8 locations in 1994; Fahler, Irricana and Lethbridge in Alberta; Outlook, (with and without irrigation), Kernen (3 reps), Saskatoon and Goodale in Saskatchewan; and Brandon in Manitoba. As a result 11 samples of each cultivar were tested for quality.

Table 1. A list of types of barley and the cultivars investigated.

Covered	Normal Hulless	Waxy Hulless
Harrington	Condor	CDC Candle
Leduc	CDC Richard	Merlin
Stein	CDC Buck	HB803
B1602	Falcon	SB89497*

* breeder's line

Any adhering hulls present on the hulless barley samples were removed using a deawner. Barley samples were passed over a 5/64" (hulless) or 6/64" (covered) screen to remove thin barley. Only plump material was used in subsequent tests, which is similar to food barley processing.

Kjeldahl was used to measure protein in barley samples and a standard oven drying method for moisture. Samples were pearled using a Satake rice pearly. Covered barley was pearly to 55% (i.e. 45% removed) and hulless barley to 60%. The colour of the pearly product was measured using a spectrophotometric method (Daun 1978). Broken and steely kernels, in the pearly product, were counted visually by an experienced grain inspector. Results were expressed in percentages of steely or broken kernels by weight in 100 grams of barley (as is moisture basis).

Results and Discussion. The waxy barley cultivars showed the best quality. (Table 3). On average they had significantly lower numbers of steely and broken kernels. The pearly waxy samples also tended to be brighter.

Table 2. Average quality results for each cultivar tested.

Cultivar	Steely %	Broken %	Brightness %	Plump %	Protein %DM ¹	Moisture %
Harrington	5.8	36.5	52.5	92.6	12.2	9.0
Leduc	5.7	60.8	54.1	88.9	12.1	8.8
Stein	6.8	52.6	52.5	89.2	12.5	9.0
B1602	3.4	31.9	54.3	88.8	12.0	8.6
Covered Average	5.4	45.5	53.4	89.9	12.2	8.9
Condor	3.8	26.7	51.8	93.1	15.5	9.0
CDC Richard	7.2	36.9	54.0	92.4	13.8	8.9
CDC Buck	2.7	24.7	54.2	83.7	13.2	8.9
Falcon	5.2	37.3	53.1	79.3	15.1	8.9
Hulless Average	4.7	31.4	53.3	87.1	14.4	8.9
CDC Candle	1.5	9.5	56.2	89.7	14.3	8.7
Merlin	3.0	14.7	54.1	93.7	15.5	8.6
HB803	5.4	17.2	52.1	94.9	15.6	8.7
SB89497	2.6	16.6	55.3	89.0	14.7	8.5
Waxy Average	3.1	14.5	54.4	91.8	15.0	8.6

¹ DM = dry matter

The hulless (normal starch) barley cultivars, on average, showed lower percentages of steely kernels and broken kernels than the covered cultivars. Brightness of the pearly products were very similar among the hulless and covered samples.

Many of the correlations among parameters were highly significant (Table 3). However, none of the parameters explained a great amount of the variability

observed in quality. Kernel moisture had the greatest effect on quality with plumpness and protein having only limited effects.

Analysis of variance (anova) indicated that growing location significantly affected all of the quality parameters investigated (Table 4). Anova also showed a significant cultivar effect which could have been the result of the differences among barley types as discussed above.

Table 3. Correlation coefficients (*r*) among parameters investigated.

	Steely	Broken	Bright	Plump	Protein	Moisture
Steely	1	0.28**	0.40***	0.24**	0.17 ^{NS}	0.62***
Broken		1	0.17 ^{NS}	0.17 ^{NS}	0.26**	0.26**
Bright			1	0.01 ^{NS}	0.14 ^{NS}	0.36***
Plump				1	0.36***	0.33***
Protein					1	0.28**
Moisture						1

^{NS}, *, **, *** = not significant or significant ($p<0.05$, $p<0.01$, $p<0.001$)

Table 4. Effect of cultivar and growing location on the barley food quality parameters as indicated by F-values determined by analysis of variance.

	Cultivar	Growing Location
Steely	2.99**	11.9***
Broken	23.2***	11.4***
Bright	2.53**	2.60**
Plump	2.48**	22.9***
Protein	45.9***	96.1***

see footnotes for Table 3

Conclusion. Waxy barley showed the best quality for food, as indicated by low numbers of steely and broken kernels plus a brighter pearled product. Plumpness, protein and moisture were all correlated with quality but explained a limited amount of the variability. Growing location had a significant effect on quality.

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β-Glucan content in German oat cultivars and in oat bran obtained from them. W. GANßMANN, Peter Kölln, Köllnflockenwerke, 25 336 Elmshorn, Germany

Introduction. The favourable dietetic effect of oat consumption in human nutrition is generally known. In this connection the important role of β-glucan in oats and especially oat bran revealed by last years scientific research. While recently the β-glucan content in oats was investigated in Ireland (8), Finland (7), Canada (5, 9) and USA (1, 3, 6) no systematical data are known for German oats - apart of first own investigations 1991 -1993 (2).

This article reports results assessing the β-glucan content of different official registered German oat varieties and of oat bran produced by grinding and sieving some of these oats. Also β-glucan contents of German industrial milling oats are referred.

Materials and Methods. Oat groats - dehulled with a laboratory air pressure huller - from 8 cultivars (12 in 1995) grown on 11 similar locations in 1994 and 1995 were analyzed for β-glucan by the method of McCleary and Codd (4). Samples derived from variety trials performed by the Agric. Board of Schleswig-Holstein and Hannover

Laboratory scale oat bran was obtained by milling 100 g groats (10 - 12 % moisture) on a Fritsch - mill „pulverisette 14 „ (2,0 mm screen) and sieving 2 x 1,5 min. over 350 µ screen; the retained coarse material results as oat bran.

Results and Discussion. The frequency distribution for all the individual values 1994 and 1995 is normal and presented in figure 1. The average β-glucan content 1994 was 4,6 % ranging from 3,7 - 5,4 % and 1995 Ø 4,7 % (3,9 - 5,6 %). Table 1 shows the β-Glucan content for the different cultivars as average of 11 locations. In both years variety typical results were observed: Cultivars *Klaus* (1994 - 5,0 %; 1995 - 5,1 %) and *Alfred* (4,8 / 4,9 %) with the highest and *Itis* (4,1 / 4,5 %) and *Bruno* (4,4 / 4,5 %) with the lowest contents. The other cultivars have an intermediate position. This range confirms our 1992/93 results (2), where the variety *Gramena* (not cultivated in the trials 1994/95) was the highest in β-glucan content (5,6 and 4,9 %) followed by *Klaus* (5,1 / 4,8 %) and *Alfred* (5,1 / 4,6 %). The new cultivars (only 1995) *Expander* and *Revisor* are obviously high β-glucan Genotypes with the highest glucan level on all locations.

Overall mean in the 2 years (1994 - 4,6 resp. 1995 - 4,7 %) is nearly equal in contrary to our first results in 1992 (5,1 %) and 1993 (4,6 %) (2).

The average values of the 8 cultivars on 11 locations ranged 1994 from 4,2 - 5,0 % and 1995 from 4,4 - 5,2 %. In spite of significant local effects on the β-glucan content no similar behavior for the individual locations in the 2 years could be observed (Table 1) as the data for location 6, 7 or 8 demonstrate.

Obviously besides genotypical effects different environmental factors gain influence on the β-glucan level. So a negative correlation between β-glucan content and time from head to harvest (8) or grain yield and grain size (7) is referred.

We observed that the high glucan level of *Klaus* was related to the lowest groat weight of all cultivars; But this correlation could not be established for *Alfred*, *Expander* or *Revisor*.

In addition to the different oat varieties we analyzed the β -glucan content of representative North-German industrial milling oats with average results of 5,0 % dmb (4,4 - 5,5) 1994 (n = 42) and 4,9 % (4,3 - 5,5) 1995 (n = 19) compared with 5,5 % 1992 and 4,9 % (1993).

The results of laboratory scale β -glucan enrichment of oat bran demonstrate (Table 2) a significant correlation between β -glucan content of groats and bran obtained from them. The bran yields varied from 40 % (Alf) to 45 % (Bruno) and were uniformly converted to 40 %. The enrichment factor is exactly 2,0 for all cultivars; i.e. to get high glucan containing bran groats rich in β -glucan should be used.

The present study results in preliminary data for β -glucan potential of German oat varieties as practical information for oat breeding and oat resp. oat bran milling.

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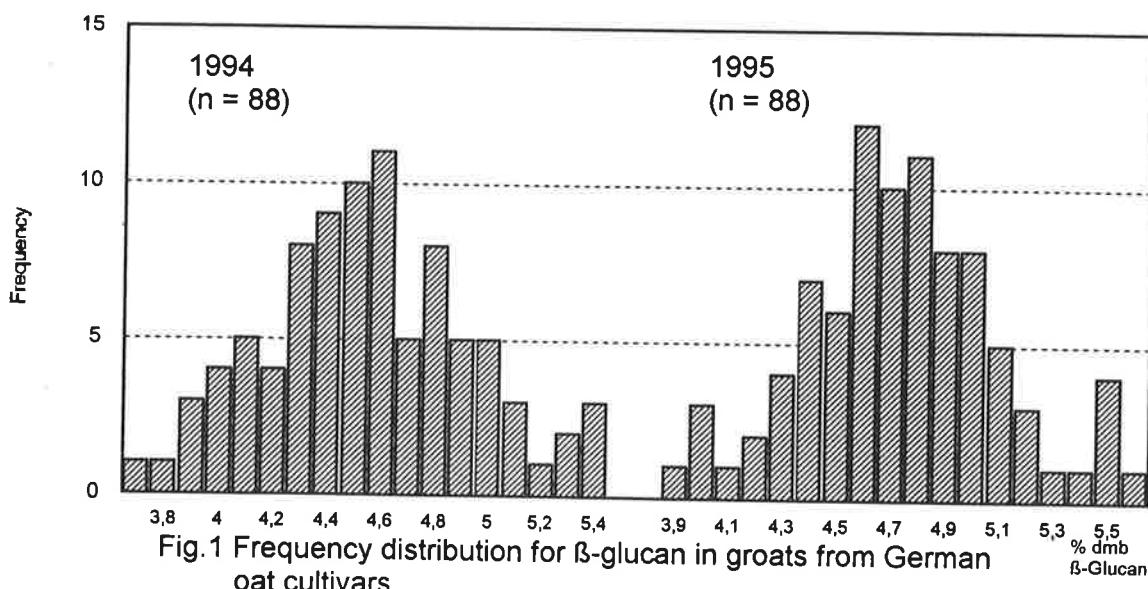


Table 1. β -Glucan content in groats of German oat cultivars (% dmb)

Cultivar	Mean ± st. dev. of 11 locations		Mean ± st. dev. of 8 cultivars		
	1994	1995	Location	1994	1995
Klaus	5,0 ± 0,34	5,1 ± 0,31	1	4,9 ± 0,21	4,4 ± 0,29
Alfred	4,8 ± 0,29	4,9 ± 0,35	2	4,7 ± 0,35	4,7 ± 0,26
Jumbo	4,5 ± 0,34	4,7 ± 0,25	3	4,7 ± 0,35	4,6 ± 0,21
Lutz	4,6 ± 0,26	4,7 ± 0,26	4	4,5 ± 0,32	4,8 ± 0,24
Bonus	4,5 ± 0,25	4,7 ± 0,33	5	4,5 ± 0,30	4,9 ± 0,44
Alf	4,5 ± 0,34	4,7 ± 0,28	6	4,5 ± 0,27	5,2 ± 0,19
Bruno	4,4 ± 0,39	4,5 ± 0,37	7	4,2 ± 0,34	5,0 ± 0,29
Iltis	4,1 ± 0,30	4,5 ± 0,36	8	5,0 ± 0,29	4,7 ± 0,38
Ø n = 8	4,6	4,7	9	4,3 ± 0,24	4,9 ± 0,30
Heinrich		4,9 ± 0,38	10	4,2 ± 0,22	4,5 ± 0,28
Expander		5,4 ± 0,29	11	4,6 ± 0,43	4,5 ± 0,26
Revisor		5,4 ± 0,34			

Table 2. Enrichment of β -glucan in oat bran from groats of different oat cultivars 1995 (Ø 4 locations, 40 % bran yield)

	Groat β -glucan (% dmb)	Bran β -glucan (% dmb)	Fines
Revisor	5,3	10,6	1,7
Alfred	4,9	9,8	1,7
Lutz	4,5	9,0	1,5
Alf	4,4	8,8	1,5
Bruno	4,3	8,4	1,5

Studies on isolation and characterization of starch from oat grains

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Introduction. In the relatively few studies conducted to date, considerable difference has been observed between the physicochemical properties of oat starch and other cereal starches and also between oat starch cultivars¹⁻³. However, there is a dearth of information on the variation in crystallinity, digestibility and retrogradation properties between oat starches isolated from different cultivars. The aim of the present investigation was to compare the thermal, digestibility, rheological and retrogradation properties of oat starches extracted from the cultivars, NO 753-2 (naked seeded) and AC Stewart (covered seeded).

Materials and Methods. AC Stewart (*Avena Sativa* L) and NO-753-2 (*Avena nuda* L) were grown at the Plant Research Center at Ottawa. Starch isolation, chemical composition, granule morphology, X-ray diffraction, differential scanning calorimetry, swelling factor, amylose leaching, pasting behavior, enzyme digestibility, acid hydrolysis, gel preparation and freeze-thaw stability were determined by procedures outlined by Hoover and Vasantha³.

Results and Discussion. The data on composition and yield are presented in Table 1. The X-ray spectra of both starches were of the "A" type. At approximately the same moisture content, the intensities of the major X-ray diffraction peaks were much higher in NO-753-2 than in AC Stewart. The results suggest that the crystallites of NO-753-2 are either more closely packed and/or are better oriented to diffract X-rays than those of AC Stewart. The swelling factor (SF) of both starches increased with rise in temperature. This was more marked between 50 and 60°C (AC Stewart > NO-753-2). Furthermore, at all temperatures the SF of AC Stewart was higher than that of NO-753-2. The results suggest that the SF of both starches is probably influenced mainly by differences in the degree of association between amylopectin chains (NO-753-2 > AC Stewart). This seems plausible, since the two starches differ only marginally with respect to their content of total lipids and amylose-lipid complexes (Table 1). The extent of amylose leaching was nearly similar in both starches. The pasting curves of the two starches were widely different. NO-753-2 exhibited a higher pasting temperature, a lower 95°C viscosity, a higher resistance to shear than AC Stewart. The difference in pasting properties is probably due to stronger associative bonding forces within the granule interior of NO-753-2 starch since the starches differ only marginally with respect to starch lipid content (Table 1) and amylose leaching. The gelatinization transition temperatures of AC Stewart were lower than those of NO-753-2. This suggest that the crystallite size and/or crystallite association

within granules of NO-753-2 are of a higher order of magnitude than in AC Stewart. The two starches differed in their susceptibility towards hydrolysis by porcine pancreatic α -amylase. AC Stewart was more extensively hydrolyzed than NO-753-2. This suggests that starch chain associations within the amorphous regions of the granule are more extensive in NO-753-2 than in AC Stewart. This seems plausible, since differences in granule size, amylose content and amylose-lipid content between the two starches are only marginal (Table 1). X-ray diffraction data showed that AC Stewart retrograded faster than NO-753-2. However, DSC data showed more extensive retrogradation in NO-753-2 starch. This is not surprising, since DSC selectively examines only the crystallization of amylopectin, whereas X-ray diffraction examines crystallization between amylose-amylose, amylose-amylopectin and amylopectin-amylopectin.

Conclusion. This study has shown that differences in physicochemical properties are influenced by differences in the magnitude of interaction between and among starch chains within the amorphous and crystalline regions of the native granule and by the chain lengths of amylose and amylopectin.

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Table 1. Chemical Composition (%) of oat starches

Characteristics	Oat Cultivar	
	NO-753-2	AC Stewart
Yield (%initial material)	35.2 \pm 0.1	34.0 \pm 0.1
Moisture	10.6 \pm 0.1	10.2 \pm 0.1
Ash ^a	0.22 \pm 0.01	0.20 \pm 0.01
Nitrogen ^a	0.001 \pm 0.01	0.001 \pm 0.01
Lipid		
Acid hydrolysed ^b	1.64 \pm 0.09	1.67 \pm 0.07
Solvent extracted		
chloroform-methanol ^c	0.35 \pm 0.01	0.30 \pm 0.09
n-propanol-water ^d	1.27 \pm 0.01	1.27 \pm 0.16
Amylose content (% of total starch)		
Apparent ^e	19.5 \pm 0.1	19.4 \pm 0.4
Total ^e	22.7 \pm 0.6	22.9 \pm 0.1
Amylose complexed with native lipid ^f	14.1 \pm 0.1	15.3 \pm 0.4

^aMoisture, ash and nitrogen were performed by the standard AACC⁴ procedures.

^bLipids obtained by acid hydrolysis (24% HCl) of the native starch (total lipids).

^cLipids extracted from native starch by chloroform-methanol 2:1 (v/v) at 25°C (mainly unbound lipids).

^dLipids extracted by hot propanol-water 3:1 (v/v) from the residue left after chloroform-methanol extraction (mainly bound lipids).

^eApparent and total amylose determined by I₂-binding before and after removal of bound lipids by hot propanol-water extraction.

^f
$$\frac{\text{Total amylose} - \text{apparent amylose}}{\text{Total amylose}} \times 100$$

Breeding adapted waxy barley cultivars for the food industry

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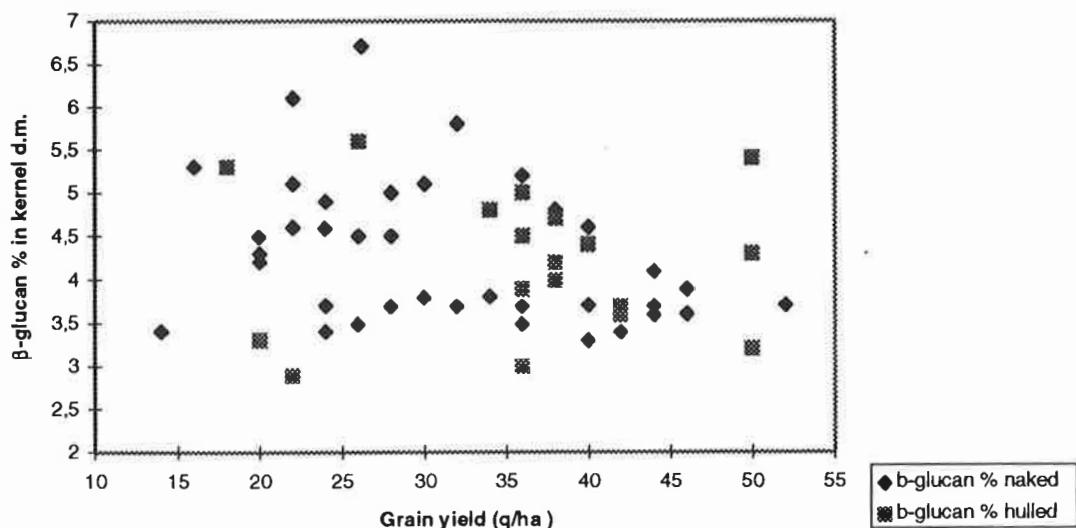
Introduction. Feeding and malting purposes are the prevailing uses of barley in France, as mostly elsewhere. However, among the utilizing industries, there is a growing interest for alternative destinations, although still on a modest scale . These concern primarily fiber, as a food adjunct, and to a lesser extent modified starches . To satisfy the needs of the food industry, efforts to improve the efficiency of extraction processes¹ or use of different raw matters -such as maize, potato, peas...- are possible solutions . Breeding of specialized barley cultivars for these specific goals is also a challenge which has been initiated since 1990 , associating INRA and industrial partners . This limited programme was launched as most foreign cultivars described for such special uses proved to be relatively unadapted in our local growing conditions² ..

Material and Methods. An initial series of 58 local and foreign cultivars , chosen in particular among those reported as having high β -glucan content in the kernel, was studied between 1990 and 1993 in INRA nursery at Clermont-Ferrand together with some INRA advanced lines ⁴ β -glucan was here considered at the crude constituent level, and not characterized in fractions as by other authors⁵. This material included classical spring naked varieties : CFL113, CFL82-657 , Godiva, Hora, Taïga as well as naked and hulled waxy cultivars from the USA (Wanubet, Shonkin) , South Corea (L236) , Australia (Waxiro) . Current agronomic traits such as plant height, heading date, disease scores, lodging resistance, were recorded . Some kernel traits were analyzed : TKW, protein (NIRS) and β -glucan content (Megazyme [®] kit) . In 1991 and later , a crossing programme involving ca. 20 genotypes was carried out, an off-season F1 generation being grown at CRD, Christchurch, New-Zealand . In the ongoing generations, the various progenies were screened for adaptation to local conditions, waxy and/or naked kernel trait when relevant, and β -glucan content . A first assessment of grain yield was performed on the more advanced F4 and F5 progenies in microplots (1994 and 1995 crops). In addition the starch gelatinizing potential of the waxy types was measured (Brabender test with 10 % flour in water, initial temperature : 50°C, reaching 95°C at 1.5°C/min;then 20 min. plateau, final cooling to 50°C) .

Results and discussion : Potential parents for crosses displayed kernel β -glucan contents (= BG) in the range 2.4 % (Mutant737 of Minerva) to 11.3 % (Arizona Hulless) . Among those with acceptable height, habit and lodging resistance, the current variation was 2.9-6.1 % in 1991 - see Figure 1 - No significant correlation existed between grain yield and BG . Despite its very high BG : 11.3 %, Arizona Hulless was not considered later in this work because of its low yield in our conditions - . Studying the genotype x environment interaction for BG in a 1991 design with 14 genotypes X 6 sites revealed a site range of

3.4-4 %, a genotype range of 3.1-4.5 % and highly significant interactions, some such effects reaching 1-1.5% sometimes .

Fig.1 - Grain yield and kernel β -glucan % of 58 various naked and hulled barley genotypes (Clermont-Ferrand ; 1991)



The accuracy of the enzymatic method for BG measurements³ was fully satisfactory in this study, with current BG standard errors of 0.2-0.3 % for one measure . Using NIR spectroscopy (Percon INFRAMATIC 8620[®]) after calibration with the enzymatic method gave determination coefficients of 0.5-0.6 : this can be sufficient to screen quite efficiently and cheaply in F3 or F4 . Some waxy genotypes, e.g. Wabet, Wanubet, Shonkin, Waxiro had regularly high BG figures above 5% over the years 1991-1994. However, other waxy genotypes such as Waxy Titan, Waxy Hector had BG seldom exceeding 4% in Clermont-Ferrand . These were hulled, but this was also the case for Waxiro, and conversely other non-waxy hulled cultivars had high BG as well : Vixen (winter 2-row)and Express (winter 6-row) for instance with a quite good yield . The presence of the waxy allele alone does not grant a high BG. Nevertheless, we could confirm its positive effect on BG trait : among 37 s.s.d. progenies (F5 kernels) of a cross (HDE83125 * Waxy Titan), the mean BG of the fixed waxy families was- significantly - 0.7 above that of the homozygous non-waxy ones , in this cross, thus somewhat lower than those reported by other authors^{7,8} .

Tab. 1 - Trial performance and β -glucan % of some INRA waxy lines in comparison with Volga (locally widespread control), CFL 113 (naked INRA line, 1973) and Waxiro controls . - Clermont-Ferrand, 1994 & 1995 -

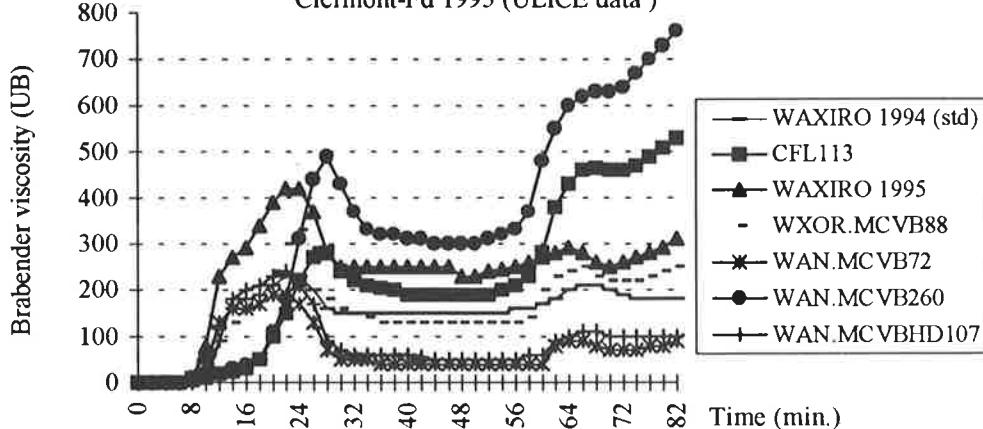
Genotypes	Naked / +.	Yield % cont.	Waxy / +	β -glucan %	Lodging (1-9)	Height (cm)
VOLGA (contrl.)	+	100 = 68q/ha	+	4.3	1.	89
CFL113	n	75	+	4.4	1	106
Waxiro	+	75	wx	5.7	3	84
Waxor*MCVB88	+	77	wx	5.9	1	86
Wanu*MCVB107	n	91	wx	5.4	1	80
Was*W16454J20	n	80	wx	5.8	3	81
Vix.Shon*Lal1	+	83	wx	7.2	2	72
Waxi*Bellissima1	+	88	wx	5.8	1	75

Preliminary results concerning the progenies from our crosses, in 1994 and 1995 microplot trials indicate -see Table 1 - that promising material can be bred : this associates satisfactory yield and stability potential with high BG and sometimes the waxy trait . A number of crosses - about two-thirds - had to be discarded from F2 onwards, due to poor lodging and powdery mildew behaviour .

Considering a species such as barley, the protein by-product of which, after kernel constituent separation, is less valorized than, say, bread wheat gluten, the opportunity of adding special starch properties, e.g. the waxy trait, may be an important economical asset . Such starches may be used as adjunct in the food industry : the Figure 2 shows some Brabender gelatinization results concerning some of our waxy lines . Further investigations in that direction are in progress .

Fig.2 - Brabender viscosity data of waxy and non-waxy barley flours;

Clermont-Fd 1995 (ULICE data)



Conclusion : This programme will be continued with a first multisite assessment of waxy and/or high BG , naked or hulled, in 1996 . Encouraging results were obtained from this initial breeding cycle, some lines now reaching 90-95 % of the best « normal » local controls, with about 6 % BG and waxy kernels . The future of such cultivars should however take into consideration the relatively narrow market open to that kind of genotypes . Other possibilities might also involve later high-amylase variants, although the impact on kernel composition seems lower⁶ .

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Analysis of kernel morphology in cultivated oat (*Avena sativa*, 2n=6x=42).

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Introduction.

Some of the most useful traits for selection in breeding are those which influence the quality of grain, the final product. Kernel physical characteristics such as shape and size can have a major impact on grain yield, milling yield, and quality of the final product. Measurement of these traits is accurate and simple using a Digital Image Analysis (DIA) system (Fulcher, 1996). Previous work on kernel morphology indicates that kernel size increased after five cycles of recurrent selection for grain yield in oat (De Kooyer et al., 1993). These results indicate an underlying genetic control for kernel size which may be associated with total grain yield. Additional work has shown that changes in kernel morphology may also impact the milling yield of oat (Fulcher, 1996). Improved grain quality and milling yield may thus be accomplished with little selection for improvement in the overall yield. Understanding the basic genetic factors which influence kernel morphology traits in oat is desirable in breeding to improve the yield and usefulness of the final product.

Here we describe the methodology used in identification of quantitative trait loci (QTLs) which consistently influence physical properties of oat kernels produced in various environments and years. We are expanding our initial QTL study to include additional lines and populations to provide biological replication.

Material and Methods.

71 F_{6,7} recombinant inbred lines (RILs) derived from the cross of *A. byzantina* cv. Kanota and *A. sativa* cv. Ogle were used for the construction of a RFLP map and the initial QTL analysis. We have now expanded the analysis to include 63 additional RILs from the Kanota x Ogle (KxO) population as well as 139 RILs from a second population derived from a Kanota X Marion (KxM) cross. Physical measurements were made on kernels of RILs grown in replicated field trials at Aberdeen, Idaho (1991 and 1992), St. Paul, Minnesota (1994) and Gore, New Zealand (1992 and 1993). These measurements were made using the Digital Image Analysis (DIA) system described by Fulcher (1996) to obtain data for the following traits: Area (average area of kernels), Dmax (mean length of kernels), Dmin (mean width of kernels), 100 x FShape (plumpness of kernels = 100 x (Dmin / Dmax)). Other measurements of kernel morphology used were %Groat (weight of groat in a sample / total weight of sample x 100), 1000KWT (1000 x (KWT / # of kernels)), Density Factor (DF = 1000 x KWT / Area), and Quality Factor (QF = (Area x FShape x %groat) / Standard deviation of Area).

The regions significantly influencing kernel morphology were originally identified using 360 co-dominantly segregating RFLP markers and the 71 RILs of the KxO population. We used both the Mapmaker QTL computer program and single factor ANOVA in this analysis. Regions which showed consistent significant associations over years and environments were deemed significant. The remaining RILs of the KxO and all of the KxM population are being genotyped with RFLP probes identifying polymorphic sequences mapping to these significant regions. The data will be analyzed using single factor ANOVA to assess biological replication of our initial QTL results.

Results and Discussion:

A high correlation has been obtained among measurements of morphological traits using DIA from such varied locations as New Zealand, Idaho and Minnesota. This consistency is due in part to the accurate measurement of these traits using the computerized system. Additionally, these traits appear to be controlled by few genes which are not drastically influenced by the environment. This hypothesis is supported by the initial QTL analysis on the first 71 RILs from the KxO population which has revealed two genomic regions, one for area and one for plumpness of kernels, that show a significant association with measurements of these traits from all years / locations (Table 1). Other genomic regions were identified which showed significant associations with area, mean length (Dmax), mean width (Dmin), plumpness (Fshape) and % groat in at least three or four out of the five environments (Table 1). These results are of interest considering that the environments and years were different in their influence on grain development in oat. Other QTL studies have been conducted on the same population grown in other environments looking at related traits such as total grain yield, test weight, and groat percentage (Siripoonwiwat et al. 1996). Three regions that influence test weight in those studies correspond to ones we have shown to influence kernel morphology. We are now in the process of further confirming these results using other populations.

Table 1. Significant RFLP-Trait Associations

Trait	LOD Score [†]	% Variance Explained
Area	2.28-4.19	24.4-28.5
	2.16-4.45 ^Y	14.6-25.5
	3.24-4.98 ^Y	22.1-28.2
Dmax	2.71-4.62 ^Y	28.9-46.8
Dmin	2.33-3.81 ^Y	19.5-41.6
100 x FShape	4.06-6.20	23.6-41.1
	2.45-2.91 ^w	17.6-25.9
	2.24-3.00 ^w	13.9-21.6
%Groat	2.03-4.8 ^Y	24.6-52.6
	2.41-6.28 ^w	38.4-58.2
	2.35-7.87 ^w	19.5-54.8
	2.49-6.59 ^w	16.2-50.9

[†] Range reflects the significant values for the various environments.

* P values for significant association identified by single factor ANOVA only.

^Y These regions were significant in four out of the five environments.

^w These regions were significant in three out of the five environments.

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THE INHERITANCE OF β -GLUCAN CONCENTRATION IN AN OAT (*Avena sativa* L.) CROSS. SOLOMON KIBITE¹ and MICHAEL J.

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INTRODUCTION. Research data spanning over the last thirty years have indicated that oat bran may reduce the risk of degenerative diseases in humans. Of particular interest has been the role of β -glucans in reducing cholesterol levels in hypercholesterolemic patients (Anderson *et al.*, 1984), their effects on blood sugar levels, especially insulin needs in diabetic patients (Jenkins *et al.*, 1981), as well as their impact on cancer of the colon and related gastrointestinal disorders (National Research Council, 1989). These findings have generated public interest in the use of oat bran. As a result, a strong demand has emerged for oat varieties that have high β -glucan concentration combined with high protein and low fat (oil) content.

The development of new oat cultivars with high β -glucan concentration would be facilitated if its mode of inheritance and genetic association with oil and protein content are well understood. Unfortunately, there is very little information on the genetics of β -glucan concentration in oats. This study was conducted: (1) to determine the heritability, mode of inheritance, number of genes and types of gene action involved in the expression of β -glucan concentration; and (2) to examine the correlations of β -glucans with oil and protein content, as well as with other economically important traits in oats.

MATERIALS AND METHODS. The oat cultivar 'Marion' was selected as the high β -glucan parent and was crossed with 'Dumont', a cultivar that is low in β -glucan concentration. F_1 plants from the Marion/Dumont cross were grown in the greenhouse, and were self-pollinated to produce the F_2 generation. The F_1 plants were also backcrossed to Marion and Dumont to produce the BC_1F_1 and BC_2F_1 generations, respectively. Seed produced by self-pollination of the BC_1F_1 and BC_2F_1 plants were composited to provide the corresponding BC_1F_2 and BC_2F_2 generations. The F_3 generation was developed by single-seed decent of randomly selected F_2 plants.

The parental lines, and the F_1 , F_2 , F_3 , BC_1F_2 and BC_2F_2 generations were grown in a spaced-plant nursery established near Lacombe, Alberta, Canada. Seeds of the parental and progeny lines were hand-planted 30 cm apart in rows 5 m long. Rows were spaced 60 cm apart. The nursery was established on a Ponoka Clay-loam (Black Orthic Chernozome) soil. Fertilizer was applied in the fall at the time of land preparation and according to soil test recommendation. Glean (*chlorsulfuron*), at the rate of 27 g ha^{-1} , was applied to control weeds when the majority of the oat plants were in the 2-3 leaf stage. Thereafter, the nurseries were maintained weed-free by mechanical means. Rainfall during the growing season (360.9 mm) was above normal (83 yr. average of 272.2 mm) and considered adequate for normal growth and development of oats in central Alberta.

The parental lines and the F_1 , F_2 , F_3 , BC_1F_2 and BC_2F_2 generations were evaluated, on an individual plant basis, for the following traits: (a) β -glucan concentration; (b) protein

content; (c) oil content; (d) grain yield; (e) number of panicles/plant; (f) number of kernels/panicle; and (g) kernel weight.

The minimum number of genes governing β -glucan concentration was estimated from the Swell-Wright formula described by Burton (1951), and the Castle-Wright formula described by Frey (1949). The types of gene action involved in the inheritance of β -glucan concentration were determined using the generation mean analysis (Hayman, 1958). Broad-sense heritability estimates were calculated using the F_2 /parental variance method (Mahmud and Kramer, 1951). Phenotypic correlation coefficients were calculated to determine the possible association of β -glucan concentration with oil content, protein content, grain yield and grain yield components (viz. number of panicles/plant, number of kernels/panicle and kernel weight).

RESULTS AND DISCUSSION. There were statistically significant differences among the parents for most of the characteristics examined in the study. As was expected, Marion was higher in β -glucan concentration and protein content, and slightly lower in oil content than Dumont. Marion also had a higher grain yield/plant and a heavier kernel weight than Dumont.

The F_1 plants were intermediate to the parents with respect to β -glucan concentration. The mean β -glucan concentration of the F_2 population was lower than the mean β -glucan concentration of the F_1 plants (Table 1). The mean β -glucan concentration of the BC_1F_2 population was significantly lower than that of Marion, but was significantly higher than that of the F_2 population. The mean β -glucan concentration of the BC_2F_2 population was significantly lower than the mean of the F_2 population, but was significantly higher than that of Dumont.

Both the Castle-Wright (Frey, 1949) and the Swell-Wright (Burton, 1951) methods indicated that a minimum of 3 factor pairs were controlling the expression β -glucan concentration in the Marion/Dumont cross. A chi-squared test of goodness-of-fit for the three-parameter model involving μ , a and d yielded non-significant χ^2 values (good fit) and suggested that dominance and epistasis were not involved in the inheritance of β -glucan concentration. This analysis also indicated that the inheritance of β -glucan concentration can be attributed mainly to alleles with additive gene effects. From these results, it would appear that any selection system that utilizes additive genetic variance should be effective in improving the β -glucan concentration of oats.

Broad-sense heritability estimates for β -glucan concentration in the F_2 , F_3 , BC_1F_2 and BC_2F_2 populations ranged from 0.37 to 0.57 (Table 1). These high estimates of heritability, together with the large amounts of additive genetic variance for β -glucan concentration (data not shown) indicate that rapid genetic advance can be made using standard selection schemes in the development of new cultivars with high β -glucan concentration.

In the majority of the populations, β -glucan concentration was positively correlated with protein content, and inversely correlated with oil content (Table 1). These associations suggest that it should be relatively easy to develop new varieties with high β -glucan concentration, high protein and low fat content for the oat milling industry, but would be relatively more difficult to breed varieties with low β -glucan, high protein and

high oil content for the animal feed industries. β -glucan concentration was not correlated with grain yield (Table 1), or with number of panicles/plant, number of kernels/panicle and kernel weight (data not shown), thus suggesting that selection for high or low β -glucan concentration would not cause correlated adverse effects on other agronomically important traits.

Table 1. Means (with standard errors), phenotypic variances, broad-sense heritability estimates and correlations of β -glucan concentration with protein content, oil content and grain yield in parental, F_1 , F_2 , F_3 , BC_1F_2 and BC_2F_2 populations developed from a Marion/Dumont cross.

Population	Number of plants	Mean \pm S.E	σ^2	h^2	Correlations of β -glucan concentration with		
					Protein content	Oil content	Grain yield
Marion	17	5.39 \pm 0.08	0.10				
Dumont	22	3.82 \pm 0.07	0.12				
F_1	4	4.36 \pm 0.09	0.08				
F_2	40	4.13 \pm 0.07	0.20	0.45	0.39*	-0.51*	-0.16 ^{ns}
F_3	40	4.34 \pm 0.07	0.21	0.49	0.39*	-0.62*	-0.27 ^{ns}
BC_1F_2	44	4.82 \pm 0.08	0.25	0.57	0.18 ^{ns}	-0.63*	-0.02 ^{ns}
BC_2F_2	53	3.97 \pm 0.06	0.17	0.37	0.57*	-0.21 ^{ns}	-0.23 ^{ns}

σ^2 = Phenotypic variance; h^2 = broad-sense heritability estimate; * = Significant at P=0.05; and ^{ns} = Not significant.

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**Food-Nutritional and Morphological
Studies on Influence of Draft Barley
Flour in Food Processing
with Scanning Electron Microscope**

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Introduction. Hayashi et al. (1991, 1992) have developed several kinds of draft barley flours by milling barley grain with a sake-rice polishing machine. These flours have desirable food processing characteristics and nutritional properties. The authors have reported the general composition and characteristics of the draft barley flour fractions (Hayashi et al., 1992; Mitsunaga et al., 1993). In addition several kinds of foods have been made by combining these draft barley flours (especially Lizza-7) into traditional wheat products, such as breads, noodles, cookies, cakes, muffins, and so on. On processing of these foods it has been known that these foods have many important texture and taste advantages (Hayashi et al., 1992; Mitsunaga et al., 1993).

The purposes of the present research are as follows;

1. To observe the change of inner structure of draft barley flour in food processing, such as bread-making, noodle-making and so on morphologically with a scanning electron microscope (SEM).
2. To observe the change of these structures during heating by several means such as boiling, steaming, burning, baking, toasting, and flying with SEM.
3. To observe the existence condition of moisture in these draft barley flours, and these processed foods made by using several moisture ratios with SEM.
4. To establish the combination ratio of any ingredients in several processed foods by the simple discrimination method.
5. To establish the combination ratio of wheat flour and draft barley flour in traditional foods by the easy discrimination method.
6. To find the optimum conditions of aging (temperature, time, and so on) in bread- and noodle-making by using several combination ratios of draft barley flour and wheat flour in order to shorten the processing time and reduce the expense as well as improve texture and taste.

Materials and Methods. Barley used for the present research was six-row, hulled non-waxy barley cultivated in Okayama, Japan. Lizza-7 was made as draft barley flour from above-mentioned barley with the sake-rice polishing mill developed especially for barley grain at Itomen Co., Ltd., Tatsuno, Japan. Bread was made by an automatical home-baking machine (Funai Electric Co., Ltd., Osaka, Japan) and noodles were made by hand at the experimental room. The scanning electron microscope used in the present research was a Hitachi model S-2150.

Results and Discussion. For the purpose of utilising draft barley flour for human food, the flour may be processed in several ways. The authors have observed how the inner structure involving subcellular particles in the barley flours can change under several processing conditions using a scanning electron microscope (SEM). In the present paper SEM images were obtained from bread and noodle made of draft barley flours by combining with wheat flour. Images obtained from mixing to baking via the fermenting stages were shown in the posters. These include pictures of bread made from 100% wheat flour, and from 80% wheat flour + 20% Lizza-7, using a magnification of x 1.500. Other figures showed sticky particles, more in some case than others. In fact, the bread made of flour containing 30% of Lizza-7 was quite sticky and had stronger elasticity. These observation by SEM of the subcellular particles of the bread's inner structure and the texture of the bread give parallel information. The wheat-like character gets stronger up to a combination rate of 20% Lizza-7 but the barley-like character, which is quite strongly hydrophilic, becomes stronger with a combination ratio of Lizza-7 of 30% or more. From these results the optimal ratio of draft barley flours in the bread may be easily distinguished.

In making noodles, Lizza-7 was combined with wheat flour at ratios of 0, 10, 30, 50, 70, and 100 %. The results of observation by SEM was that distinct pictures were unable to be obtained, but sticky-like particles seemed to increase when Lizza-7 of more than 30 % was used. At the same time the texture of the noodles made of 30% Lizza-7 flour was more elastic and stronger. From these results observation of subcellular structures of processed foods by SEM may be used to distinguish the combination ratio of draft barley flour in processed foods.

Conclusion. Bread and noodles were made of the draft barley flour, Lizza-7, by combining several ratio of Lizza-7 into wheat flour. The structural change of the subcellular particles in three stage of bread-making and noodles were observed with a scanning electron microscope. When the combining ratio of Lizza-7 was over 30%, the texture of bread and noodle were elastic, and more sticky particles were observed in the subcellular structure. These latter phenomena were in parallel. These results may suggest the following:

1. Discrimination of the combined ingredients may be possible by observing the structural changes of the subcellular particles with SEM.
2. The texture of the processed foods can be improved by using the draft barley flours.
3. Shortening of the processing time and reducing of the expense in the food processing may be possible by combining the draft barley flours with wheat flour.
4. It is possible to introduce these highly nutritional draft barley flour fractions into processed foods.

By developing the present research more, it would be possible to contribute to overcoming specific food problems as well.

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Distribution of alleles of *Itc1* locus controlling trypsin inhibitor in barley. M.P. LADOGINA and B.A. KALABUSHKIN, Vavilov Institute of General Genetics, Russian Academy of Sciences, Gubkin St. 3, Moscow 117809, Russia.

Introduction. Trypsin inhibitors revealed in endosperm of cereals play important role in protection of seeds from pathogens and pests. In cultivated barley, the locus *Itc1*, controlling the main trypsin inhibitor, was located on chromosome 3 [1], and three alleles of this loci were identified [2, 3]. Allelic variants of barley trypsin inhibitor were designated as BTI-CMe1, CMe2, and CMe3. Therefore, as a polymorphic protein, barley trypsin inhibitor (BTI) can be used as a biochemical marker of important agricultural traits. That is why the aims of this work were: to investigate diversity of BTI in cultivated barley, to analyze frequencies of alleles of the locus *Itc1*, and to study correlations between allele frequencies and climatic parameters.

Materials and Methods. We studied 76 spring barley cultivars (*Hordeum vulgare* L.) including 12 land-races from Uzbekistan (Middle Asia). The seeds were kindly provided by Dr. Pomortsev.

Barley cultivars were joined into five groups according to their geographic origin: I - Denmark, Finland, Great Britain, Norway, Sweden, Baltic countries, and northern and central Russia; II - Belgium, Czechoslovakia, Germany, Poland, and Netherlands; III - Ukraine; IV - the Urals and Siberia; V - low Volga, southern Kazakhstan, and Uzbekistan. The regions differed from each other in their moisture indices (ratio between rainfalls and evaporation per year) and sum of temperatures above 10°C during a season.

Trypsin inhibitors were extracted from individual embryoless grains and separated by electrophoresis in 6% polyacrylamide gel in tris-Na-EDTA-borate system (pH 8.3) according to [4]. After electrophoresis, zones of trypsin inhibitors were developed by the method proposed by Konarev [5]. Proteins were transferred from a gel to a gelatine layer of the Micrat film. The film was dried, put on 1% agarose gel containing trypsin, and incubated at 39°C for 60 minutes. Trypsin digested gelatine layer; therefore, bands of undigested gelatine corresponded to trypsin inhibitors.

We analyzed 10-15 grains in each cultivar. Allele frequencies were calculated from the assumption that sum of all alleles in each region is equal to 1. Number of homogeneous cultivars was doubled; each heterogeneous cultivar was considered as 1 with the first allele and 1 with the second.

The results obtained were processed by SYSTAT (version 5.0).

Results and Discussion. Using one-dimensional native electrophoresis with subsequent development of trypsin activity, we revealed four BTI variants in cultivated spring barley and one variant in wild barley *H. spontaneum*. We designated variants of inhibitors as A-E according to their electrophoretic mobility: A and E were the fastest and the slowest variants, respectively (B was revealed only in *H. spontaneum*).

Analysis of cultivars with BTI variants, previously identified in [3], showed that A variant corresponded to CMe2 and D - to CMe1.

Study of four crosses between cultivars with different BTI variants demonstrated that A, C, D, and E variants are controlled by alleles of the single locus *Itc1*. The corresponding alleles were designated as *Itc1a*, *Itc1c*, *Itc1d*, and *Itc1e*.

Frequencies of the alleles varied considerably (Table). The allele *Itc1d* (BTI-CMe1) was the most frequent: it occurred in 50% of the cultivars studied. The allele *Itc1c* was the rarest (9% of cultivars). *Itc1a* and *Itc1e* were found in 17 and 24% of cultivars, respectively.

Table. Characteristics of regions and frequencies of alleles of the locus *Itc1*

Region	Moisture index	Sum of temp. above 10°C	<i>Itc1a</i> (CMe2)	<i>Itc1c</i>	Alleles (variants) <i>Itc1d</i> (CMe1)	<i>Itc1e</i>	Total cultivars
I	>1	<2200	0	0	0.74	0.26	54
II	>1	2800	0	0	0.82	0.18	22
III	1 - 0.33	2800	0.08	0.25	0.42	0.25	24
IV	1 - 0.55	<2200	0	0	0.33	0.67	18
V	<0.33	3100	0.715	0.205	0.06	0.03	34
Total			0.17	0.09	0.50	0.24	152

Note that distribution of the alleles was uneven in the regions studied. The alleles *Itc1a* and *Itc1c* were found only in cultivars from dry areas; the other two alleles occurred everywhere, but their frequencies varied in the regions (Table). Chi-square test confirmed heterogeneity of allele frequencies ($\chi^2 = 157.45$, df = 12, P < 10⁻⁵).

To study relations between allele frequencies and climatic parameters, we performed correlation analysis. The results showed significant correlation between moisture and two *Itc1* alleles. Correlation coefficients were -0.892 for *Itc1a* (P < 0.042) and 0.947 for *Itc1d* (P < 0.015). Relations of the other two loci with moisture index were not significant. However, based on increased frequency of *Itc1e* in the region IV, characterized by dry spring, we can assume that this allele may be related with drought resistance in spring.

We did not find significant correlations between alleles frequencies and temperature.

It is interesting to supplement our results with the data obtained by Moralejo et al. [6]. They have shown that BTI-CMe2 variant is more frequent in Spain (nearly 33% of cultivars) than in other European countries (nearly 8% of cultivars). Besides, this variant is widespread in *H. spontaneum* from Morocco [7]. All these facts allowed the authors to conclude that CMe2 is of almost exclusive western Mediterranean origin [6]. In our work, this variant was frequent in cultivars and land-races from the region V (low Volga, southern Kazakhstan, and Uzbekistan), which is geographically distant from the Mediterranean countries. The region V, southern Spain, and Morocco are characterized by similar climate (dry summer) and high frequency of *Itc1a*. Thus, we can conclude that BTI-CMe2 variant (*Itc1a*) is widespread among cultivars from arid regions. This fact confirms our data on relationship between moisture and frequencies of this allele.

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Effect of disease control and plant growth regulator treatment on quality characteristics of spring oat cultivars. Alex McGarel and Ethel White, Department of Agriculture for N. Ireland, Plant Testing Station, Crossnacreevy, Belfast BT6 9SH, United Kingdom

INTRODUCTION. Oats in the UK are utilised in both human and animal food products. Currently there is much interest in the chemical and nutritional properties of oat grain and in developing new food and industrial processes exploiting many of the unique features of oat grain. However, physical characteristics of oat grain are of primary importance when oats are used for milling.

Oat cultivars differ in, and crop management affects, grain weight, husk: kernel ratio (kernel content), hectolitre weight and grain size distribution. Of these characteristics, kernel content relates most closely to the measure of output from the milling process which can be termed 'extract yield'. However, kernel content has traditionally been expensive and time-consuming to determine and, in practice, the industry has used hectolitre weight as an indicator of value for milling.

Growers exercise some control over the quality of grain produced by crops through choice of cultivar and management decisions namely - sowing date, seed rate, fertiliser applications, disease control, plant growth regulator (pgr) application. In this paper, effects of disease control and plant growth regulator application on quality characteristics on a number of spring oat cultivars are described.

MATERIALS AND METHODS. In Northern Ireland oat cultivar trials are conducted following UK agreed protocols for Cereal Variety Recommended List Trials. Each year, two spring oat trials with two or three replications of plots of 40 m^2 are grown under three management regimes in a randomised block design.

The three management regimes are:

- (1) no disease control or pgr treatment
- (2) complete disease control, no pgr treatment
- (3) complete disease control plus pgr treatment.

Following harvest grain weight is determined by automated counting of samples of 250 grains from the dried grain used in moisture content determination for each plot. Hectolitre weight is determined using a sample of grain whose moisture content has been equilibrated to about 15%. Two sub-samples of 150 'good' oat grains are selected from the dried sample and husks and kernels separated by hand and weighed to determine kernel content. Grain size distribution is determined by sieving good oats over 1.0, 2.0 and 2.2mm sieves.

RESULTS. Results are presented for seven cultivars included in trials during the period 1991-1995.

Grain weight (mg) (s.e. = ± 0.91)

	- fungicide -pgr	+ fungicide - pgr	+ fungicide + pgr
Aberglen	34.4	36.6	34.8
Bruno	34.5	36.7	36.3
Dula	31.0	35.4	33.4
Melys	32.2	35.1	34.6
Minerva	34.4	36.0	36.9
Piper	30.9	32.9	34.1
Valiant	32.4	35.8	34.2
mean (s.e. = 0.35, 79d.f.)	32.8	35.5	34.9

Kernel content (% kernel) (s.e. = ± 0.44)

	- fungicide -pgr	+ fungicide - pgr	+ fungicide + pgr
Aberglen	75.2	76.5	75.4
Bruno	74.4	75.6	73.6
Dula	76.6	77.8	75.9
Melys	74.8	76.0	75.6
Minerva	75.6	77.2	76.2
Piper	73.9	74.8	72.8
Valiant	74.3	76.0	74.9
mean (s.e. = 0.17, 79d.f.)	75.0	76.3	74.9

Whole Grain size distribution (% whole grain <2.2 mm) (s.e. = ± 1.76)

	- fungicide -pgr	+ fungicide - pgr	+ fungicide + pgr
Aberglen	15.9	11.8	12.0
Bruno	17.8	10.3	19.1
Dula	21.6	9.5	13.6
Melys	9.4	7.1	8.3
Minerva	14.4	7.9	11.8
Piper	13.3	6.5	15.5
Valiant	11.6	10.0	11.6
mean (s.e. = 0.66, 79d.f.)	14.7	9.0	13.1

Hectolitre weight (kg/hl) (s.e. = ± 0.85)

	- fungicide -pgr	+ fungicide - pgr	+ fungicide + pgr
Aberglen	52.5	52.6	54.9
Bruno	51.2	51.3	54.5
Dula	51.1	51.3	51.8
Melys	52.2	51.5	54.4
Minerva	51.0	52.9	55.0
Piper	49.9	51.3	54.5
Valiant	50.5	51.2	52.7
mean (s.e. = 0.32, 79d.f.)	51.2	51.7	54.0

Percentage of grain lots with Hectolitre weight >50kg/hl			
	- fung. - pgr	+ fung. - pgr	+ fung. + pgr
Aberglen	67	71	75
Bruno	60	100	100
Dula	57	71	60
Melys	71	71	80
Minerva	83	100	100
Piper	33	100	100
Valiant	57	71	60

Kernel content and grain size distribution were significantly better when the crop was kept free from disease and pgr was not applied. There was no difference in grain weight between the '+ fungicide - pgr' and the '+ fungicide + pgr' regimes but both were better than where fungicide was not applied. Hectolitre weight was not affected by application of fungicides but increased when pgr was applied. However, the Cultivar x Regime interaction was significant for hectolitre weight unlike the other three characteristics.

DISCUSSION. Large beneficial effects of disease control on grain yield and grain weight were obtained. Extract yield, i.e. the yield of groats, would be markedly improved because of the increase in the proportion of the total weight comprised by the kernels and also because of their increased dimensions, leading to lower sieving losses. Disease control also increased the likelihood that grain lots would meet the millers' 50kg/hl acceptability standard for hectolitre weight although the effect on mean hectolitre weight was both much smaller and more erratic from cultivar to cultivar than on other performance and quality characteristics.

Application of a plant growth regulator in addition to disease control generally depressed grain weight, kernel content and grain and groat dimensions. Although hectolitre weight increased in most cultivars, the likelihood of the grain lots meeting the millers' 50 kg/hl standard was only improved in two of the seven cultivars.

Kernel contents and hectolitre weights of each cultivar were averaged across all three management regimes. The relationship between mean kernel content and mean hectolitre weight accounted for only 19% of the variation in kernel content, indicating that hectolitre weight is not a good predictor of kernel content or, therefore, of extract yield.

CONCLUSIONS

- Hectolitre weight is not a reliable indicator of value for milling.
- Disease control leads to benefits for both the oat grower because yields are increased and the oat miller because extract yields are improved.
- Application of a growth regulator in addition to disease control reduced quality for milling compared with when only disease was controlled.

An application of barley flour substitution on breadmaking. N. MORITA¹, H. ANDO², M. SHIMIZU³, S. HAYASHI⁴ and T. MITSUNAGA³, ¹College of Agriculture, Osaka Prefecture University, Sakai 593, ²Kyoto Bunkyo Junior College, Kyoto 611, ³Department of Food and Nutrition, Kinki University, Nakamachi, Nara 631, ⁴Barley Food Research Center, Kobe 651, Japan

Introduction. A *DBG* (Draft Barley Grading) *Sieve Method*, a new method of preparing barley flour, has been introduced by Hayashi et al.¹⁾ The method is quite different from the conventional method. Prior to the introduction of this *DBG Sieve Method*, barley grains and the flour from them had been conventionally used as animal feed. As we face the increase of the world population today, the matter of how to feed these people has also become a serious concern. We are faced with the change of having to find alternate food stuff that we can hopefully harvest in abundance and that is nutritious at the same time. Some properties of bread baked with wheat flour containing barley flour as a supplement was reported introductory.²⁾ This paper deals with some rheological and baking properties; it also deals with a sensory evaluation of bread baked with barley flour substitute.

Materials and Methods. Two kinds of barley flour were used for this experiment. They were both fractionated by Draft Barley Classifying Method.²⁾ One of them was Lizza 7, a trade name by Itomen Co. of Tatsuno, Japan. Lizza 7 is the classifying degree indicating 30 to 70 % inner layer. The other was Lizza 3, which is the central layer to 30 % of the whole barley weight. Wheat flour used were a hard type-*Midorisango* provided by Masuda Flour Milling Co. of Osaka, Japan. The ingredients for the bread were 2 g dry yeast, 5 g sugar, 1 g NaCl, 5g butter (without NaCl), 65 ml water per 100 g wheat flour. Barley flour (100 g) was determined to possess a larger water absorbing capacity of 170 ml by farinograph test; so the amount of water was decided from the ratio of barley flour substitution. The apparatus used for breadbaking was an automatic breadmaker sold by Matsushita Electric Co. (Osaka) as SD-BT6 Home Bakery.³⁾ The volume of the loaves was measured by rapeseed displacement. The sensory evaluation for testing breads, containing various amounts of barley flour with varying water content and the control bread was carried out by the method of paired comparisons, as reported.⁴⁾ Breads were prepared the day before the sensory evaluation; the crusts were removed and pieces of crumb were blind-coded.

Analytical methods. Viscoelastic properties of bread dough mixed for 30 min in the "home baker" and the bread crumb were measured using a Yamaden creep meter (Tokyo, Japan). The speed of the plunger (4-cm diameter) to the sample (2.5 cm i.d. x 1.5 cm) was 30 cm/min.

Results and Discussion. Baking results. Different mixtures of wheat and barley were prepared in the afore-mentioned "home bakery". The barley flour was mixed to constitute 5%, 10%, 20%, 30% and 40% of the entire mixture. The barley flour was prepared according to the Lizza 3 and 7 methods. The moisture content of bread crumb or crust ranged from 40% to 55% even in the increasing amount of barley and so the differences were not significant. No significant differences were observed between the moistures of Lizza 3 and 7 except in the crust, in which Lizza 3 had lower moisture than 7. The figure 1 shows the specific volume of bread baked with Lizza 7 as the ratio of barley flour changes from 5 to 40% and the ratio of water content within the barley changes from 90 to 120 and 170 ml. Up to 20% barley flour content, it was observed that no specific volume decrease occurred. In the case of water content of 90 and 170, the specific volume became smaller especially with 30% to 40% barley flour content, with the exception of water content of 120.

Rheological Test. Viscoelastic properties of dough mixed in the baker containing four different amounts of water (90, 100, 110 and 120) were tested. Up to 20% of the barley ratio, the modulus of elasticity and viscosity coefficient were similar to that of control's, except for 20% barley with 90 ml water content. With more amount of barley substitute, the value of modulus of elasticity increased. Likewise, up to 20% of the barley substitute, the viscosity coefficient showed similar values to that of

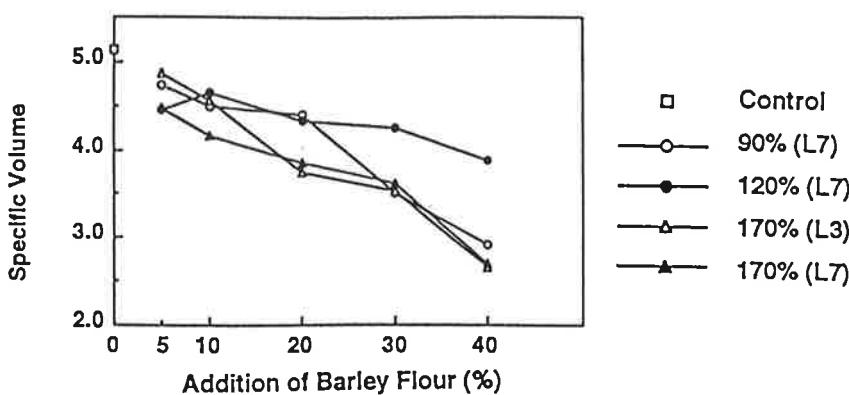


Fig. 1. Changes in specific volume of bread baked with various amounts of barley flour

the control's. From the firmness test of the bread crumb, the bread baked with 40% barley substitute with the water content of 170 showed the lowest value of modulus of elasticity. For water contents of 100, 110, 120 and 130, the modulus of elasticity did not change so much, except for higher amount of barley flour (30% and 40%) with the water content of 100 and 110 ml. But, no distinct relations were observed on the elasticity of dough.

Sensory test. An evaluation of bread baked with barley flour was made. The ratio factor of barley flour and wheat flour for baking was carried out as follows: (1) control (only wheat flour); (2) barley 10 and wheat 90; (3) barley 20 and wheat 80; (4) barley 30 and wheat 70; (5) barley 40 and wheat 60. Three different amounts of water content - 90 ml, 120 ml and 170 ml - were added to the ingredients per 100 g of barley flour added. In the case of wheat flour, it was 65 ml per 100 g. Four tests were conducted and evaluated as follows: 1. -2 Very Bad; 2. -1 Bad; 3. 0 Neutral; 4. +1 Good; and 5. +2 Very Good. Since we used 4 levels of barley flour, 3 levels of water content, and one control without barley and 65 ml of water, the total combination is calculated to be 156. However, taking into consideration the number of panellists and also the increase of evaluation error, we decided to do it almost in the half scale, by selecting 6 most important levels. So we reduced the number of experiments to 60. Table 1 shows the summary of one of the results of sensory scores

Table 1. Sensory evaluated value of bread baked with barley flour

Characteristic	A (0-65)	B (20-120)	C (40-120)	D (20-170)	E (40-170)	F (20-170) ^b	LSD ^a
Gas cell distribution	0.3750	-0.0208	-0.0208	0.3750	-0.7080	-0.2917	0.5724
Flavor	0.3750	-0.0208	-0.0625	-0.1458	-0.3542	0.1667	0.5423
Taste	-0.4583	-0.1875	0.1458	0.1875	-0.8330	0.2292	0.5939
Texture	0.3333	0.0625	-0.2083	0.3333	-0.7917	0.2808	0.6017
General evaluation	0.3958	-0.1042	0.2083	0.3542	-0.8333	0.1875	0.5658

^a least significant difference at p = 0.05. ^b barley flour 20%, water content 170ml to barley.

obtained from two different groups of panelists. From the analysis of the variance of the result, the main effects were significant, but the effect of the order and the combination were not significant. There were no significant differences between two different groups of panelists. From the multiple regression analyses of the sensory scores, the regression variances observed from each characteristic were highly significant. Table 2 shows the summary of multiple regression equation and multiple

correlation coefficient of each characteristic of baked bread. As is obvious, the lowest value of

Table 2. Multiple regression equation on sensory evaluation

Characteristic	Multiple regression equation	Multiple correlation coefficient
Gas cell distribution	$y = 2.1688 + 0.0052 X_1 - 0.0279 X_2$	0.8257
Flavor	$y = 0.7797 - 0.0118 X_1 - 0.0064 X_2$	0.9257
Taste	$y = 1.4553 - 0.0066 X_1 - 0.0159 X_2$	0.8356
Texture	$y = -0.1651 - 0.0340 X_1 + 0.0111 X_2$	0.8201
General evaluation	$y = 1.5806 - 0.0065 X_1 - 0.0172 X_2$	0.7903

multiple correlation coefficient was 0.7903, and the other coefficient values and their regression equation were presumed to be reliable, since about 63% of the data regarding to the scattering information obtained from the predicted values is explainable.

Fig. 2 shows the summary of sensory scores obtained from the panelists. The control A shows good scores in all characteristics, to be followed by barley 10 - water 90, barley 20 - water 170.

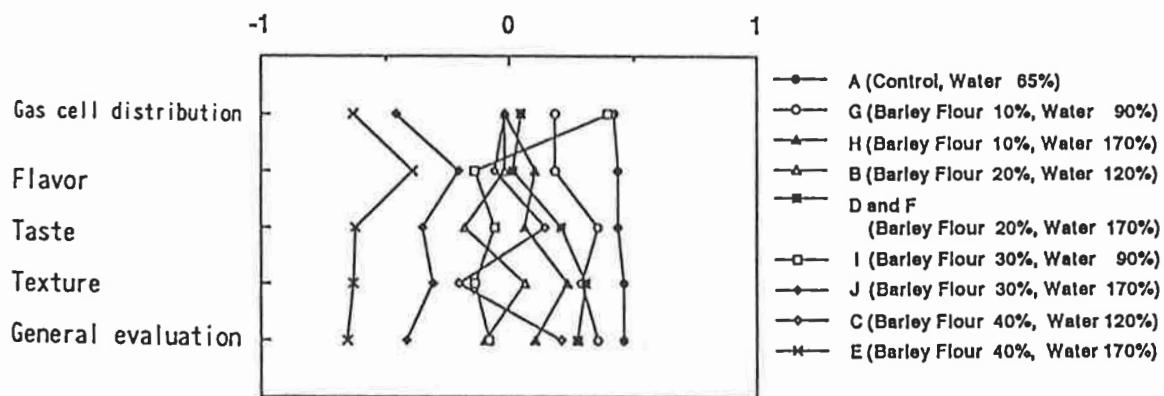


Fig. 2. Average values of sensory evaluation of bread baked with barley flour

Barley 40- water 120, barley 10 - water 170, barley 20 - water 120, and barley 30 - water 90 were almost in the average values. In the case of water 170, bread baked with barley 30 or 40 were not favorable. The barley 40 - water 170 was the most inferior. From these results, the bread baked with only wheat flour of water content of 65 was the most favorable in all characteristics that were tested. Barley 40-water 170 was the most inferior in all the characteristics tested. But as the amount of water decreased from 170 to 120, results improved. In the case of barley 20, the reduction of water content from 170 to 120 resulted in an unfavorable tendency in taste, texture and the general evaluation. However, the bread baked with barley 10 and 30, containing 90 ml of water, showed a favorable result.

In the Sensory Test, the most favorable quality of the bread baked with barley flour was found in the range of the water content of around 100 ml, but it depended on the amount of barley flour. Note also that the water absorbing capacity of 170 ml, which was obtained from farinograph test, can not be applied to the present breadmaking method with barley flour.

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Some characteristics of malted hulless oats. DAVID M. PETERSON, Cereal Crops Research, ARS, USDA and University of Wisconsin-Madison, 501 Walnut St., Madison, WI 53705, USA.

Introduction. Malted barley, in addition to its major use for brewing beer and distilled spirits, has long been used in the food industry as a source of flavor, color, sweetness, enzymes, and other nutritional components (1). The purpose of this research was to determine the characteristics of several malted hulless oat genotypes, so that they can be evaluated for similar or other food uses. Previous studies on malting oats examined the malt characteristics of covered varieties relative to barley malt for brewing (2, 3). In this initial phase of the project, 31 malted and unmalted samples of hulless oat cultivars and lines were analyzed for lipid and free fatty acids (FFA) content. Based on the results, malted and unmalted samples of eight genotypes were selected for further analyses: lipase activity, N, starch, and β -glucan content.

Experimental. Single samples of 31 hulless oats were obtained from the Cooperative Naked Oat Test grown in Ottawa, Canada in 1993. The samples included two cultivars and 29 lines (one subsequently released as a named cultivar) from plant breeding programs in several of the United States and Canada. Samples were ground in a Retsch ZM-1 Centrifugal Mill (Brinkman, Westbury, NY) to pass a 0.5-mm sieve. All analyses were done in duplicate. Petroleum ether-soluble lipid content was measured by standard gravimetric analysis. FFA were extracted with isoctane and analyzed by a copper soap method (4). Starch content was measured by digestion with thermostable α -amylase and amyloglucosidase followed by colorimetric determination of glucose using a kit from Megazyme (Sydney, Australia). β -Glucan content was also measured enzymatically by digestion with lichenase and β -glucosidase, and the resulting glucose determined colorimetrically using the glucose oxidase procedure (Megazyme). Total N was measured by combustion with a nitrogen analyzer (Leco, model FP-428, St. Joseph, MI). Carbohydrate soluble in 80% ethanol was measured by the phenol-sulfuric acid procedure (5).

Approximately 85 g of oats were steeped in 16°C water to 45% moisture (16 h) with a half-hour air rest at 12 h. They were germinated 6 d at 16°C, 100% humidity. Germinating oats were hand mixed twice daily. The green malt was kilned 22 h, with increasing temperatures from 49°C to 85°C. The malt was stored in a -20°C freezer.

A paired *t* test was used to compare differences between mean values for unmalted and malted oats for each parameter.

Results and Discussion. Mean lipid content of the 31 genotypes was 7.3%, with a range of 5.4 to 9.1%. The mean value was similar to that obtained from analysis of 4000 oats from the "World Collection" (6). Total lipid in the malted samples decreased an average of 1.1%, although two Idaho lines showed a decrease of > 2%. The decrease in lipid content probably results from lipase-catalyzed degradation of triacylglycerides to FFA and glycerol. Malting increased FFA significantly in all genotypes, from an average of 5.3 to 10.2% of lipid. The samples from Idaho

developed the least quantity of FFA. Lipid and FFA contents of unmalted and malted oats were considerably higher than a sample of hulless barley.

Eight genotypes were selected for further analysis. These included the varieties Pennuda and AC Baton and lines from breeding programs at Agriculture Canada (Ottawa), Pennsylvania (Marshall Farm), and Idaho (ARS). The mean differences between oats and malt for each parameter were significant at $P < 0.01$ (Table 1). Lipase activity decreased by 37 to 72 % in the malted samples, as compared to unmalted. Malt lipase activity and oat lipase activity were correlated with the decrease in lipid content during malting ($P=0.04$ and 0.08 , respectively), but neither oat nor malt lipase activity appeared to be related to the development of FFA in the malt. Lipase activity was much lower in groats of three hulled cultivars (1995 crop) than in these hulless genotypes.

N increased slightly in the malt, probably a result of some dry matter loss due to respiration. Undoubtedly, storage proteins were degraded during malting, with an increase in enzyme proteins and free amino acids, but these were not measured. Starch decreased by an average of 4.5%, which is similar to that observed during barley malting (7). β -Glucan was nearly completely degraded, averaging $< 0.3\%$ in the malt. This also is similar to experience with barley malt (unpublished data). Soluble carbohydrate levels in the malt averaged about 8%, accounting for most of the loss of starch and β -glucan.

Conclusions. The increase in FFA observed is not desirable, as this can lead to oxidative rancidity due to the subsequent action of lipoxygenase and other enzymes. The wide range in values indicate some possibility for identifying genotypes with even lower development of malt FFA. The next steps will be to search for genotypes with the least development of FFA and to devise malting conditions that minimize their development. The importance of lipase activity to development of FFA is undetermined. Further research is needed to determine if the malt has acceptable flavor characteristics and is stable.

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Table 1. Analysis of Selected Hulless Oat Samples and their Malts¹

Sample	Lipid (%)	FFA (% of lipid)	Lipase (Units) ²	N (%)	Starch (%)	β -glucan (%)	Soluble CH_2O (%)
	Oat	Malt	Oat	Malt	Oat	Malt	Oat
AC Baton	7.98	6.70	3.75	11.26	38.9	24.4	2.32
NO3-6	8.43	6.55	3.45	11.02	60.0	23.8	2.33
Pennuda	5.75	4.79	5.81	11.12	26.3	13.3	2.87
MF9018-5901	5.77	4.87	7.33	12.41	58.5	17.4	2.67
MF9018-11801	5.43	4.48	8.76	14.91	—	13.0	2.93
87Ab5932	8.19	6.18	4.38	8.35	75.2	21.4	2.51
88Ab3073	8.54	6.40	3.96	8.83	59.7	22.1	2.62
90Ab1500	9.06	7.86	4.59	8.64	29.4	15.4	2.66
Mean	7.39	5.98	5.25	10.81	49.7	19.7 ³	2.61
Std. Dev.	1.48	1.16	1.90	2.21	18.3	4.3	0.22
Mean Diff.	1.42	-5.56	30.0	—	—	—	—
Std. Dev. Diff.	0.51	1.40	16.2	0.09	—	4.5	4.57
<i>t</i>	7.79**	11.22**	4.91**	9.02**	8.00**	—	66.68**

¹ All data expressed on a dry weight basis except FFA; ² μ moles oleic acid equivalents $\text{h}^{-1} \text{g}^{-1}$; ³ Excluding MF9018-11801;

** Significant difference at P=0.01.

Breeding Barley for Functional Food-Starch. Å. Ståhl, Carbamyl AB;
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Introduction. Starch products in the food industry contribute to viscosity, texture, proper gel formation and solution stability. In order to prevent starch retrogradation when storing the products, the starch can be modified chemically by incorporating blocking groups. Starch products having suitable amylose/amyllopectin proportions can partly substitute for artificial processing to meet the demands from international trends to reduce use of chemicals in food production. Specifically the freeze/thaw stability can be attained if high amylopectin starch (waxy starch) is used. Waxy starch has been identified in several agronomic crops. In barley two genes controlling amylose/amyllopectin proportions were identified. Amo-1amo-1 - enhancing the amylose content and Wxwx - reducing the amylose content. This paper reports on the effect of waxy starch in industrial products, and on breeding waxy barley for commercial production.

Material. Several genetic sources carrying the waxy gene were introduced. Six are being backcrossed to adapted Scandinavian germplasm. The six genotypes are: Waxy Compana, Waxy Hulless Compana, BZ 489-30, Azhul, Waxy Titan and Waxy Oderbrucker. Several breeding lines and Golf barley were used as recurrent parents.

Methods. A backcross program including five backcrosses is in progress. Backcrossed lines were tested for agronomic characters in randomized complete blocks trials.

Screening for low amylose plants during backcrossing was made using a simple iodine test on crushed seeds. The amylose content was determined by a Technicon InfraAlyzer 400 with calibration constants using the wavelengths: 2139, 2100, 1982, 1940 and 1445 nm. For advanced lines the amylose content was estimated by amperometric titration with potassium iodate according to BeMiller (1). The quantitative starch determination of grains was carried out according to Salomonsson et.al. (2).

Starch extraction from ground barley was performed according to Vasanthan et.al. and South et.al. (3;4). Brabender profile was run with a 450 g sample of 5 % starch in distilled water using a 350 cmg measuring head. The test run started at 25 °C and was heated to 95 °C with an increment of 3 °C/ min. and then keeping the temperature at 95 °C for 5 min.

For freeze/thaw stability test 50 g cooled solution from the Brabender test was transferred to each of two 50 ml. centrifuge tubes. The tubes were centrifuged at 1500 g for 10 min. then liberated water was pipetted off and weighed. The tubes were kept in a freezer at -20 °C over night. After thawing the samples were centrifuged as described above and the freeze/thaw cycle repeated four times. The cumulative % water separated was plotted versus number of cycles. The water separation was found to follow a second degree equation. The coefficient b in $y=a+bx+cx^2$ correlated linearly to amylose concentration and was a quantitative estimation of solution stability of the starch. The correlation of the viscosity peak versus the amylose percentage follows the function: "Peak viscosity = $1782e^{-0.1176x}$, where x stands for % amylose".

Table 1. Yield data and seed characters for Waxy barley lines in field trials.

Variety	Yield kg/ha	Rel yield %	Tgw g	Starch %	Amylose %	β -glucan %
Golf	8312	100	47,5	60,5	30,2	4,7
SW 1541 BC ⁵	8158	98	47,5	61,1	7,4	6,3
SW 1543 BC ⁵	8223	99	47,5	59,1	6,2	6,2
SW 1546 BC ⁵	8287	100	45,0	59,3	5,3	5,7
SW 1547 BC ⁵	8216	99	45,0	58,7	6,0	6,0
SW 1549 BC ⁵	8157	98	45,0	58,8	6,0	5,8
SW 6129 BC ²	6970	84	45,0	57,6	6,1	5,5

Fig.1

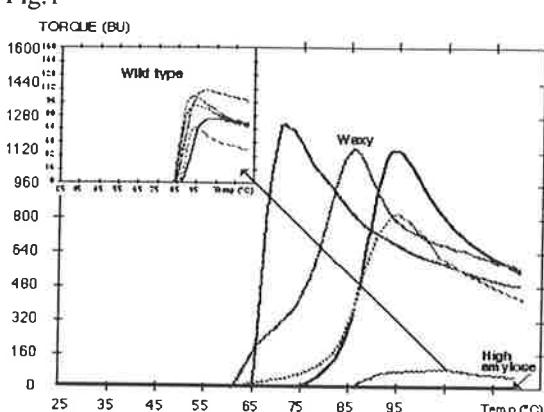


Fig.2

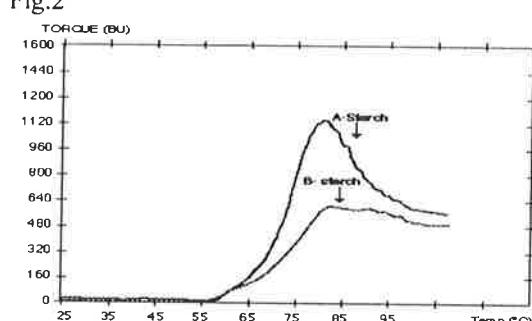


Fig.3

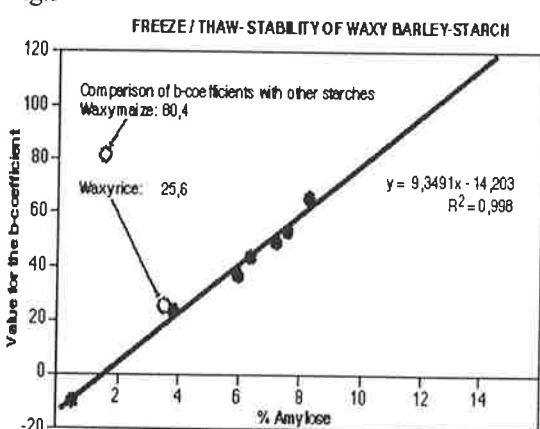
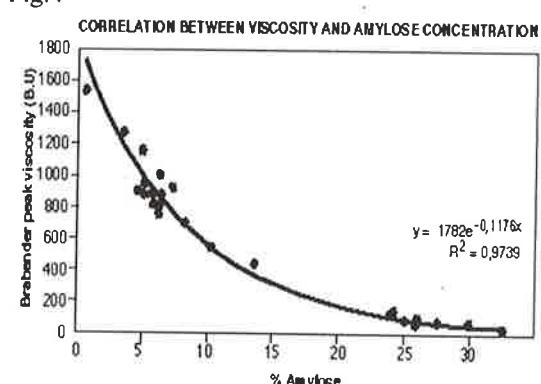


Fig.4



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Results. Five backcrosses were completed for the waxy gene sources Waxy Compana, Waxy Hulless Compana, Waxy Titan and Waxy Oderbrucker. One BC² line was isolated and multiplied for large scale experiments. Field experiments including five BC⁵ lines, one BC² line and the standard Golf gave information about agronomic and chemical characters (Table 1):

1. The BC² line yielded 84 % of the standard. The BC⁵ lines yielded close to 100 % of the standard.
2. Thousand grain weight for all lines were fully acceptable, some are as high as the standard.
3. The BC⁵ lines show an average loss of starch content of about 1 %. The BC² line had 3 % less starch than the standard.
4. The amylose content of breeding lines amounts to about 6 % which is equal to the values for the gene sources.
5. The high β -glucan contents in the gene sources were retained in the backcross lines.

Starch samples extracted were very pure and all within the following specifications: protein < 0.3 % and fat < 0.4 % estimated on dry matter starch.

A wide variation in granule swelling between different genetic backgrounds was observed. Three major categories could be identified among the brabender profiles. Waxy starch demonstrated the highest viscosity followed by normal amylose starch and high amylose starch showing no swelling at all. Within the normal and particularly within the waxy types a variation was observed (Fig. 1). Peak viscosity was found to correlate to the amount of residual amylose (Fig. 4). A- and B-starch granules showed totally different profiles, the B-starch being much more cooking stable (Fig. 2).

Freeze/thaw stability in cooked waxy barley starch was found to be a function of the amount of amylose containing starch (Fig. 3). Waxy barley starches have been shown to have a superior stability at the same amylose content when compared to other amylopectin starches like waxy maize starch (1.5 % amylose) and waxy rice starch (3.5 % amylose).

Discussions. Some statements and concluding remarks are made for waxy genotypes:

1. The waxy gene does not seem to interfere with yield potential.
2. Thousand grain weight can easily be retained during the backcross procedure.
3. The waxy gene does not seem to affect the starch content seriously.
4. The amylose content in waxy barley is not affected by the genetic background.
5. The genetic background has influence on granular swelling, which has impact on cooking resistance of starch in food production.
6. Waxy barley starch gives an excellent freeze/thaw stability, which has great importance for shelf life.
7. The studies confirm that the waxy starch character is correlated to β -glucan content.

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Fuel alcohol production by high gravity fermentation: Oats as a base for double mashing with wheat or barley. K. C. THOMAS and W. M. INGLEDEW, Department of Applied Microbiology and Food Science, University of Saskatchewan, 51 Campus Drive, Saskatoon, Saskatchewan S7N 5A8, Canada.

Introduction: Oats have not received serious attention as a raw material for fuel alcohol production because of their low starch and high β -glucan contents. One of the practical difficulties in using oats is the development of viscosity during mashing through solubilization of β -glucan and pentosans and the subsequent formation of gels. Considerable difficulties are experienced in handling mashes of higher specific gravity (pumping) and in fermentation (trapping of CO₂ and consequent mash lifting) because of high viscosity. Raising the dissolved solids content to high levels and applying very high gravity (VHG) fermentation technology for fuel alcohol production can be achieved only if the viscosity of the mash can be reduced. In this communication we report that both hulled and hullless oats can be processed to yield VHG mashes with dissolved solids contents in excess of 30 g per 100 ml and that oats can be used as a basal mash to prepare double mashes using wheat or barley or other materials as adjuncts.

Materials and Methods: Hulled oats and hard red spring wheat were purchased from a local store. A hullless oat (variety Terra) obtained from Dr. P. D. Brown, Agriculture Canada, Winnipeg and a hullless barley (SB 90354, an experimental cultivar with normal starch content) were used. The ' β -glucanase' was a crude enzyme preparation derived from *Aspergillus niger* (GNC Bioferm, Saskatoon SK.) and it was formulated as a powder. High temperature α -amylase (High-T), glucoamylase (Allcoholase II), and active dry yeast were all obtained from the Alltech Biotechnology Center, Nicholasville, KY.

Oats were ground and mashed as described previously (Thomas and Ingledew 1995). Oat basal mash to be used in the preparation of double mash with wheat or barley as adjunct materials were prepared in small scale as follows. Two liters of water was warmed to 45°C and 9 ml of 2 % (w/v) glucanase suspension in water was added, followed immediately by 1 Kg of ground oats. The slurry was incubated with stirring for 30 min. The temperature was then raised to 60°C and the mashing conducted as described for wheat (Thomas and Ingledew 1990), barley (Thomas *et al.* 1995) or oats (Thomas and Ingledew 1995). The liquefied mash was strained through a stainless steel strainer (24 mesh) and the residue washed twice with 800 ml portions of sterile distilled water to recover soluble dextrins. Depending on the concentration of dissolved solids desired in the mash all or part of the washings were pooled with the mash. In some cases the washings were used as make up water in the preparation of a second mash. The basal mash thus obtained was used to prepare double mashes as described previously (Thomas *et al.* 1996). Wheat or hullless barley was used as the adjunct material; other grains or concentrated sugars could be used. Double mashes thus prepared contained 35 to 36 g dissolved solids per 100 ml.

The double mash was then saccharified with glucoamylase and fermented at 20 °C with active dry yeast as described for wheat (Thomas and Ingledew 1990). Fermentation progress was monitored and viscosity measurements and analyses were conducted as described previously (Thomas and Ingledew 1995).

Results and Discussion: The viscosities of mashes prepared from hulled and hullless oats without the aid of viscosity-reducing enzymes was considerably higher than that observed with wheat mash prepared under similar conditions. By treating

the water slurry of oats with β -glucanase and by adjusting the water to oats ratio it was possible to select a particular dissolved solids content for the prepared mash. A few examples are given in Table 1.

Table 1. Dissolved solids contents and viscosities of mashes prepared from hulled and hulless oats processed with the aid of β -glucanase.

Type of oats	Water to grain ratio	Water adjusted ^a	Viscosity (BU)	Dissolved solids in the mash (g/100 ml)
Hulled	3:1	yes	<10	15.9 ± 0.5
	3:1	no	<10	21.6 ± 0.4
	2:1	yes	<10	23.9 ± 0.5
	2:1	no	<10	29.4 ± 0.6
Hulless	3:1	no	110	28.4 ± 0.4
	2.5:1	no	280	33.6 ± 0.3
	2:1	yes	100	26.9 ± 0.6
	2:1	no	520	42.3 ± 0.4

^a volume lost through evaporation made up by adding sterile water.

Since the viscosity of the mash prepared from hulled oats was very low it was used as basal mash to prepare double mash with wheat or hulless barley. The VHG mashes prepared all contained 35 g or more dissolved solids per 100 ml. The wheat and barley mashes prepared with oat mash as the basal first mash were all less viscous than corresponding VHG mashes prepared from one kind of grain (wheat or barley) alone. The double mashes were fermented at 20° C with 16 mM urea or 1 % yeast extract as nutrient supplements.

Table 2. Dissolved solids concentration and fermentation of double mashes (oats and wheat or oats and barley) with and without nutrient supplementation

Grains	Supplement	TS supplied g/100 ml	TS consumed g/100 ml	Hours	Ethanol % (v/v)
oats/wheat	none	36.4 ± 0.2	32.5 ± 1.0	216	16.2 ± 0.4
oats/wheat	YE	37.0 ± 0.1	35.8 ± 0.3	144	17.1 ± 0.3
oats/wheat	U	36.6 ± 0.1	36.6 ± 0.0	<96	18.0 ± 0.1
oats/barley	none	35.0 ± 0.0	35.0 ± 0.0	<96	17.2 ± 0.3
oats/barley	YE	35.8 ± 0.1	35.1 ± 0.1	<120	16.7 ± 0.2
oats/barley	U	34.9 ± 0.2	34.7 ± 0.1	<96	17.0 ± 0.0

Abbreviations: TS = Total dissolved solids; YE = yeast extract; U = urea

Results presented in Table 2 show that in all cases fermentation was stimulated by nutrient supplementation. Urea supplementation stimulated the rate of fermentation more than yeast extract. This applied to oat double mashes with either wheat or barley as adjunct materials.

Since oat mashes contained more free amino nitrogen (FAN) than wheat or barley mashes alone, it was assumed that no nutrient supplementation would be required or if required supplementation would be minimal. With yeast extract in the medium the amount of FAN consumed more than doubled and, as expected, this stimulated the fermentation. It is assumed that all of the urea was taken up by the yeast. From the rate of fermentation it appears that urea is a far better nitrogen supplement than yeast extract in such double mashes.

There was more cell proliferation in urea-supplemented fermentors irrespective of whether wheat or barley was used to raise the gravity of the oat mash. This higher cell number corresponded with the fast rate of fermentation observed with urea-supplementation. At the end of fermentation, the time of which varied with the type of nutrient supplementation, the viability of yeast cell population decreased to a greater extent than in unsupplemented controls.

In normal grain fermentations 30-35 % of grain weight is insoluble and plays no part in fermentation. Insolubles must be cooled in fermentation, heated in the still and make production of >18 % v/v ethanol almost impossible unless they are removed prior to fermentation. When hulless varieties are used, no need exists for the removal of particulates. Consideration should be given to contract growing of such substrates for this industry.

Ethanol concentrations obtained from these mashes were considerably below values calculated on the basis of dissolved solids initially present. A VHG mash prepared entirely from wheat and containing 35 % dissolved solids may yield 21 to 22 % ethanol by volume. The apparent lower ethanol yields in oat mashes or double mashes containing oats is most likely related to the low carbohydrate contents of these mashes. It is apparent that a significant part of the dissolved solids derived from oats is not carbohydrate but is soluble protein instead (data not shown). Ethanol yields calculated on the basis of starch are comparable to those obtained with wheat mashes.

The double mash procedure (a batch process) provides flexibility to alcohol plants that wish to use non-typical grains because of price fluctuations of the preferred substrates (wheat in western Canada). The procedure appears to work with any substrate. Rye and triticale are currently under study and we also hope to examine jet cooking for preparation of VHG mashes from more than one grain. VHG technology allows alcohol plants to produce more alcohol in the same physical plant at a lesser cost /L of ethanol than conventional technology (Thomas *et al.* 1996)

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Barley developing for human food in the Czech Republic. K. VACULOVÁ¹⁾, J. EHRENBERGEROVÁ²⁾, J. ZIMOLKA²⁾, ¹⁾Agricultural Research Institute Kroměříž, Ltd., Havlíčkova 2787, 767 41 Kroměříž, Czech Republic, ²⁾Mendel University of Agriculture and Forestry, Zemědělská 1, 613 00 Brno, Czech Republic.

Introduction. Specific barley breeding programs for food use were initiated widely in the Czech Republic after barley grain had been reported to have a positive effect on healthy human nutrition (Newman et al., 1989). Owing to rich tradition in malting barley growing and breeding, no non-malting cultivars have been released in our country until now. New food barleys are expected to have a higher grain yield and/or significantly better nutritional quality. At present, the research and breeding activities are focused on: 1) naked barley with high field performance, plump grain and white aleurone, and good lodging and leaf disease resistance, 2) barley with naked or covered grain and higher soluble dietary fiber content, and 3) barley with high nutritional quality for special food purposes.

Methods. 1) Naked barley breeding started in the 1970s, but only for feeding purposes. Some interesting genotypes of spring barley have been developed by crossing with Hiprolý, Abyssinian wild barleys and chemical mutations. Seven new naked barleys developed on their basis (BR 1117, BR 1119, KM 1057, KM 1771, MU 1331, MU 1333, MU 1335) were cultivated after two growing factors (forecrops, fertilizers and pesticides), in randomized blocks with four replications in comparison with standard (Ladik) and malting (Rubin) cultivars under dry conditions (Žabčice, Moravia) for two years (1994,1995).
 2) The performance and the breeding suitability of high β-glucans spring barley donors (HGB) from Montana State University (dr. C.W. Newman) was evaluated through their hybrids with high-yielding cultivars and local new selected lines in F2 and F3 generations.

Results and Discussion. The field experiment showed significant differences mainly for grain yield (Y), TGW, protein content (P%) and starch (S%) not only in comparison with standard cultivars but as well as among individual naked lines (Table 1).

Table 1. Field performance of new naked barley lines

Cultivar, line	Yield, t/ha	TGW, g	Protein, %	β-glucan, % DM	Starch, % DM	Fat, % DM	C16:0, % ²⁾	C18:1, %	C18:2, %
Ladik	7.48e ¹⁾	44.2e	13.0a	3.75ab	61.2a	2.20ab	21.3bc	15.1a	51.8b
Rubin	6.80d	41.5d	12.8a	3.93bc	61.4ab	2.15ab	21.5c	15.4a	52.0b
BR1117	5.95bc	37.8bc	14.3cd	3.65ab	62.2bc	2.32b	20.3a	16.3ab	52.7b
BR1119	5.64a	36.2a	13.9b	3.77ab	63.6d	2.21ab	20.3a	16.4ab	52.4b
KM1057	5.69ab	36.1a	14.6d	3.43a	60.9a	2.68c	21.4c	18.0c	48.8a
KM1771	6.17c	39.1c	14.0b	4.21c	62.4c	2.29ab	20.4ab	16.7b	52.0b
MU1131	5.78ab	37.6b	14.2bc	3.79ab	62.3c	2.24ab	20.6abc	16.4ab	53.2b
MU1133	5.81ab	39.1c	14.4cd	3.74ab	62.9cd	2.16ab	21.0abc	16.4ab	51.8b
MU1135	5.71ab	38.4bc	14.6d	3.75ab	62.9cd	2.11a	20.6abc	15.5ab	53.4b

¹⁾ Numbers in same column with different superscripts differ (P<0.05)

²⁾ Fatty acids: 16:0 - palmitic, 18:1 - oleic, 18:2 - linoleic.

Naked barleys also exhibited significant responses to weather conditions of the year (Y, TGW, P%, fat and fatty acids-FAs), a forecrop (TGW, P%, S%, fat and FAs), and fertilization (Y, P%, S%). A positive correlation of grain yield and TGW ($r=0.51^{**}$) was found for lines, while in hulled standards the main effect was assessed for grain weight per m^2 ($r=0.82^{**}$). According to the overall correlation coefficient the parameters of grain nutritional value (P%, S%, β -glucans= β -G, fat) did not show any effects on grain yield ($R=0.1487$, $n=56$); only generally-known negative relationships between P%, TGW and S% were confirmed. Results obtained in breeding the hybrids derived from crosses of these lines with high-yielding Western-European cultivars confirm possibilities of increasing grain yield in these so-called „normal“ naked barleys.

Among the studied naked lines KM 1057 is of a special character. It had the lowest β -G content, and on the other hand, the highest content of fat with significant different composition of FAs. The earlier studies (Hubík, Vaculová, 1990) report that this line is characterized by both untypical spectrum of C and B hordeins, higher content of protein and amino acids, lysine and threonine. This is also confirmed by the experiments with laboratory rats where weight gains were up to 29.7 % higher in comparison with the malting cultivar Rubin during three years. Pearling products from KM 1057 are noted for higher concentration of the components (Table 2), only β -G content is expected to be low. After processing, these products were used in a small human clinic experiment ($n=9$) which showed, on average insignificant, but at participants with a higher initial level (>8 mmol/l) significant decrease in total serum cholesterol (Vaculová et al., in press). In accordance with Weber et al. (1991) it may be caused by the effect of higher total content of α -tocotrienol in grain.

Table 2. Characteristics of pearling products from barley line KM 1057

Product, component	Value	Whole grain	Pearl barley	Embryos+pearl	Brans
Protein	%	13.62	11.00	17.31	19.25
Starch	% in DM	60.60	69.70	62.60	48.50
Fat	% in DM	4.12	1.86	7.04	9.73
Vitamin E	mg α -TE	2.84	0.92	4.94	no data
Tocopherols (sum)	mg/100g	7.28	3.61	8.21	no data
α -tocotrienol	% of sum	48.40	53.80	19.20	no data

The complex hypocholesterolemic influence of barley grain consisting in a combination of endogenic and exogenic effects can be expected only from special HGB which have genetically based high β -G content. Under our conditions, adverse agronomic traits, especially lodging, susceptibility to powdery mildew and lower yield, make their growing impossible. A hybridization program aimed at suitable partners has started since 1993. Comparing the performance of 15 combinations with HGB in F2 (average and individual selected plants) and F3 generations showed that grain yield was affected by grain weight per ear (0,57*) and TGW (0,43*) most, while lodging was influenced only by plant height (0,47*). The first results are illustrated in Fig. 1. No line combining the required high

parameters has been selected until now and therefore, further recurrent selection will be necessary.

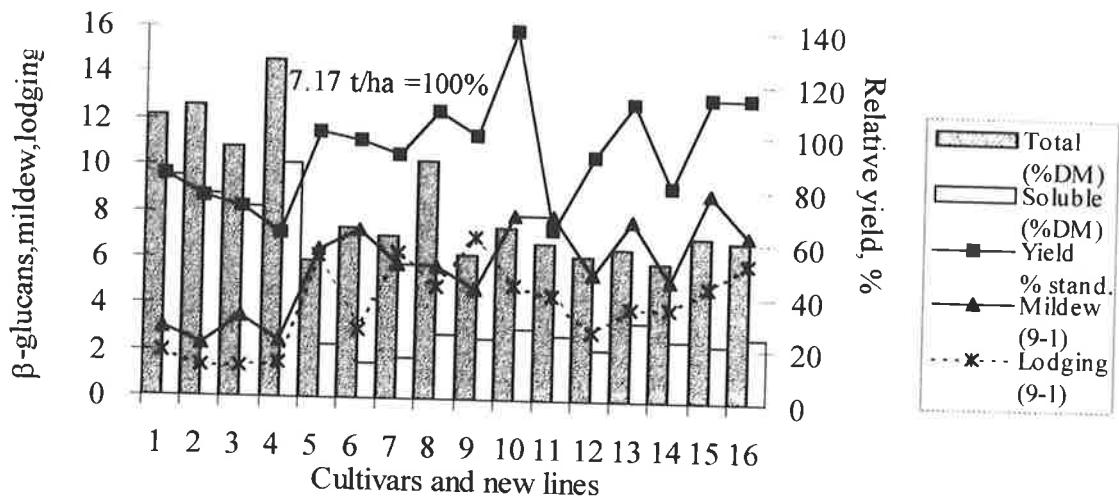


Fig.1. Total and soluble β -glucans, relative yield, powdery mildew infection and lodging in Wabet (1), Wapana (2), Wanubet (3), Washonubet (4), standard cultivar Akcent (5), new naked barley lines (6-9), and hybrids with HGB in F3 generation (10-16).

Conclusions. In case that no naked barley is grown, only the highest-yielding materials have chances to be used for food purposes. Considering the state of breeding in individual outlined directions, it seems that the most suitable materials for practical utilization will be the „normal“ naked barleys, in which grain yield could be increased by both breeding and right cropping practices. For traditional production of pearl barley genotypes with covered grain and high β -G content could also be perspective, but low resistance to lodging and powdery mildew is a serious problem in developing new cultivars. Breeding barley for special food purposes (functional, designer foods) will meet better practical responses only after the first human food cultivars are released.

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Isolation and cationization of barley starches in the laboratory and at pilot plant scale.
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Introduction. Cationic starches are widely used as wet end additives in the pulp and paper industries to enhance starch and filler retention during paper making. Although, there is an abundance of barley on the Canadian Prairies as a starch source, imported cationic corn and potato starches are used by Canadian pulp and paper mills. Vasanthan and Bhatty (1995) reported that pin-milling and air-classification of barley concentrated large granule (prime) starch into a fine fraction (F3); further purification of F3 fraction by a short wet procedure yielded almost pure large granule starch.

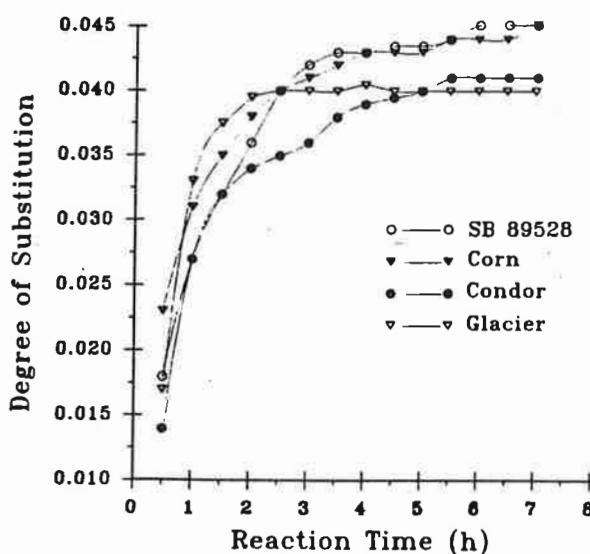
The objective of the present study was two fold: a) to evaluate the suitability of laboratory cationized prime barley starches as wet end additives in paper making by comparing their paper strength characteristics with those of a commercial grade cationic corn starch; and b) to investigate the technical feasibility of prime barley starch isolation and cationization at a pilot plant scale.

Materials and Methods. The laboratory scale study included barleys containing regular (Condor), waxy (SB 89528) and high amylose (Glacier) starches, prime corn starch and a commercial grade cationic corn starch (Cato 15). For the pilot scale study, Condor barley, was purchased locally. Two methods of starch cationization were used; laboratory scale preparation of cationic starches was done by an alkaline alcoholic semi-aqueous procedure described by Bhirud et al. (1994); for the pilot plant preparation, the alkaline aqueous procedure of Carr and Bagby (1981) was used. The degree of substitution (DS) of cationized barley and corn starches was calculated from the difference in the nitrogen content of native and cationized starches (Wurzburg 1986). The cationized barley and corn starches were evaluated in paper making at the Dept. of Paper Science and Engineering, Miami University of Ohio, Oxford, OH, USA. Paper sheets were prepared according to Method 205 of Technical Association of the Pulp and Paper Industry (TAPPI) (1981) and tested for breaking length and burst index using methods described in TAPPI (1981); and for Scott bond according to Reynolds (1974). Pilot scale milling and air-classification of Condor barley were done at Parrheim Foods Ltd. Saskatoon. The wet process for prime barley starch isolation and cationization was carried out at the POS Pilot Plant Corporation, Saskatoon.

RESULTS AND DISCUSSION. Cationic starches are produced by covalent bonding of quaternary ammonium groups to starch hydroxyl sites; the common reagent used is 3-chloro-2-hydroxypropyltrimethylammonium chloride (CHPTAC). The magnitude of cationic groups binding to starch is calculated from the difference in the nitrogen content of native and cationized starches and reported as the DS.

The prime starches isolated from waxy, regular and high amylose barleys, corn starch and Cato-15 were included in the laboratory scale investigations. Cato-15, had a DS of 0.036. All the three barley starches and corn starch were cationized in alkaline semi aqueous media; the increase in DS with reaction time was measured up to 7 h (Fig. 1).

Fig. 1. The increase in degree of substitution with reaction time during cationization of barley and corn starches in the alkaline alcoholic semi aqueous media.



The suitability of laboratory prepared cationic barley and corn starches as wet end additives in paper making was evaluated by comparing their paper strength characteristics with those of Cato-15 which was used as a reference. The DS of the laboratory cationized barley and corn starches was identical to that of Cato-15 (0.036); the reaction time required to attain this DS in barley and corn starches was obtained from Fig. 1. The data on strength characteristics such as breaking length, burst index and Scott bond of Cato-15 and laboratory modified cationic corn and barley starches are given in Table I and indicated that the functionality of cationic barley starches was comparable to Cato-15 and thus may be substituted for cationic corn starches in paper making. These results warranted a pilot scale investigation to evaluate the technical feasibility of barley starch isolation and cationization.

Table I
Paper Strength Characteristics of Cationic Starches

Cationic starch	DS	Basic weight (g/M ²)	Breaking length (m)	Burst index (KPa.m ² /g)	Scott bond (ft.lb)
Blank ^a	-	73.5	5806	2.39	109
Cato-15 ^b	0.036	72.4	6725	3.05	177
Laboratory modified:					
Corn ^b	0.037	71.6	6985	3.24	193
SB 89528 ^b	0.036	71.5	7090	3.28	230
Condor ^b	0.035	69.1	7338	3.41	209
Glacier ^b	0.036	65.0	7370	3.20	214

Values are means of three determinations; ^a No starch added; ^b Added 1% dry pulp wt. basis.

The pilot scale study was conducted in two phases: The Phase-I involved milling and air-classification; Condor barley (1000 kg) was passed through a dehuller to remove some of the outer grain material; the bran yield was ~7% (wet basis). The polished grain was finely milled and air-classified twice to produce a starch-rich coarse fraction (yield, 491kg and composition, 74% starch, 7% protein and 3% β -glucan). The objective of phase-II was two fold: a) purification of prime barley starch from the starch-rich coarse fraction; and b) cationization of prime barley starch in the alkaline aqueous media. The coarse fraction (491 kg, dry weight) was homogenized in water to form a slurry. The viscosity of the slurry was ~220 cps and was primarily due to the presence of soluble β -glucan. Such a high viscosity was likely to decrease the efficiency of centrifugation and the screening process. This viscosity was reduced to 40 cps by the addition of lichenase which depolymerized β -glucan. The slurry was then treated with 0.05N NaOH and centrifuged to yield heavy and light phases. The light phase was discarded but had dry matter that was 15% of the coarse fraction; the dry matter had 8% starch, 37% protein and 12% β -glucan. The heavy phase was re-slurried and screened with a 325 mesh (45 μ m). The starch that passed through the screen was recovered as starch cake-I that was 52% of coarse fraction and had 94% starch, <1% of protein and β -glucan. The residue remaining on the screen was re-slurried in water and re-screened to yield bran (yield 9%; composition 60% starch, 2.5% protein and <1% β -glucan) and starch cake-II (yield 14%; composition 92% starch, <1% of protein and β -glucan).

The efficiency of starch recovery from coarse-2 [total starch in starch cakes I and II as a percentage of total starch in coarse-2] was ~84%; this was comparable to the value (~86%) obtained in the laboratory study (Vasanthan and Bhatty 1995). The starch cake II was not combined with starch cake I before cationization as it contained too many bran particles; only the starch cake-I was cationized in an alkaline aqueous media. The target DS was 0.036; the cationization reaction time required was 2 h obtained from a study between DS vs reaction time. The yield of cationized prime barley starch powder was 213 kg which was ~59% of the total starch in coarse-2 fraction. The cationized barley starch had a DS of 0.044 which was higher than the desired DS; this was due to the longer processing time taken for the recovery of cationized starch from the reaction mixture. However, the reaction time could be adjusted to obtain the desired DS in future preparations. The cationized starch was off white colour due to some contamination of fine barley bran that passed through the 325 screen. This problem can be minimized by polishing barley grains to a greater extent before milling and air-classification, removal of up to 20% of outer grain material may be required. This study suggested that prime barley starch can be isolated and cationized for use in the pulp and paper industries by a combination of dry milling, air-classification and a wet alkaline process, and offers possibilities for industrial utilization of barley in the prairies.

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Genotype and environment effect on oat protein and beta-glucan content.

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Introduction. Oats were grown in Hungary on an average area of 220,000 hectares in the 1930s, but this area has now dropped to an estimated 50,000 ha. Due to its relatively large water requirements, the cultivation of spring oats is justified in the western part of Hungary, which has the most rainfall, or in the northern hills. Despite many attempts to introduce it, the cultivation of winter oats has not been successful. Winter oats sown by Cserháti in 1901, Gyárfás in 1912 and Legány in 1918 were frozen out (Kükedi 1958). Researchers in this field have reached the conclusion that if they overwinter, winter oats yield substantially more than spring oats in Hungary, but cultivation cannot be recommended until varieties with better frost resistance are available.

The beneficial dietary effect of oats in human consumption is due to the great biological value of the nutrients it contains. The well-known nutrient components of oat flakes and oatmeal make it the richest protein source of all the cereals. Among the vitamins it has a particularly high content of vitamins B₁, B₂, B₆, nicotinic acid and pantothenic acid (Peterson et al. 1975; Gould et al. 1980).

Thanks to its dietary fibre content, oats reduce the cholesterine level and have a favourable effect on the blood sugar level. The soluble dietary fibre content of oats consists mainly of β-glucan (Shinnick et al. 1987). In experiments on chickens, Fisher and Griminger (1967) found that animals fed on oats had a significantly lower cholesterine level than the control group. In human nutrition studies carried out over the last two decades several research teams have confirmed the cholesterine-reducing effect of oats (Anderson et al. 1984, Van Horn et al. 1988). The β-glucan content of cultivated varieties ranges from 2.5 % to 6.5 %, thus enabling this property to be improved by breeding and optimised by cultivation (Peterson 1991). The present investigations were aimed at determining the protein and beta-glucan contents of oat varieties grown at various growing sites and how these were correlated with the yield.

Materials and Methods. Plant Material. The experiments were carried out in Martonvásár, Lászlópuszta and Kaposvár in 1995. The climatic and soil characteristics of these three growing sites within Hungary show considerable deviations. Spring oats bred in Hungary and in several other European countries were sown in four replications in a random block design with 8 m² plots per variety and replication. At all three sites sowing was carried out on March 26th, which is the optimum sowing date in Central Europe. After harvesting, 1 kg samples from the yield measured for each plot were sent from each growing site to Martonvásár for analysis.

Determination of β-glucan. The seed was dehulled and ground in a Retsch ZMI ultracentrifugal mill through a 0.5 mm screen. Approx. 50 mg samples were weighed into centrifuge tubes and boiled with 10 ml water for 1 hour. After the addition of 10 ml 0.075 M H₂SO₄ the samples were boiled for a further 12 min. The extracts were analysed for β-glucan concentration by flow injection analysis (Peterson 1991). For the analysis 20 µl aliquots were injected into a stream of 0.2 M glycine buffer at pH 10.0 including 25 mg/l Calcofluor. The combined stream was passed through a Waters 474 fluorometer set at 350 nm excitation and 420 nm emission values. Peak heights were measured and barley β-glucan was used as the standard.

Protein analysis. The protein contents of oat varieties originating from the different growing sites were analysed using an automatic KJELTEC 1035 ANALYZER system with a sample quantity of 2 g and a factor of 6.25.

Results and Discussion. The β -glucan contents for each cultivar in each location are shown in Table 1. There were significant differences between the cultivars at each location. Cultivars 10787 cn, Somesan, Muresan, 10861 cn and 11125 cn gave the highest averages across locations and ranked 1 to 5 at the individual locations. Dragon and GK Pillangó gave the lowest averages. The mean values for β -glucan concentration were highest in Martonvásár and lowest in Kaposvár (Table 1).

**Table 1. Protein and β -glucan contents of oat cultivars at various growing sites
(in terms of 100 % dry matter)**
Hungary, 1995

Cultivar	Protein			β -glucan (without husks)		
	Kaposvár	Lászlóp.	Martonvásár	Kaposvár	Lászlóp.	Martonvásár
Bakonyalja	8.98	12.92	12.50	3.76	3.51	3.76
GK Pillangó	8.80	12.08	12.52	3.80	3.03	3.15
Kwant	9.10	11.67	11.51	4.29	3.05	4.14
STH 74/7	9.70	11.60	11.96	3.98	3.26	4.17
STH 173/1	10.17	12.48	13.07	4.22	3.86	4.00
Dragon	9.49	14.10	13.84	3.54	3.32	3.03
Jawor	9.43	11.30	12.38	3.42	3.59	4.42
Komes	9.61	10.81	13.37	3.69	4.05	5.07
CHD 692	9.81	11.50	12.22	3.69	4.16	4.62
CHD 792	10.38	13.85	14.26	3.71	4.96	5.03
CHD 894	9.23	10.56	11.99	3.51	4.46	4.91
Somesan	11.07	13.10	13.58	4.64	5.27	5.26
Muresan	10.73	12.99	14.63	4.61	5.01	5.59
10861cn	10.83	13.89	13.38	4.54	5.03	5.57
10787cn	11.13	12.22	13.51	5.27	4.82	5.55
11125cn	12.31	13.35	12.97	4.65	5.37	5.01
Mean	10.05	12.40	12.98	4.08	4.17	4.58

LSD ($p : 0.05$) = 1.113

in case of varieties

LSD ($p : 0.05$) = 0.476

in case of locations

LSD ($p : 0.05$) = 0.723

in case of varieties

LSD ($p : 0.05$) = 0.309

in case of locations

The combined analysis of variance across all locations indicated highly significant differences for location and cultivar. Significant differences were also found between the protein contents of the different varieties (Table 1). When averaged over the three experimental sites the highest protein contents were found for 11125 cn, CHD 792, Muresan, 10861 cn and Somesan, while the lowest values were given by CHD 894 and Kwant. The average protein content of the cultivars was highest in Martonvásár and lowest in Kaposvár. It can thus be seen that for both the indices examined the cultivars grown in Martonvásár gave the best results and those grown in Kaposvár the

worst. However, there was no significant correlation either between the protein and the β -glucan content or between the two chemical components and the yield (e.g. in Martonvásár the correlation coefficients were $r = 0.185$ for yield - β -glucan content, $r = 0.172$ for protein - yield and $r = 0.391$ for protein - β -glucan).

The cultivars were chosen without prior knowledge of their β -glucan contents. The range was not great, but significant differences were found within each location and across all locations. The ranking of the cultivars was fairly consistent across locations. This shows that selection for higher β -glucan content in plants grown in one or a few environments will probably indicate relative performance in other environments.

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Preprocessed Barley as a Feedstock for Fuel Ethanol Production. S. Wang, K. Sosulski, M. Ingledew* and F. Sosulski**, Saskatchewan Research Council, 15 Innovation Blvd., Saskatoon, SK S7N 2X8, Canada., *Departments of Applied Microbiology and Food Science, and **Crop Science and Plant Ecology, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK S7N 5A8, Canada.

Introduction. The fuel ethanol plants in western Canada operate mainly on wheat feedstock, with rye constituting up to 15% of total grain entering the mash cooker. Barley, lower in starch than wheat and rye and high in β -glucan, was not a feedstock of choice. However, increasing wheat prices have forced fuel ethanol plants to evaluate low-priced grains, among them feed barley, as starch feedstock for ethanol production. A method for grain preprocessing, originally developed for preprocessing of wheat to increase starch content for ethanol production (Sosulski and Sosulski, 1994), was evaluated for preprocessing of barley. The effect of preprocessed barley on ethanol production and reduction in energy requirements per litre of ethanol are discussed in this paper.

Materials and Methods. Two-row barley, CDC Dolly, from the 1994 harvest, was obtained for this study from Dr. Rossnagel, Crop Development Centre, University of Saskatchewan. Barley, tempered to 12.5% moisture, was preprocessed on a SATAKE abrasive mill (model TM05, SATAKE Co., Tokyo, Japan) to remove hull and to reduce bran content in milled endosperm.

Ground barley or preprocessed flour were dispersed in water at 60°C, the ratio of grain or flour to water being 1:3. The α -amylase enzyme, Maxallic (International Bio-Synthetics, Inc., Charlotte, NC) was added to mash in two portions, each at a concentration 0.5% (v/w enzyme to grain/flour). Starch hydrolysis with the first portion of enzyme was carried out at 95°C for 45 min. After lowering the temperature to 80°C, the second portion of enzyme was added and liquefaction was continued for an additional 30 min. Barley or barley flour mash was then transferred into a jacketed fermenter (Wheaton Instruments, Millville, NJ) containing 4% yeast extract (AYE 2200). Saccharification with Alcoholase II (International Bio-Synthetics, Inc., Charlotte, NC) was carried out at 30°C for 30 min at an enzyme concentration 0.8% (v/w enzyme to grain/flour). Inoculum (10^7 cells/ml mash) of *Saccharomyces cerevisiae* yeast (Altech Inc., Nicholasville, KY) was added to mash cooled to 27°C. Fermentations were carried out for 72 hr.

Ethanol was distilled from beer and the concentration of ethanol in the distillate was measured by an alcohol dehydrogenase assay, Kit # 332-A (Sigma Chemical Co., St. Louis, MO). Stillage was freeze-dried for further analysis.

Barley flour, hull-bran fraction removed during grain preprocessing and stillage were analysed for starch, protein, total dietary fibre (TDF), fat and ash.

Results and Discussion. Preprocessing of barley by abrasion on the SATAKI mill removed hull and bran, and reduced the weight of flour to 80.5 % of the original grain weight (Table 1). Starch concentration in flour was increased from 58.7%

69.9 % of dry matter, without substantial change in concentration of protein, fat and ash. As expected, the preprocessed flour was substantially lower in TDF, as compared to grain.

Table 1. Composition of barley and barley preprocessed products, % dm

Product	% of Grain	Starch	Protein N x 5.7	TDF	Fat	Ash
Grain	100.0	58.7	11.6	19.1	2.3	2.5
Flour	80.5	69.9	11.3	12.1	1.9	1.4
Hull-Bran	19.5	14.3	13.1	46.3	4.2	6.0

The hull-bran fraction contained 14.3% of starch representing a loss of 7.7% of starch.

Table 2. Ethanol concentration in the beer and ethanol yield

Product	Ethanol Concentration		Ethanol Yield L/tonne	Increase %
	% w/v	% v/v		
Grain	8.7	11.0	322	-
Flour	10.5	13.3	395	22.5

The increased concentration of starch in feedstock for fermentation increased the concentration of ethanol in beer from 8.7% (w/v) to 10.5% (w/v), and yield of ethanol from 322 to 395 L/tonne (Table 2). Thus an ethanol plant operating on the preprocessed barley would increase production by 22.5% without addition of new fermentation equipment (Table 2).

Table 3. Stillage composition, % dm

Product	% of Grain	Starch	Protein N x 5.7	TDF	Fat	Ash
Grain	44.4	0.5	27.3	41.1	3.4	6.5
Flour	36.1	0.3	36.4	24.8	4.3	5.4

The weight of stillage from the preprocessed barley flour was reduced from 44.4% to 36.1% of the original grain weight (Table 3). Stillage protein was substantially increased while TDF was reduced as compared to stillage from barley grain. Thus the stillage from preprocessed barley should be more valuable as animal feed.

Table 4. Energy use (MJ/L) and savings (%)

Product	Cooking Gas	Distillation Gas	Drying Gas	Total Gas	Total Electricity
Grain	2.2	7.9	21.0	31.1	2.9
Flour	1.9	7.5	18.2	27.6	2.3
Savings	13.2	5.4	13.2	11.4	20.6

Preprocessed barley required less energy for mash cooking, ethanol distillation and stillage drying (Table 4). The total reduction in gas requirement was 11.4%. Use of electricity was also reduced, mainly due to removal of bran which decreased energy requirements for milling.

Table 5. Effect of preprocessing on product yields and revenue,
based on a 10 million L ethanol plant

	Grain	Flour
Grain processed (tonnes)	31,056	38,579
Flour (tonnes)	-	31,056
Hull-bran (tonnes)	-	7,522
Stillage (tonnes)	13,789	11,211
Ethanol (million L)	10.00	12,27
Ethanol production increase (%)	-	22.7
Barley cost (\$ 134/tonne)	4,161,504	5,169,586
Revenue:		
Hull-bran (\$ 100/tonne)	-	752,200
Stillage (\$ 180* or 280**/tonne)	2,482,020*	2,802,750**
Ethanol (\$ 0.25/L)	2,500,000	3,067,500
Total (\$)	4,568,350	6,622,450
Grain cost : revenue	1:1.1	1:1.3

Preprocessing of grain generated a new coproduct, the hull-bran fraction, assumed to be used as ruminant feed. The stillage from the preprocessed barley flour is of superior quality, due to higher protein and substantially lower TDF than stillage from barley. Thus, the ethanol plant operating on preprocessed barley would increase total revenue and improve its grain to revenue ratio (Table 5).

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Evaluation on the nutrient quality characters of Qingke barley (*H. vulgare* var. *nudum* HK.) landraces from Sichuan province, China. W. R. YANG¹⁾ Z. Y. FENG²⁾ S. P. YE³⁾ T. W. XU²⁾ X. R. HU^{1),1)} 1)Crop Institute of Sichuan Academy of Agricultural Sciences, Chengdu, Sichuan 610066, P. R. China; 2) Department of Crop Genetics & Breeding, College of Agriculture, Sichuan Agricultural University, Yaan City, Sichuan 625014, P. R. China; 3)Sichuan Agricultural Management College,Chengdu,Sichuan 610072,P. R. China.

Introduction. Qingke(*H. vulgare* var. *nudum* HK.) is a kind of edible naked-grain barley,especially for the Zang(Tibetan) people in China. It is a major crop and a staple food of the Zang people in Ganzi and Aba Zang Autonomous prefectures of Sichuan province,in Xizang(Tibet) Autonomous Region and in Qinghai province,China.Qingke is usually used to make a special food called Zanba by the Zang people. Some historical proofs showed that Qingke has been cultivated by the Zang for over 2 thousand years. Through thousands of years of natural and artificial selection,Qingke has been evolved into an important gene pool of barley in China,which has very rich genetic variation. Since 1988,we have collected Qingke landraces and developed varieties from Ganzi and Aba Zang Autonomous Prefectures of Sichuan province. In present,most of the landraces are maintained in the Chinese National Gene Bank(Sun Lijun et al,1994). In recent years,genetic evaluations of Sichuan Qingke germplasms have been carried out. In the present paper,the nutrient characters of 62 Qingke landraces were evaluated.

Materials and Methods. Sixty-two Qingke landraces originated in Ganzi and Aba Zang Autonomous Prefectures of Sichuan province were employed in the present experiment. The nutrient quality characters including protein content (PC),lysine content(LC)and starch content (SC)were determined by the central laboratory of Shandong Academy of Agricultural Sciences Using the methods described by previous authors(Huang Ziliu et al. 1990;Li Hongen et al. 1995;Wang Linji et al. 1986). The original data were provided by Sun Lijun from the Institute of Crop Germplasm Resources, the Chinese Academy of Agricultural Sciences. These nutrient quality traits were classified according to the following standards. PC: I . PC<13% , II . 13%≤PC<16% , III . 16%≤PC<18% , IV . PC≥18% ;LC: I . LC<0. 4% , II . 0. 4%≤LC<0. 5% , III . LC≥0. 5% ;SC: I . SC<

50%, II. $50\% \leq SC < 55\%$, III. $SC \geq 55\%$.

Results and Discussion. Among the Qingke landraces from Sichuan examined, significant differences in mean values for PC, LC and SC were observed. The mean of PC was 15.71% with a range of 11.15%~18.62%, while the coefficient of variation(CV) was 9.54% (Table 1). Table 1 showed that nearly half of 62 accessions belonged to the type II and type IV. Two cultivars, 'Abazhuonaxi (ZDM8716)' and 'Lixianjiashanliunen (ZDM8799)' showed the highest protein content, over 18%. Therefore, the use of either of the two cultivars as a parent in crosses is recommended, thus one might obtain segregates for higher grain protein content.

Table 1 The variation for PC,LC and SC of 62 Sichuan Qingke barley landraces

Type	PC		LC		SC	
	No.	Percentage	No.	Percentage	No.	Percentage
1	4	6.45	12	19.35	1	1.61
2	29	46.77	46	74.19	51	82.26
3	27	43.55	4	6.46	10	16.13
4	2	3.23				
Mean	15.71%		0.43%		53.16%	
Range	11.15%~18.62%		0.29%~0.52%		49.49%~56.45%	
CV(%)	9.54		11.61		3.93	

The range of variation for LC was slightly wider than that of PC. The average of LC was 0.43% with a range of 0.29%~0.52%, while the CV was 11.61% (Table 1). The accessions with LC exceeding 0.5% were 'Abazhuonaxi', 'Heshuizegailiunenbei (ZDM 8788)', 'Lixianjiashanliunen' and 'Maerkangzhumuzhuqingke(ZDM 8813)'. As mentioned above, the cultivars 'Abazhuonaxi' and 'Lixianjiashanliunen' had also the highest protein content, so they should be excellent germplasms for high quality Qingke breeding.

The variation of SC was not as wide as that of both PC and LC. The average of SC was 53.16% with a range of 49.49%~56.45%, while the CV was 3.92%. Almost all of the accessions fell into the type II and type III (Table 1).

The results of simple correlation analysis showed that very significant positive correlations were present between PC and LC ($r=0.9190$), while SC was signif-

icantly and negatively correlated to PC($r = -0.7350$) and LC ($r = -0.6090$). Anon(1980) suggested that there was a negative relationship between LC and PC in wheat. This important information indicated that high PC and high LC in Sichuan Qingke landraces could be simultaneously transferred into the derived varieties to improve the nutrient quality.

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Section V: Nutritional Quality 2 – Livestock Feed

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Progress in Breeding Forage Oats Cultivars

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INTRODUCTION:

India accounts for 2 percent of world's geographical area and 15 percent of livestock population indicating excessive animal pressure on the limited land resources. Present level of fodder production is meeting only 46 percent of the fodder requirement. Merely an area of 8.3m.ha.(4.4% of total cultivated area) is under fodder crops. There is no scope to increase area under fodder because of existing pressure of food and cash crops. Forage production has to be maximized per unit area and time. Oats (Avena sativa) are grown as a cultivated fodder in India during the Rabi season. Oats are grown either as a sole crop or mixed with other crops either as a single cut/multicut crop. Varietal development in India has started with the introduction of Kent and Algerian varieties. Before 1947 prominent varieties under cultivation were Weston-II, Brunker-10, Fos 1/29 and Algerian-17 etc. Hybridization methods had led to the release of varieties OL-9, OL-125, UPO-212, OS-6 and JHO-822 for different agroclimatic zones of India.

OBJECTIVES:

Oats breeding programme is based on the needs and problems of the farmers. Major goals are to obtain high forage and seed yielding cultivars, good in quality and resistance to biotic and abiotic stresses.

MATERIALS AND METHODS:

Good parents were selected from the germplasm constituting of exotic and indigenous collections. Pedigree method of breeding was followed. The bulked F_6 progenies were tested in

progeny row trials. Promising entries were promoted to initial and advanced multilocation trials in the Punjab State and All India Co-ordinated Centres.

Cultivars OL-9, OL-125 and OL-529 were developed from the crosses: N.P.HybridxKent, ApplexIRC-63 and Kent x OL-87 respectively. The State Variety Release Committee approved the release of OL-9 for the Punjab State after proper testing at farmers' fields. Subsequently OL-9 was released at All India Level too by Central Variety Approval Committee. OL-125 has been released for North - Western, North-Eastern and Southern zones of India. A new promising strain OL-529 has been identified and recommended for testing at farmers' fields during 1995-96. It is at pre-release stage.

RESULTS AND DISCUSSIONS

Distinguishing features of the varieties along with the check (Kent) are depicted in Table: I

Table-I: Distinguishing characters of Oats varieties

<u>Variety</u>	<u>Dry Matter Yield (t/ha)</u>	<u>Plant Height (cm)</u>	<u>Days to Flower</u>	<u>Tillers per meter</u>
Kent	9.3	127	113	60
OL-9	10.7	145	117	56
OL-125	9.8	140	116	62
OL-529	11.4	165	118	66

The cultivars released had shown superiority over the check cultivar in yield and other related trials. In 31 experimental trials OL-9 had outyielded kent in green fodder yield by 15 percent. On the basis of average of 26 trials, OL-9 had surpassed kent by 15.5 percent in dry matter yield. OL-125 had shown superior performance both in green fodder and dry matter yields. In North-Western Zone of India OL-125 produced 8.9 percent more

dry matter than kent. OL-529, the strain at pre-release stage had shown higher dry matter yield of 11.4 percent in multicut system and by 10.5 percent in single cut experiments (six trials conducted during 1992-95). Total digestible nutrients were higher under both single and multicut conditions exceeding OL-9 by 22 and 24 percent respectively.

The future breeding programme is being boosted by enriching the germplasm base and introgressing desirable traits of quality and disease resistance.

Quality of German oats. J. HAMPSHIRE and W. GANSMANN,
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Germany.

Introduction. The production of oats in Germany resulted in a total harvest of about 1,4 millions tons (1995). Oat production in Germany has been falling about 10-15% in the last year. The acreages of oats decreased within the period 1984 to 1995 from 550000 to 311000 hectares. About 180000 t oats are used for human consumption. The major part of oats used by German oat milling industry is processed to rolled oats.

The quality factors for milling oats are laid down in raw material specifications:

Groat colour	white to cream, clean
Flavour	sound, fresh, characteristic
Moisture	max. 15 %
Test weight	min. 53 kg/hl
Thousand grain weight	min. 27 g (dry basis)
Hull content	max. 26 %
Grading (plumpness)	min. 90 % > 2mm
Double oats	min. 0,8 %
Foreign material	max. 1%
Foreign grain	max. 3%

The quality status of oat lots usually handled in the market as industrial oats is important for the milling industry.

Materials and Methods. We investigated the quality of oat lots of different origin:

- Oats grown 1980-1995 in regions of North Germany
- Oats imported from Australia (1980, 1983, 1987-1993), England (1991-1992), Finland and Sweden (1994-1995) used in German oat mills.

Results and Discussion. Investigations of German oat samples during a period 1980 to 1995 indicate that the mean values of thousand grain weight (TWG) ranges from 27-32 g (figure 1) and thousand kernel weight (TKeW) from 20-25 g. In 2 years -1989 and 1992- the TWG (about 27 g) and TKeW (19,6-19,8 g) were low compared to the other years. The grain and kernel weights

were unsufficient because of dry weather conditions during the vegetation period. For the mean hull content of German oats a range of 23-28% has been found (figure 2). Normally German oats consist of 23-26 % hulls.

In Germany nearly every year about 10000 t Australian oats were imported because of its excellent grain quality and appearance. The TGW and TKeW values of Australian oats are higher compared to German oats. A range of 31-34 g for TGW and 22-25 g for TKeW has been found. In spite of higher hull content in Australien oats (26,6-29,6 %) the TKeW is much better than for German oats. This is due to the excellent TGW and the lower moisture content (about 9-10 %) in comparison with German oats (about 13-15 %).

Oats imported from England (1991-1992) have low levels of TGW (25-26 g) and TKeW (19,0-19,4 g).

Since Finland and Sweden entered into the European Market in 1995 Scandinavian oats were imported into Germany. The average values of TGW and TKeW for Finnish and Swedish oats are equal to German oats. Finnish oats have slightly lower hull contents (about 23%).

The groat colour is important for the appearance of the final food product. The appearance of European oats varies in accordance with the weather conditions. Often rainy weather at harvest time cause shades of grey-striped and black groats. Because of this fact, German milling industry have a demand for oats with excellent appearance. Australian oats are characterized by their light, clean and pure appearance.

In commercial samples from German oats mean protein content is about 14-15 % on dry basis. The variation was 14,3-17,6 % respectively. In 1989 and 1992 protein level was high (> 17,0%). Dry weather conditions caused higher protein contents. English oats contain about 14 % protein (1991-1992). The protein level of Australien oats are lower (12-14 %) in comparison with German oats. This depends on lower fertilisation in Australia.

The mean lipid content on dry basis vary between 6,6 to 7,5 % for German oats, compared to about 7% for Finnish and Swedish oats, 8 % for English oats and 9-10% for Australian oats.

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Fig. 1: Thousand grain weights of German and Australian oats

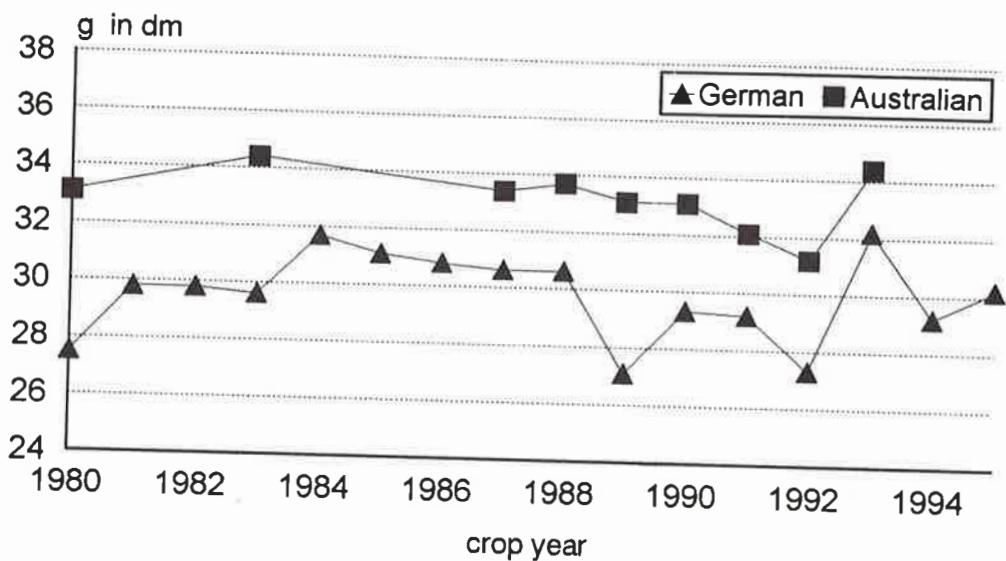
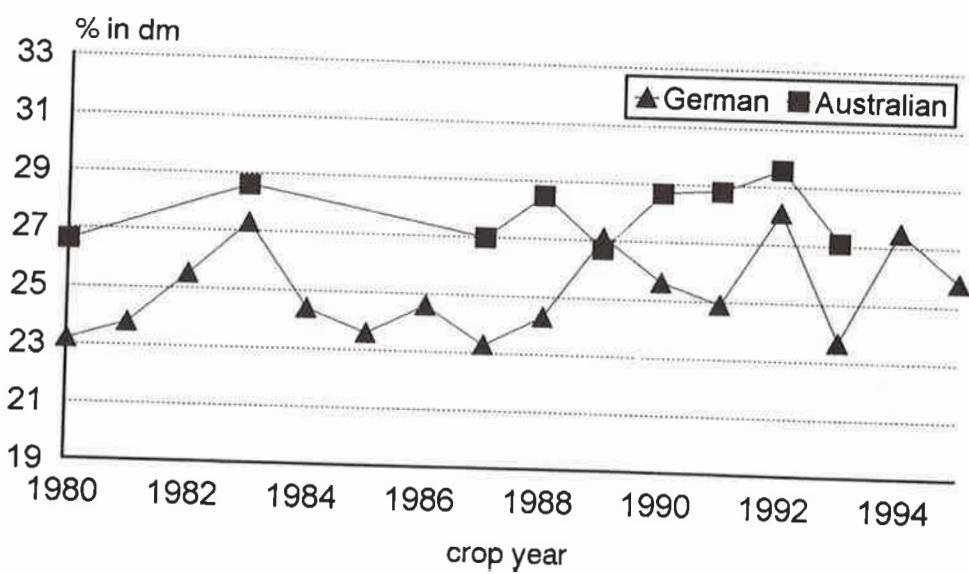


Fig. 2: Hull contents of German and Australian oats



The use of the mobile nylon bag technique and near infrared reflectance to determine digestible energy and protein content of hulless barley for pigs. J.H. HELM, W.C. SAUER AND L.A. HELBERG, Alberta Agriculture, Food and Rural Development, Field Crop Development Centre, 5030 50 St., Lacombe AB T4L 1W8, Canada. Dept. of Agriculture, Food and Nutritional Science, Univ. of Alberta, 410 Agriculture/Forestry Centre, Edmonton, AB T6G 2P5, Canada.

Introduction. Barley is the primary feed ingredient for pig production in western Canada yet historically, little attention has been paid to the feed quality of this grain. The development of new barley varieties requires that a large number of lines be screened inexpensively for agronomic, quality and disease resistance traits. It would also require large comparative samples to test even one line. It is presently both time consuming and very expensive to try to select for feed quality characteristics. Often chemical tests on the grain have been used to indicate quality such as crude protein and gross energy. However, these measurements do not take into account the animals' ability to digest and utilize these factors. The use of the mobile nylon bag technique in pigs (MNBT) as described by Sauer (1983) gives the plant breeder a new opportunity to study the effects of genetics and environment on feed quality as measured by the animal. Still, this technique too is expensive to utilize in a plant breeding program. The purpose of this study was to develop a method based on Near Infrared Reflectance Spectroscopy (NIRS) to determine protein digestibility (PD) and energy digestibility (ED) in the pig. This method would allow the breeder to screen thousands of lines in a breeding program.

Materials and Methods. Ten cultivars of barley which included nine hulless types and one hulled control, Harrington, were grown in randomized trials at ten locations in western Canada in 1993 and 1994. One site was lost in 1994 due to hail. A 1 kg sample of clean seed was derived from each site by a composite of the three replications in the test. This sample was further subsampled to derive the samples for MNBT, laboratory tests and NIRS tests. All samples were used in all tests to give us a total of 190 working samples.

Five barrows(Camborough x Canabrid), with an average initial body weight of 40kg, were housed individually in metabolic crates 1 week prior to insertion of the cannulas. The pigs were fed a 16% crude protein diet (Sauer et al., 1983).

After a 7-day adjustment period to the metabolic crates, the pigs were fitted with a simple T-cannula in the duodenum according to procedures described by Sauer et al. (1983). The cannulas were prepared according to methods described by Sauer et al. (1983), modified slightly according to de Lange et al. (1989). The pigs were immediately returned to the metabolism crates after surgery and fasted that same day. The next day, they were given approximately 100 g of the grower diet, twice daily, The feed allowance was increased slowly until the pigs consumed 2 kg/day. Water was freely available at all times. The pigs were allowed a 14-day recuperation period.

Nylon bags, measuring approximately 25x40 mm with a pore size of 53 μ m, were prepared from monofilament nylon (Nitex mononylon cloth #3-270-53 ASTM, Thompson and Co. Ltd., Montreal, Canada). The percentage open area in the cloth was 36%. The bags were sealed on three sides with heat provided by a sealing device (Seal Master, Packing Aids Corporation, San Francisco, CA).

A 1g sample of finely ground feed (1.0 mm mesh screen) was placed into a nylon bag, and the open end of the bag was then sealed. The bags were grouped in blocks of 8 and placed in a beaker containing 500 ml of a solution made up of deionized water, 0.01 N HCL and purified pepsin powder (Fisher Scientific Company, Fairlawn, NJ). The activity of pepsin in this solution was 377.4 IU/l and the pH was maintained at 2.0. The beaker was placed in a waterbath (37°C) and agitated at a rate of 90 oscillations/min for 2.5 h. Thereafter, the bags were removed from the beaker, washed with deionized water and frozen until required.

The thawed bags were inserted during a 5-min period while the pigs were eating, eight during the morning meal (0800 to 0830) and eight during the evening meal (1600 to 1630). The bags were retrieved in faeces during three daily searches at 0800 to 0830, 1200 to 1230 and 1600 to 1630, respectively, carefully isolated and immediately frozen.

A total of 40 bags with 1g contents were prepared for each feedstuff. Eight bags of each feedstuff were inserted into each of five pigs.

Following freeze-drying, the bags were carefully pried open. The contents were pooled within pig and feedstuff (pooled from eight bags) and analysed for nitrogen (LECO FP-428) Nitrogen Analyser; LECO Corporation St. Joseph, MI) and energy (Parr 1241 Adiabatic Bomb Calorimeter; Parr Instruments, Moline, IL). The feedstuffs were also analysed for nitrogen and energy.

NIRS spectra were collected from whole barley samples with a 6500 NIR scanning monochromator (NIRSystems, Silver Springs, MD). Equations for the NIRS were developed using Intrasoft International NIRS 2.0, version 3.0 calibrate program. The calibration was developed using modified Partial Least Squares Regression (Shenk and Westerhaus, 1991a), 1,4,4,1 math treatment and setup for two outlier elimination passes (Shenk and Westerhaus, 1991b). A repeatability file was used to minimize the effects of temperature and packing. All constituents were predicted on a dry matter (DM) basis. There were considerable differences in seed size and shape. The hulled variety Harrington was removed from the calibration where it was distinct in its reaction.

Results and Discussion. The equations developed had R² values ranging from 0.98 for crude protein (CP) to 0.88 for ED (Table 1). The constituent equations had corresponding 1-VR³ values of 0.97 to 0.86. Equations for digestible energy content (DEC) and ED are based on fewer samples due to the increased variability for these values from the pig. It is well known that particle size in the ground sample will have a major effect on the MNBT results. We also know that the Detrend scaling factor (particle size)

of the samples ranged from 3.69 to 7.38 as calculated by the ISI Symmetry program for NIRS. We are unsure of these effects on the results but we did observe that the pig results had a greater variability and therefore more of the samples were discarded from the calibration. There was also a greater variability within sample and between samples in 1993 than in 1994.

This preliminary data indicates that NIRS has good potential for the screening of whole grain samples for relative feed value in a plant breeding program where the goal will be to select the top 10 to 20%. It is still questionable if it will be accurate enough to be used for feed formulation. The data will be used to develop and estimate the influence of plant genetics and environment on the variability between samples for these feed quality characteristics.

This is a part of a larger study entitled Genetic and Environmental effects on feed quality of hulless barley that is still being carried out. At this time only two of the three years of testing have been completed. An additional set of samples collected in the 1995 will be added to the calibration set.

Table 1. NIRS equations developed for hulless barley.

Variable	n	Mean	SEC ¹	R ²	SECV ²	1-VR ³
CP	175	13.34	.30	.98	.36	.97
DM	109	88.98	.25	.92	.32	.86
Energy	156	4017.9	21.12	.90	26.29	.85
PD	113	79.81	1.55	.91	2.21	.81
DEC	61	3414.98	21.05	.91	24.57	.88
ED	45	84.98	.46	.88	.50	.86

¹ Standard Error of Calibration

² Standard Error of Cross Validation

³ Explained Variance

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GRAIN YIELD AND QUALITY IN OAT SPRING CULTIVARS

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INTRODUCTION: Oat is an important plant species all over the world. This species is very adaptive on different environmental condition. The favorable growing conditions are these with more rainy climate and with acid soils (Nikolic, 1982). The oat production areas in Yugoslavia are not big and are environmentally diverse in precipitation, soil types and crop management practice. Although the marketing of oat has become increasingly linked to quality standards, the effect of genotype, environment and their interaction on oat quality have received little research attention (Kibite and Edney, 1992). So now, little is known about genetic variability that exists among oat cultivars and potential for manipulating their quality through plant breeding.

The production amount (tons/year) is decrease (Nikolic, 1982). Mainly this species use as feed and very small quantity use as a food. Oat grains used as horse feed and sheep feed. Except of more favorable protein content the lipid composition have advantage. Most of the lipid acids in triglycerides are unsaturated oleic and linoleic acids. This characters is very important for feeding cows which gave milk for high quality butter production (Banks et al., 1988). The oat grain consists beta-glucon which has positive role in decreasing glucose and cholesterol in blood what is very important for the nourishment of patient with diabetes and cardiovascular disorders.

MATERIAL AND METHODS: Four different spring oat cultivars (Rajac, Mediteran, Slavuj and Condor) in seven year environmental conditions were grown and analyzed for grain yield and quality. Experiments of each year were carried out on experimental field of ARI "SERBIA", Center for Small Grains in a randomized block trial on plot size 5 m² with five replication.

Grain quality was analyzed at the Center for forage Plants in Krusevac and Center for Small Grains in Kragujevac. The oat cultivars are originated from cross combination of different parents namely: Rajac (Mustang x Astor), Mediteran (Astor x Gambo), Slavuj (UPBS-3024 x UPBS-3074).

RESULTS AND DISCUSSION: The average values of yield of Yugoslav spring oat cultivars and check Condor cultivar are presented in Table 1. In average Slavuj cultivar had highest grain yield and 0.65 t/ha higher than check Condor cultivar. During period of analysis the average grain yield for assayed oat cultivars was highest in 1988. and 1989. year. In average plant height of Mediteran and Slavuj cultivars was lower than in Rajac cultivar.

During this seven year of investigation of spring oat cultivars (Rajac, Mediteran and Slavuj) showed higher yield than check Condor cultivar. The similar results in four year assayed oat cultivars were found (Maksimovic et al., 1994).

Table 1. Yield of new Yugoslav spring oat cultivars tested in microtrials (1986-1992)

Year	Grain Yield (t/ha)				
	Rajac	Mediteran	Slavuj	Condor	Mean
1986	5.43	5.90	6.28	5.40	5.76
1987	5.32	5.59	5.43	4.71	5.26
1988	7.72	7.54	7.68	6.96	7.48
1989	7.74	7.48	7.85	7.00	7.52
1990	4.90	5.13	5.80	5.27	5.28
1991	3.79	4.20	3.55	3.29	3.68
1992	6.41	6.28	6.80	6.22	6.43
Mean (t/ha)	5.90	6.02	6.20	5.55	5.92
+differences (t/ha)	+0.35	+0.47	+0.65	0.00	

Table 2. 1000 kernels weight of Yugoslav spring oat cultivars tested in microtrials (1986-1992)

Year	1000 kernel weight (g)				
	Rajac	Mediteran	Slavuj	Condor	Mean (g)
1986	31.00	30.80	30.50	28.40	30.17
1987	31.10	29.60	29.80	29.30	29.95
1988	26.50	27.00	25.00	26.50	26.25
1989	30.00	26.50	30.00	25.50	28.00
1990	22.00	21.50	20.00	19.00	20.62
1991	27.00	24.00	22.00	26.00	24.75
1992	25.00	27.00	27.00	27.00	26.50
Mean (g)	27.51	26.62	26.32	25.95	26.60
+Differences (g)	+1.56	+0.67	+0.37		

Table 3. Mass of hectoliter at Yugoslav spring oat cultivars tested in microtrials (1986-1992)

Year	Mass of hectoliter (kg)				
	Rajac	Mediteran	Slavuj	Condor	Mean (g)
1986	52.30	52.70	54.55	45.35	51.22
1987	49.85	49.00	46.35	47.70	47.47
1988	43.50	43.90	39.60	41.45	42.11
1989	51.75	49.85	45.55	47.80	48.73
1990	51.90	55.15	51.25	46.75	51.26
1991	49.65	48.80	41.85	45.55	46.46
1992	48.40	51.90	51.05	47.80	49.78
Mean (kg)	49.62	50.18	47.17	45.65	48.15
+Differences (kg)	+3.97	+4.53	+1.52		

Table 4. Height of plants of Yugoslav spring oat cultivars tested in microtrials (1986-1992)

Year	Height of plants (cm)				
	Rajac	Meditaran	Slavuj	Condor	Mean (cm)
1986	107	102	100	106	104
1987	95	90	80	80	86
1988	111	100	110	107	107
1989	160	130	160	140	148
1990	99	102	100	101	101
1991	100	98	96	90	96
1992	110	90	100	110	103
Mean (cm)	111.7	101.7	106.6	104.9	106.2
+Differences (cm)	+6.9	-3.1	+1.7		

Table 5. Chemical analysis of grain of Yugoslav spring oat cultivars from microtrials (Krusevac and Kragujevac)

Characteristics of grain	1985		1986		1988	
	Rajac	Condor	Meditaran	Condor	Slavuj	Condor
Content of crude proteins %	13.2	11.5	13.2	11.5	14.2	12.9
Content of crude lipids (%)	2.4	2.5	3.4	3.7	6.3	4.9
Content of crude cellulose %	10.1	13.5	14.8	12.7	12.6	14.1
Nonnitrogen extr. subs. (%)	63.6	62.4	59.8	60.9	54.9	58.5
Content of mineral matter %	3.1	3.4	2.9	2.9	3.6	4.2
Content of organic matter %	90.5	90.5	87.4	87.4	96.4	95.9
Dry matter content (%)	93.6	93.9	90.3	90.3	91.6	94.5

The 1000 grain weight and hectoliter mass had higher value than check Condor cultivar (Table 2. and 3.).

Grain protein content in Rajac cultivar (13.2%), Mediteran (13.2%), Slavuj (14.2%) was higher than in check Condor cultivar which values of protein content varied from 11.5% to 12.9%. (Table 5.)

The variation of assayed properties in oat cultivars indicating influence of year environmental conditions.

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Grain Quality Aspects of Oat Brazilian Genotypes. S.C.K. MILACH, L.C. FEDERIZZI, G.C.H. THOMÉ, C.R.A. BOTHONA, C.B. CABRAL, M.T. PACHECO and M.C.C. TEIXEIRA, Departamento Plantas de Lavoura, Faculdade de Agronomia - UFRGS, Av. Bento Gonçalves, 7712, Cx.P. 776, Porto Alegre, RS, 90012-970, Brazil

Introduction. Oat grain physical and chemical quality has become an important goal in breeding programs around the world. In Brazil, it became of greater significance with the MERCOSUL, the official term signed by several countries opening the trade market in Latin America. Oat varieties ought to have high grain quality standards to be competitive in the Latin America market. Grain quality characterization of oat Brazilian genotypes has been limited due to difficulties of evaluating grain characteristics as length, width, area in breeding programs. New instruments including a digital image analyzer are now available at Federal University of Rio Grande do Sul allowing a precise characterization of physical grain aspects of oat Brazilian cultivars. Despite its superior protein quality, the oat grain is deficient for some essential aminoacids such as lysine, threonine and methionine. These aminoacids have to be supplemented in human or animal diets made with oat. Oat varieties with higher levels of such aminoacids can become an important food source, especially in third world countries. In higher plants, these aminoacids are synthesized from the aspartate metabolic pathway and their final production inhibited by the accumulation of lysine and threonine in the cell (Dougall & Fulton, 1967). Based in this knowledge, Phillips et al. (1981), suggested an *in vitro* selection system to identify high methionine corn lines. In the presence of lysine and threonine seed germination is inhibited because of methionine starvation. Genotypes with higher methionine level are less inhibited during seed germination. The objectives of this study were: a) characterize grain physical quality of fifteen elite oat cultivars; and b) determine the response of these cultivars on culture media supplemented with lysine and threonine to identify differences for grain methionine content.

Materials and Methods. Fifteen oat elite cultivars were evaluated in this study and are listed in Table 1. The image of three samples with twenty-five dehulled seeds were captured with a video camera for digital image analysis. The images were transferred to a high speed portable computer through an external video capturing card and were calibrated with a milimetric scale. The Sigma Scan/Image software from Jandel Scientific Corporation was used to analyze the digitalized images. Grain characteristics evaluated were area, length, width, perimeter and shape factor (which varies from zero to one, where one is the perfect circle). The same cultivars were evaluated for methionine levels. Seeds of each cultivar were desinfested with 70% alcohol, 2% sodium hypochlorite and washed three times in sterile water. Seventy-five seeds of each genotype were placed on five plates with MS (Murashige & Skoog, 1962) media supplemented

with 15 mM lysine plus 15 mM threonine and the same amount placed on MS not supplemented (control). All plates were incubated at 20°C in the dark. Primary root length of seven-day-old seedlings was measured for all genotypes from both treatments.

Results and Discussion. Phenotypic variability was identified for all physical grain traits evaluated (Table 1). The most recent released varieties have a larger grain, indicating that the local breeding programs have been successful in selecting visually for this trait. Variability was also identified for shape factor. Cultivars from the Federal University of Rio Grande do Sul breeding program are rounder than others. The digital image analysis shown to be an useful and precise method for physical grain evaluation of oat Brazilian genotypes. Symons & Fulcher (1988) were also able to identify significant differences among six oat genotypes for physical grain traits using a similar technique. Moreover, De Koyer et al. (1993) used image analysis to show changes in grain morphology through several cycles of recurrent selection for grain yield in oat.

Table 1. Grain physical quality aspects of fifteen oat Brazilian cultivars.

Cultivars	Área (mm ²)	Length (mm)	Width (mm)	Perimeter (mm)	Shape Factor
CTC2	20,2 A*	8,9 BCD	2,9 AB	21,3 AB	0,56 CD
UPF13	20,1 A	9,3 A	2,7 CD	21,9 A	0,53 FG
UFRGS16	20,1 A	8,6 CD	2,9 AB	20,7 BCD	0,59 B
UPF17	20,1 A	9,2 AB	2,7 CDE	21,7 A	0,53 EFG
UPF16	19,6 AB	9,3 A	2,5 EFG	21,7 A	0,52 GH
UFRGS15	19,5 AB	8,1 EF	3,0 A	19,9 D	0,62 A
UPF15	18,9 ABC	8,8 CD	2,7 CD	20,7 BCD	0,55 DE
UFRGS14	18,7 ABC	8,5 DE	2,8 BC	20,2 CD	0,57 BC
UFRGS10	18,1 BC	8,8 CD	2,6 DEF	20,7 BCD	0,53 EFG
CTC3	17,8 CD	9,0 ABC	2,4 G	21,0 ABC	0,50 H
UPF7	17,5 CD	8,6 D	2,6 DEF	20,1 D	0,54 DEF
UFRGS7	16,5 DE	8,5 D	2,4 G	19,8 D	0,52 FGH
CTC5	16,5 DE	7,8 F	2,7 CDE	18,7 E	0,59 B
UPF14	15,6 EF	8,0 F	2,4 FG	18,8 E	0,55 DE
CTC1	14,8 F	7,8 F	2,3 G	18,3 E	0,55 DE

* Means followed by the same letter do not differ significantly at 5% by Tukey's test.

There was difference for primary root length among the genotypes grown on the control media (Table 2). For this reason the relative growth of primary root on MS and MS plus lysine and threonine was more informative than the absolute treatment and control values. Phenotypic variability was observed for this trait. Root growth was inhibited from 30 to 50% in relation to the control. The cultivars UFRGS 15, UFRGS 16 and CTC3 were the least inhibited genotypes.

According to Phillips et al. (1981), this result indicates that these varieties have higher methionine content in the grain.

Table 2. Means of primary root length (cm) of fifteen oat Brazilian cultivars grown in MS medium supplemented or not with lysine plus threonine.

CULTIVARS	MS	MS + LYS + THRE	DIFFERENCE	RELATIVE
				GROWTH%‡
UPF 14	6,1 A	3,8 A*	2,4 A	61
CTC 5	5,4 B	3,2 B	2,1 AB	60
UFRGS 16	4,2 CDE	2,9 BC	1,6 BCDE	70
UPF 16	5,3 B	2,9 BC	2,4 A	54
UPF 13	5,4 B	2,8 BC	2,5 A	53
UFRGS 7	4,1 CDE	2,6 CD	1,4 CDE	64
UPF 7	4,4 C	2,6 CD	1,9 ABCD	58
UFRGS 15	3,6 DEF	2,6 CD	1,1 E	71
UPF 15	4,7 C	2,5 CD	2,2 AB	53
CTC 1	4,5 C	2,5 CD	2,0 ABC	55
CTC 3	3,6 EF	2,4 CDE	1,1 E	68
UPF 17	4,6 C	2,4 CDE	2,2 AB	52
CTC 2	4,3 CD	2,4 CDE	1,9 ABC	56
UFRGS 14	4,3 CD	2,2 DE	2,0 ABC	52
UFRGS 10	3,2 F	1,9 E	1,3 DE	60

* Means followed by the same letter do not differ significantly at 5% by Duncan's test.

† Mean difference between primary root length of control and treatment.

‡ Relative root growth in % of treated versus control medium.

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Beta-glucan and hordein contents of developing grain in Spain and Scotland.

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Introduction. The barley cultivar, Troubadour, and the mutant, TR49, derived from it, have proved to be widely adapted and suited to comparing the effects, on quality, of diverse environments. Differences between Spanish and Scottish grown grain have been observed in characters such as grain size, nitrogen content, relative proportions of hordein polypeptides and levels of soluble and insoluble beta-glucan (Swanson *et al.*, 1993), (Molina-Cano *et al.*, 1995). During malting, Spanish grown grain modified more rapidly, despite higher nitrogen levels, due to faster and more extensive enzyme development (Swanson *et al.*, 1995).

These data suggest that Spanish and Scottish grown grain differ both in structure and hormone levels and that the genotypes used may be well suited to studying the nature and timing of environmental effects, during grain filling. Meteorological data has also been obtained and efforts are being made to interpret the role of climatic differences in varying patterns of grain filling. Here, beta-glucan and hordein contents and mean temperatures, from one season of experimentation, are considered.

Materials and Methods. Plots of the genotypes Troubadour and TR49 were grown at the Scottish Crop Research Institute (SCRI), Dundee, Scotland and Bell-Lloc, Lleida, Spain in 1993. Samples of approximately 30 ears (more during early stages) were taken at twice weekly intervals and oven-dried (Riggs and Gothard, 1976), over the period from approximately two weeks after anthesis until harvest ripeness. Dried grain was finely milled and hordein content of the flour was determined by an h.p.l.c. method (Griffiths, 1987). Results are presented in arbitrary units, based on the sum of the areas under the peaks. Total beta-glucan content was measured by the method of McCleary and Glennie-Holmes (1985). To enable comparison between sites, development of grain constituents was considered against 'degree days after anthesis', the product of number of days and average temperature.

Results and Discussion. In the Dundee area, spring barley is sown in March, ears emerge in June and harvest is in August. By contrast, in northern Spain, the same barleys are autumn-sown and harvested in June. Weather data, for the last 3 months of growth (Fig. 1) show that the ripening period, for Spanish barley, was accompanied by a steadily increasing temperature. At harvest, temperature was much greater than at any point during the growth period in Scotland, where mean weekly temperatures did not increase between anthesis and harvest ripeness. On the scale of degree days after anthesis, however, harvest ripeness is attained at a similar point in both locations.

Analysis of variance (data not shown) indicated no significant differences between the two genotypes for any character measured here, so results for Troubadour and TR49 could be used as replicates. At harvest ripeness, Spanish grown samples had considerably higher levels of total beta-glucan (Table 1), although the insoluble proportion was lower. This is consistent with the view of Morgan and Riggs (1981), who

TABLE 1. Mean beta-glucan and hordein contents at harvest ripeness in 1993.

Location	Beta-glucan (%)		Hordein Content (arb. Units)	B:C Ratio
	Total	Insoluble		
Spain	4.71	2.88	1111.4	1.39
Scotland	3.94	3.07	755.9	2.76

suggested that beta-glucan, especially the soluble portion, was increased in hotter, drier environments. Differences between Spain and Scotland, in beta-glucan, were also evident in the pattern of development (Fig. 2). Smith *et al.* (1987) reported that total beta-glucan synthesis continued until approximately 6 weeks after anthesis, with levels then fairly constant until harvest ripeness. A similar pattern was observed in Dundee but beta-glucan levels in Lleida rose virtually throughout the period to harvest.

Total hordein content showed a similar pattern of development to beta-glucan, so differences in nitrogen levels of the mature grain, between Spain and Scotland, may be due to accumulation of storage protein in the later stages of development. This will have implications for spacial location of these proteins and relative proportions of their constituent polypeptides. Here, B:C hordein ratio was very different between the two environments (Table 1). Development of storage protein will also affect grain structure and malting quality, with such areas being considered in subsequent, more detailed publications of this research.

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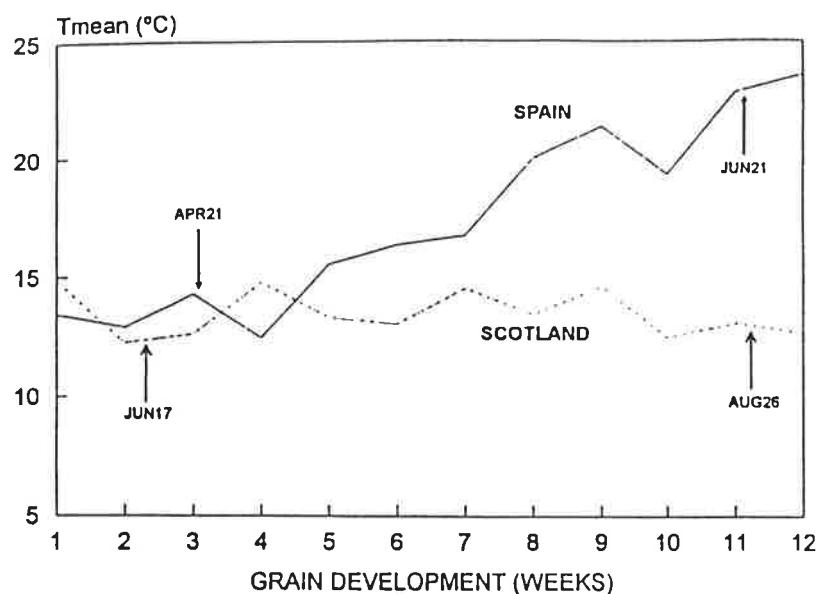


FIGURE 1. Mean weekly temperatures ($^{\circ}\text{C}$) during the development of the barley grain in Scotland and Spain. Arrows indicate common anthesis and maturity dates of two genotypes, Troubadour and TR49. Periods of grain development: Spain, April - June; Scotland, June - August.

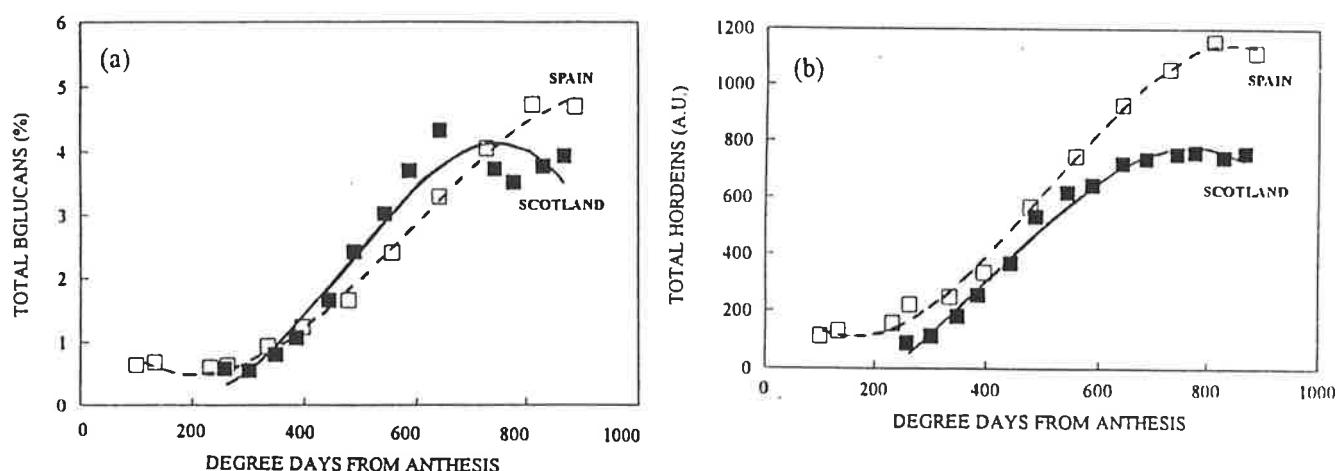


FIGURE 2. Changes in content of total beta-glucan (a) and total hordein (b), during barley grain development in Scotland and Spain:

Estimated breeding values of semi-dwarf, tall and naked (hull-less) oat genotypes for grain quality traits. S.D. Pelham¹, A.R. Barr², A.B. Frensham¹, P.K. Zwer¹ and B.R. Cullis³, ¹South Australian Research and Development Institute, Urrbrae, SA, 5064, Australia; ²Waite Agricultural Research Institute, Urrbrae, SA, 5064, Australia; ³Agricultural Research Institute, Wagga Wagga, NSW, 2650, Australia.

Introduction. The evaluation of parental material and the determination of its value in achieving the objectives set by a breeding program is often difficult. Information about particular performance attributes of a variety or breeders line can be used to predict its "breeding value" using a technique developed by Cullis et. al. (1996). By accounting for known sources of genotype x environment (G x E) variance this technique can more reliably predict the inherent "breeding value" (EBV) as well as the "specific value" (ESV) of a genotype for any measured characteristic. For example, a variety with stem rust resistance may have a high ESV for yield. This reflects the yield increase gained by selecting for this particular characteristic. The EBV reflects the yield gain due to genotypic factors other than the known specific traits which are included in the analysis. A variety or breeders line may have both a high ESV and a high EBV. It is the addition of both the ESV and EBV which will determine the best variety for any one characteristic.

This paper compliments a paper by Frensham et. al. (in preparation) which used a database set of 3073 mean grain yields from 174 trials conducted across southern Australia, spanning the period 1985 to 1994 to analyse the components of G x E variance. Using this technique, Frensham et. al. found that differences in plant type, stem rust resistance and Barley Yellow Dwarf Virus (BYDV) resistance explained a significant amount of the G x E variance. The same technique was used to analyse physical and chemical grain quality information to determine sources of variance. An ESV and EBV for the same set of varieties was calculated for each of the grain quality characteristics analysed.

Materials and methods. To accurately predict the "value" of a line it is first necessary to understand the G x E interactions which are influencing its performance and to account for as much of the unknown sources of variability as possible. A mixed model analysis as outlined by Frensham et. al. (in preparation), was performed on the physical and chemical grain quality characteristics most commonly measured by the South Australian oat breeding program; namely hectolitre weight, screenings percentage, 1000 grain weight, protein, and oil percent. Genotype traits included in the model were plant type (which compared dwarf with tall non-naked genotypes and naked with non-naked dwarf genotypes), maturity, BYDV, and stem rust resistance. The ESV therefore reflects the combined effect of all of these traits. The inclusion of their interactions with environment lead to an estimation of the unexplained G x E variance (data not presented).

Hectolitre weight (kg/hl) was measured by weighing a known volume of grain poured from a fixed height (calibrated against a standard chondrometer) and gives an indication of the density of a given sample. Screenings percentage (%<2mm) was measured by shaking a 50g sample over a 2mm sieve for 30 seconds. Grain size (g) was measured by weighing 1000 grains. Protein and oil percentage were measured using near infra-red (NIR) techniques.

Results and discussion. Plant type, maturity, BYDV, and stem rust resistance had varying effects on each of the different grain quality characteristics (Table 1). Hectolitre weight and grain weight were significantly effected by maturity and plant type. Early maturing, naked varieties had the highest hectolitre weights and early maturing, tall types had the best grain weights. BYDV resistance, maturity, and plant type had a significant effect on screenings percentage. Dwarf non-naked and tall varieties

which were earlier maturing and BYDV resistant had significantly lower screenings percentages. BYDV, stem rust resistance, and plant type had a significant effect on protein percent. BYDV susceptible, stem rust resistant, dwarf naked types had the highest protein. Plant type also had a significant effect on oil content with naked types producing lines with the highest oil content.

A number of lines were identified with high EBV for use as parental material (Table 2) to improve oat quality through improvements in grain density, grain weight, screenings percentage and to select high protein and high and low oil types. Euro, Carrollup, Kalgan and the breeders lines OX87;072-12 and OX82;042-48 had the highest EBV for hectolitre weight. The naked oat variety Bandicoot had the highest ESV because dwarf, naked plant types were highly significantly better for hectolitre weight compared with dwarf non-naked plant types (Table 1). Both Kalgan and OX87;072-12 had a low ESV for hectolitre weight because they are dwarf, non-naked plant type. Within tall plant types, Mortlock and Bulban differ in their ESV. Mortlock has a higher ESV because it is earlier maturing than Bulban. Maturity had a significant effect on hectolitre weight but this effect was not as large as the effect of plant type. Swan and Yarran had the highest EBV for grain weight. All tall plant types had higher ESV's than the dwarf plant types. Yarran and Bulban had the lowest EBV for screenings percent. Dolphin, West, Mortlock, and Yarran had the highest EBV for protein. Wallaroo, Kalgan, and Pallinup had the best EBV for high oil, whereas Euro and Bettong had the best EBV for low oil.

This study is complimentary to the one by Frensham et. al. (in preparation) and is a statistical technique which can confirm a breeders expectations which are often based on field experience. It can also be used to predict the value of parental material for a particular end use. Key plant characteristics can also be identified in a breeding program. This technique is invaluable in reducing the G x E interaction in any assessment of breeding trials by explaining a portion of the previously unexplained G x E variance.

Table 1: Estimated effects of key plant traits on the grain quality and statistical significance.

Trait	Hectolitre weight	Grain weight	Screenings percent	Protein	Oil
BYDV	-0.25 ns	-0.03 ns	-3.44 *	-0.32 *	-0.30 ns
Stem rust	0.24 ns	0.33 ns	-1.07 ns	0.28 ***	-0.12 ns
Maturity	0.35 **	0.49 ***	-1.88 ***	0.07 ns	-0.03 ns
Dwarf,non naked-dwarf, naked	-11.45 ***	9.44 ***	-39.43 ***	-3.46 ***	-2.31 ***
Dwarf,non naked-tall	-1.21 ns	-1.84 *	-3.20 ns	-0.53 *	0.30 ns

* P<0.05

** P<0.01

***P<0.001

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Table 2: Estimated specific value (ESV) and estimated breeding value (EBV) of a range of oat varieties and breeders lines grown in Australia for a number of grain quality characteristics.

Variety/breeders line	Origin*	Type#	Hectolitre weight	Grain weight	Screenings %	Protein	Oil
		ESV	EBV	ESV	EBV	ESV	EBV
Echidna	SA	D	-4.24	1.06	2.43	-13.23	2.17
Dolphin	SA	D	-5.44	-1.19	1.21	-0.87	-13.89
Swan	WA	T	-3.53	1.53	3.86	3.14	-11.80
West	WA	T	-3.67	0.51	3.43	-0.51	-7.84
Mortlock	WA	T	-2.17	1.08	5.50	0.42	-14.96
Bulban	VIC	T	-5.00	-0.76	1.73	0.71	-3.08
Marloo	SA	T	-4.00	0.52	3.02	0.34	-6.69
Wallaroo	SA	T	-2.02	-1.70	5.91	-1.73	-18.61
Kalgan	WA	D	-5.01	1.82	1.40	-1.17	-9.94
Cypress	NSW	T	-4.48	0.11	2.25	-2.65	-2.78
Bandicoot	SA	DN	6.76	-1.03	-7.60	1.39	-13.14
Dalyup	WA	D	-4.56	0.45	2.02	1.04	-12.18
Yarran	NSW	T	-1.97	1.30	5.49	2.77	-11.69
Potoroo	SA	D	-3.89	-1.17	2.97	-1.73	-15.76
Euro	SA	T	-3.15	2.43	4.26	1.08	-11.88
Bettong	Quaker	T	-3.15	0.66	4.34	1.32	-13.02
Carrolup	WA	T	-2.15	2.50	5.61	-2.04	-16.60
Pallinup	WA	T	-2.40	0.51	5.31	0.15	-15.59
OX82;042-48	SA	D	-3.12	2.39	4.22	1.64	-10.84
OX87;072-12	SA	D	-5.36	2.26	0.89	1.53	-7.67

* SA = South Australia, WA = Western Australia, VIC = Victoria, NSW = New South Wales, Quaker = derived from Quaker Oat Nursery for South America

D = Dwarf, T = Tall; DN = Dwarf, naked

North American oats as forage in Australia. G.J. PLATZ and R.G. REES,
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Introduction. In the subtropical areas of eastern Australia (Queensland and northern New South Wales) oats is grown for forage for cattle and, to a lesser extent, sheep. Oats is usually planted in late summer-autumn to provide autumn-winter-spring forage. Rusts are major limitations to reliable oat production in this area. Crown rust (*Puccinia coronata*) is a particular problem in forage crops and can be devastating in autumn and spring while stem rust (*Puccinia graminis avenae*) is more a problem in seed or grain crops in late spring. Major widespread epidemics in 1984 and 1988 caused extensive losses and increased efforts to reduce the problem. Historically, oats has been a neglected crop in this region with very limited research input and no breeding until recently. The potential value of North American oat varieties has been recognised for some time and Minhafer and others have been popular for forage. Although North American varieties have been developed for grain production under American conditions they have generally performed well for forage production in subtropical Queensland. The advent of Australian Plant Breeders Rights (PBR) on oats in the early 1990s has increased interest in oat varieties with release of a series of introduced varieties by private and public organisations.

Current varieties. The 14 varieties released between 1990 and 1996 and two potential releases for 1997 are listed in Table 1. All these varieties had their origin in North America although two reached Australia via Brazil or New Zealand. At the time of release each variety appeared to possess effective resistance to crown rust. Many were also resistant to stem rust. Virulent pathotypes of both rusts were rapidly selected on most of the resistances with only three varieties, including the new release Barcoo, remaining resistant to both rusts. Major developments in the rust populations include virulence on *Pc38+Pc39*, *Pc58+Pc59+Pc60+Pc61* and *Pg8+pg9+Pg13*. All varieties now resistant to stem rust appear to possess *pga* and hence all are vulnerable to a single new pathotype.

Future varieties. The mild climate of the subtropics of Australia allows plant growth all year and oat rusts survive freely on wild oats and self sown oats. While the perfect stage is non-functional, field rust populations are extremely diverse and widely virulent (J.D. Oates and D.G. Bonnett, pers. com.; G.J. Platz and R.G. Rees, unpublished). Pathotypes of *P. coronata* virulent on up to 18 or 20 recognised resistance genes occur (G.J. Platz and R.G. Rees, unpublished). Of particular concern is the presence in the population of virulence on resistances such as *Pc50*, *Pc63*, *Pc70* and *Pc71* not yet exposed in commercial varieties.

Simple resistance is unlikely to remain effective and hence the continued usefulness of many varieties imported directly from North America is in doubt. The two new releases proposed for 1997 with *Pc68* resistance are likely to have a short commercial usefulness. Virulence on *Pc68* has not been detected in Australia but is likely to develop as the area planted to these varieties increases. The need for a different strategy to control oat rusts in subtropical Australia has been recognised for some time.

Studies on slow-rusting resistance (Brake and Irwin 1992) and experience in Queensland suggest that presently recognised levels of slow-rusting resistance are unlikely to be adequate under the population pressures generated in this region. Recurrent selection may, however, be of value in enhancing this resistance.

Pyramiding genes for crown rust resistance is an approach being followed in Queensland in expectation that combinations of individually effective resistance genes will provide increased effectiveness to resistance and help conserve the supply of resistances available. Selecting the desired combinations of resistance genes is difficult with conventional techniques and molecular markers offer considerable potential and are to be used to facilitate selection. In time, locally developed varieties largely based on those from North America but with complex rust resistance should provide a greater degree of stability to the oat variety situation in subtropical Australia.

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Table 1. Recent forage oat varieties for subtropical Australia

Variety	North American notation	Origin	Australian release by	Release year	Crown rust**	Stem rust**
Cluan	Q0 220-29	Quebec	DPIF Tasmania	1990	S	S
Quamby	Q0 215-19	Quebec	DPIF Tasmania	1990	S	S
Panfive	CI 8456	Minnesota	Qld. University	1990	S	S
Amby	82 Quaker 197	Texas	DPI Queensland.	1991	S	R
Culgoa	82 Quaker 225	Texas	DPI Queensland	1991	R	R
Cleanleaf*	ND 811386	N. Dakota	Pacific Seeds	1992	R	R
Enterprise *	CDA 10,01	Manitoba (N.Z.)	Heritage Seeds	1993	S	S
Riel*	Riel	Manitoba	DPI Queensland	1993	S	S
Condamine*	UPF 86 S071	Texas (Brazil)	Pacific Seeds	1994	S	R
Graza 50*	Valley	N. Dakota	Pioneer Hi-Bred	1994	MR	S
Graza 60	Dumont	Manitoba	Pioneer Hi-Bred	1994	S	S
Graza 70*	Robert	Manitoba	Pioneer Hi-Bred	1994	S	S
Nobby*	82 Quaker 207	Texas	DPI Queensland	1994	S	R
Barcoo*	88 Quaker 129	Texas	Pacific Seeds	1996	R	R
	Dumont 68	Manitoba	DPI Queensland	1997	R	S
	Robert 68	Manitoba	Pioneer Hi-Bred	1997	R	S

* PBR protected

** Response to current pathogens: S = susceptible, R = resistant, MR = moderately resistant

Mapping of loci that affect carbohydrate content in barley grain. S.E. ULLRICH¹, F. HAN¹, J.A. FROSETH¹, B.L. JONES², C.W. NEWMAN³, D.M. WESENBERG⁴, NORTH AMERICAN BARLEY GENOME MAPPING PROJECT; ¹Washington State Univ., Pullman, WA 99164; ²USDA-ARS, 501 Walnut St. Madison, WI 53705; ³Montana State Univ., Bozeman, MT 59717; ⁴USDA-ARS, P.O. Box 307, Aberdeen, ID 83210, USA.

Introduction. The concentration of the various carbohydrate components in barley (*Hordeum vulgare* L.) grain impact both agronomic and end-use characteristics. This study involved starch, β -glucan, and acid detergent fiber (primarily cellulose) contents. Starch concentrated in the endosperm contributes positively to grain yield and other physical kernel traits, malt extract, brewer's wort, and food/feed energy content. β -glucans concentrated in endosperm cell walls contribute positively to hypocholesterolemia in humans and other animals and negatively to brewing and poultry feed quality. Acid detergent fiber (ADF) concentrated in the pericarp and hull (palea and lemma) contributes positively or negatively to physical grain characteristics, embryo protection, beer filtration and nutrient utilization and intestinal health (MacGregor and Fincher, 1993).

An understanding of genetic control of these carbohydrate components should improve breeding effectiveness of the various traits affected by their concentrations. The objective of this study was to map genes associated with these quantitative traits (starch, β -glucan, ADF contents) from the six-row spring barley cross, 'Steptoe' x 'Morex'. A 150 F_1 derived doubled haploid line (DHL) mapping population and comprehensive molecular marker linkage map were developed by Kleinhofs et al. (1993). The β -glucan mapping data were published previously (Han et al. 1995) but included here to complement the other carbohydrate mapping data.

Material and Methods. Chemical component data were collected from grain samples of the 150 DHL mapping population and parents grown at two to four western USA locations in 1991 and/or 1992. Starch, β -glucan and ADF analyses were performed as described by Åman and Hesselman (1984), Han et al. (1995), and AOAC (1980), respectively. Quantitative trait locus (QTL) analyses were based on MAPMAKER/QTL (Lincoln et al. 1992) as described by Han et al. 1995.

Results and Discussion. *Starch.* Although the difference between mean starch percentage of the parents was slight, the starch distribution in the 150 DHLs was relatively broad and normal (Fig. 1a). The DHL population frequency distribution indicates there are genetic differences between Steptoe and Morex for starch content. The QTL analysis identified a locus on chromosome 2 and another on chromosome 6 (Table 1). These two loci account for about 21% of the total variation. The small number of genes and low multilocus r^2 are probably due to the relatively low polymorphism between the parents. A parallel study in Montana identified the same chromosome 6 locus (J.G.P. Bowman, 1996, personal communication).

β -glucan. The low parent polymorphism and broad normal mapping population frequency distribution situation for β -glucan content (Fig. 1b) is similar to that of starch content. Three loci were identified; two on chromosome 2 and one on chromosome 5

(Table 1). The three loci together account for 34% of the total variation in β -glucan percentage.

Table 1. Mapping summary: QTL genotypic difference (Diff), variation explained (Var%), and LOD score for carbohydrate component content in barley grain.

Chromosome	Marker Interval (cM between marker)	Starch			ADF			Barley β -glucan		
		Diff	Var%	LOD	Diff	Var%	LOD	Diff	Var%	LOD
2	ABG703- <i>Chs1B</i> (11.0)									
	<i>Rbcs</i> -ABG002 (11.5)				0.67S	28.0	9.1	0.23M	10.5	3.2
	<i>Adh8</i> -ABG014 (26.6)	1.13S†	12.3	4.0	0.68S	28.4	8.8	0.32M	19.2	5.8
	<i>His3C</i> -ksu15 (11.9)				0.64S	24.7	7.2			
4	WG622-ABG313B (10.5)				0.39S	9.3	2.9			
5	AGA006- <i>Hor1</i> (13.0)				0.61S	23.6	8.1			
	<i>Glb1</i> -ABC160 (8.8)							0.28S	15.0	4.9
6	PSR154- <i>Nir</i> (12.3)	1.00S	10.7	3.5						
Multilocus r^2 (%)			20.7				64.5			34.0

† The letter suffix indicates the parent contributing the larger value allele . S - Steptoe; M - Morex.

ADF. The parents were very different in ADF content and the DHL frequency distribution was broad and normal (Fig. 1c). Five loci were identified; three on chromosome 2 and one each on chromosome 4 and 5 (Table 1). Nearly 65% of the variation in ADF content is explained cumulatively by these five QTLs.

Relationships among QTL. Interestingly, a QTL was identified for each trait in the same *Adh8*-ABG014 interval on chromosome 2. This could mean there is really only one gene in this interval that controls a major carbohydrate synthesis function. Alternatively there may be a cluster of closely linked genes. The fact that not just one parent contributes the higher value allele complicates the one gene pleiotropy theory . It is possible the β -glucan locus in the *Glb1*-ABC160 interval is actually *Glb1*, a gene for β -glucanase isoenzyme EI (Han et al. 1995), and the ADF locus on chromosome 2 might be related to the rubisco gene (*Rbcs*) that flanks the locus. The cluster of ADF loci and overlap of starch and β -glucan loci on chromosome 2 might be just that; a cluster of loci. But we are dissecting the region further with near isogenic lines to attempt to sort out the actual number of genes.

In conclusion, progress has been made to identify genes that affect grain carbohydrate content. The flanking markers of these loci could be used for molecular marker assisted selection in breeding. It would be desirable to go further and analyze other major carbohydrate components such as hemicellulose and arabinoxylans that affect barley agronomic and end use traits.

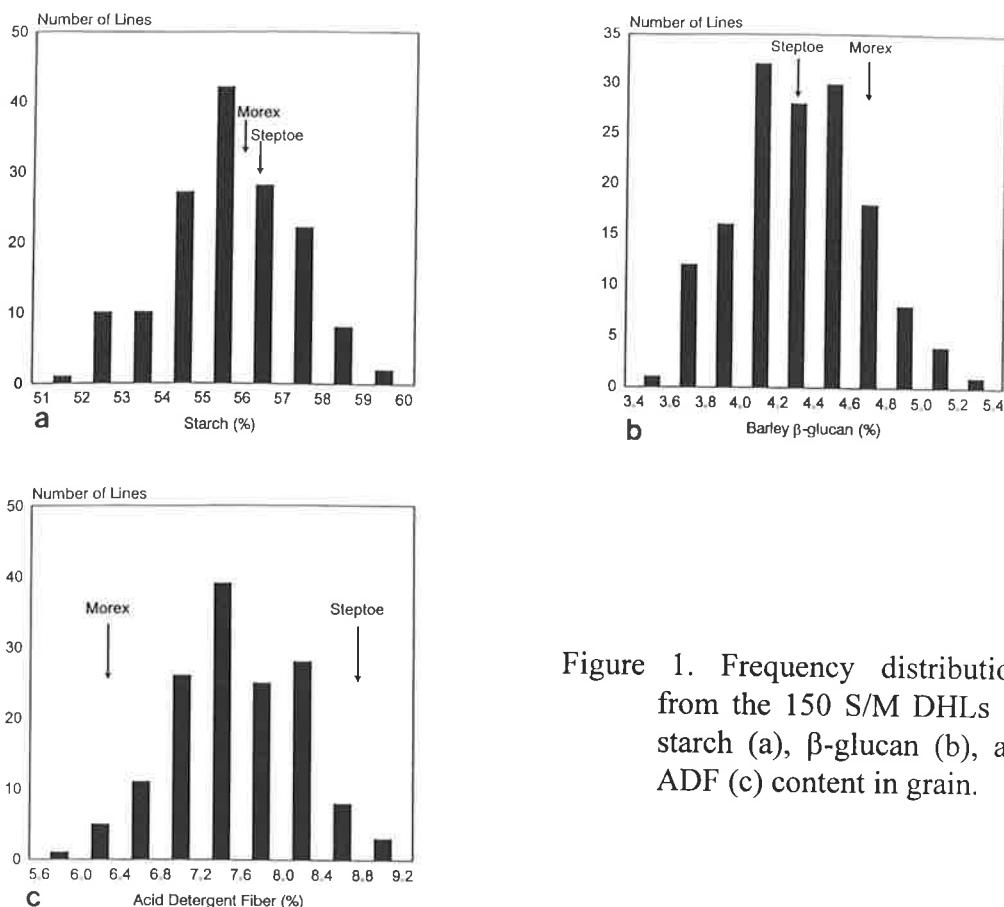


Figure 1. Frequency distributions from the 150 S/M DHLs for starch (a), β -glucan (b), and ADF (c) content in grain.

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Ruminal degradation characteristics and feeding value of hulless barley relative to barley and corn for dairy cows. W.Z. YANG, K.A. BEAUCHEMIN and L. M. RODE, Agriculture and Agri-Food Canada, Lethbridge, AB.

INTRODUCTION. Barley (B) is a predominant grain in dairy concentrates in western Canada. However, B is lower in digestible energy than corn (C) due to its hull, so cows fed B based-diets usually produce less milk than cows fed C based-diets (Overton et al., 1995), unless B diets contain a greater proportion of concentrate. High concentrate B based-diets are rapidly fermented in the rumen following ingestion (McAllister et al., 1990). This increases the acidity of the rumen which may induce a series of metabolic disorders.

Hulless barley (HB) cultivars have been recently commercialized and may provide an alternative means of increasing the digestible energy content of the diet without altering forage to concentrate ratios. The objective of this study was to compare the effects of HB with B and C on dry matter (DM) intake, total tract digestibility and milk production of lactating dairy cows. A second objective was to evaluate the ruminal digestion kinetics of these grains as affected by processing technique.

MATERIAL AND METHODS. Twenty-four lactating Holstein cows, 12 primiparous and 12 multiparous, were used in an experiment designed as eight 3×3 Latin squares with three 21-d periods. Three concentrates were formulated to contain mainly steam-rolled HB (cv. Condor), B or C. Each period, cows received one of three diets consisting of 60% concentrate, 30% barley silage and 10% cubed alfalfa hay (DM basis). The diets were isonitrogenous but differed in estimated net energy for lactation (NE_L) (HB, 1.68; B, 1.64 and C, 1.67 Mcal/kg). Feed intake and milk yield were recorded daily. Milk samples were analyzed for milk fat, protein and lactose. Apparent digestibility was determined by adding Cr_2O_3 to the diets.

The effective ruminal degradability of HB, B and C grains was determined in situ using six ruminally cannulated Holstein cows. Each cereal grain was tested using two physical forms: ground to pass a 2-mm sieve or steam-rolled. An additional temper-rolled HB with 21% moisture was also tested. Experimental details and analytical procedures are described in Beauchemin et al. (1994). Data were analyzed using the general linear models procedure of SAS (1989). Differences among means were tested using the PDIFF option in SAS (1989).

RESULTS AND DISCUSSION. Generally, no interactions were observed between parity and diet, therefore, data were pooled from primiparous and multiparous cows. Daily DM intake (kg/d) was higher for C (22.0) than for B (18.6), which was similar to HB (18.9) (Table 1). Lower intake of cows fed barley may be related to its rapid rate of ruminal fermentation (Nocek and Tamminga, 1991).

Total tract digestibility was consistently lower for HB than for B or C for DM (57.6 vs. 63.1, 64.1%), starch (85.1 vs. 95.6, 92.2%) and CP (53.3 vs. 60.8, 62.5%) (Table 1). Low digestibility of the HB diet was likely due to ineffective grain processing, as a considerable amount of whole grain was observed in the feces. Poor digestion of whole barley by cattle was noted by Beauchemin et al. (1994).

Higher milk yield was observed for C than B or HB diets (30.1 vs. 28.7, 28.3 kg/d) (Table 1). Differences in milk yield between cows fed HB and C were unexpected because these diets were estimated to be isocaloric. This difference probably resulted from higher DM intake of cows fed C combined with lower digestibility of the HB diet. Higher feed efficiency was observed for HB than for B and C when expressed as kilograms of milk yield per kilogram of digestible DM intake (2.81 vs. 2.50, 2.45), but not when expressed per kilogram of DM intake (1.50 vs. 1.54, 1.53). These results suggest that, once digested, HB is more efficiently used for milk production than B or C.

Higher milk yield of cows fed C compared with B is in agreement with Overton et al. (1995). In addition to higher DM intake for the C diet, a shift in the site of starch digestion to the small intestine probably increased the availability of glucose for lactose synthesis. Nocek and Tamminga (1991) postulated that absorption of glucose derived from the hydrolysis of starch in the small intestine may spare glucose derived from gluconeogenesis for gut metabolism, which may indirectly increase the amount of glucose available to the mammary gland.

Milk composition was generally not affected by grain source as shown in Table 1. The higher lactose percentage of milk from cows fed C than B may be due to more glucose absorbed from the small intestine resulting in increased availability of glucose for lactose synthesis.

Ruminal DM degradability of ground grains was higher for HB than B (82.9 vs. 77.7%), indicating high potential digestibility due to the absence of hull (Table 2). Dry matter, starch or CP degradability of ground grains was lower for C than B or HB. The protein matrix in the horny endosperm of C is extremely resistant to digestion by ruminal microorganisms (McAllister et al., 1990).

As expected, steam rolling reduced ruminal degradability compared with grinding (Table 2). In contrast to ground samples, steam-rolled HB had lower DM and starch degradability than B. This indicates that steam-rolling may be inappropriate for HB. Although low degradability was also observed for steam-rolled C, this was compensated for by high digestion in the small intestine so that total digestibility was similar for B and C. Temper-rolling significantly improved ruminal digestion of HB compared with grinding or steam-rolling.

In conclusion, HB may increase dairy cow productivity if HB is adequately processed to ensure high ruminal and intestinal digestibility. Ruminal digestion of grain was affected by the processing technique used. Steam rolling was ineffective for HB but temper-rolling improved the ruminal digestion of HB.

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Table 1. Dry matter (DM) intake, digestibility, and milk yield and composition.

Item ²	Diet ¹			
	B	HB	C	SE
DM intake, kg/d	18.6b	18.9ab	20.0a	0.5
Digestibility, %				
Dry matter	63.1a	57.6b	64.1a	2.0
Starch	95.6a	85.1b	92.2a	1.8
Crude protein	60.8a	53.3b	62.5a	2.2
Milk yield, kg/d				
Actual	28.7b	28.3b	30.1a	0.4
4% FCM	26.1b	26.1b	27.5a	0.5
Milk composition, %				
Fat	3.46	3.55	3.50	0.04
Protein	3.19	3.13	3.18	0.03
Lactose	4.83b	4.85b	4.89a	0.01
Milk efficiency				
Milk/DM intake	1.54	1.50	1.53	0.03
Milk/digestible DM intake	2.50b	2.81a	2.45b	0.10

¹B = barley, HB = hulless barley, C = corn. ²FCM = fat corrected milk.

a,b Means in the same row with unlike letters differ ($P < 0.05$).

Table 2. In situ ruminal effective degradability¹ of hulless barley (HB), barley (B) and corn (C) in dairy cows.

	Grain			
	B	HB	C	SE
Dry matter				
Ground	77.7b	82.9a	56.6c	0.7
Steam-rolled	43.3a	37.4b	24.9c	1.4
Temper-rolled	-	69.0	-	-
Starch				
Ground	93.1a	90.1a	62.1b	2.3
Steam-rolled	53.2a	41.6b	32.5c	1.7
Temper-rolled	-	-	-	-
Crude protein				
Ground	79.8a	76.6a	48.8b	2.3
Steam-rolled	30.7a	29.1a	8.0b	2.3
Temper-rolled	-	65.8	-	-

¹Calculated using equation $a+bc/(c+k)$, k was assumed to be 6%/h.
a,b,c Means in the same row with unlike letters differ ($P < 0.05$).

WINTER BARLEY MUTANTS FOR FODDER PURPOSES IN UKRAINE

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Introduction

Increase in fodder and protein production is one of the guidelines in the world-wide development of agriculture. Higher productivity of fodder crops due to new highly productive varieties, winter barley for green fodder in particular, is the means to gain the aim in Ukraine.

Materials and Methods

Newly created varieties of winter barley for green bulk were the object of our investigation. The varieties were produced as a result of affecting winter barley seeds by water solutions of chemical mutants (O.M.Zayats, M.M.Pavlyshyn, N.I.Bobyleva, 1985, 1993, 1995). Varieties were tested 4-6 times according to the method by the State Committee for Progeny Testing of Agriculture Crops (1971). The dispersion analysis, described by B. Dospechov (1978), was the basis for statistical data processing.

Results and discussion

New varieties of winter barley for green fodder are selected in the Institute of Agriculture and Animal Husbandry of Western Ukraine. The winter barley varieties were investigated in different climatic zones of Ukraine : Steppe, Forest and steppe and Polissya to prove their efficiency. The Steppe climate is mostly continental and droughty. Despite high annual temperatures, precipitation amount to 250-300 mm. The climate of the Forest-steppe zone is temperate continental with average annual temperatures 7-8°C and precipitation of 500-700 mm from the west to the east. The climate of Polissya is temperate and humid with precipitation of 500-600 mm/per year.

State Progeny Testing and Variety Protection Committee of Ukraine has conducted testing of winter barley varieties in different climatic zones based on recommendations of scientists from the institute. According to the recommendations 2,5-3,5 million germination seeds of winter barley per 1 hectar were sowed in optimal terms. The number of seeds per 1 hectar was diminished by 20% for running to seeds. The green bulk yield of different varieties was determined at the initial stage of coming into ears (because green bulk usage of winter barley varieties, according to observations after plants growing, starts at the early stage of coming into ears and continues for 5-7 days). Grain yield is determined during the

stage of full ripening. Crops were sown on promising fields after various predecessors. The results are shown in Tables 1,2.

Table 1

Field progeny testing of winter barley varieties for green bulk yield in 1993-1995

Variety designation	Yield of dried fodder t/ha	Days to ripening	Plant height sm	Stems per plant	Leaves %	Resistance to falling rating	Resistance to drought rating	Survival after winter, rating
Steppe zone								
Broad-leaved	6.15	244.5	105.0	1.9	43.7	5.0	5.0	5.0
Fodder	6.88	246.0	100.0	2.1	46.4	5.0	5.0	5.0
LSD 0.05	0.39							
Forest and steppe zone								
Broad-leaved	6.52	244.7	146.0	2.4	37.1	5.0	4.0	3.5
Fodder	6.25	244.0	109.5	2.4	38.9	5.0	4.0	3.5
LSD 0.05	0.55							
Polissya zone								
Broad-leaved	7.82	243.3	116.6	3.2	42.9	5.0	4.8	4.9
Fodder	7.83	233.4	117.0	3.4	42.5	5.0	4.8	4.8
LSD 0.05	0.40							

Table 2

Field progeny testing of winter barley varieties for grain yield in 1993-1995

Variety designation	Grain yield, t/ha	Weight of 1000 grains, gram	Days to ripening	Resistance to falling, rating	Resistance to crumbling, rating	Resistance to drought, rating	Survival after winter, rating
Steppe zone							
Broad-leaved	2.08	30.4	262.7	4.0	5.0	4.0	5.0
Fodder	3.09	31.8	263.3	4.3	5.0	4.0	5.0
LSD 0.05	0.44						
Forest and steppe zone							
Broad-leaved	3.04	44.7	289.5	4.5	4.5	4.0	4.0
Fodder LSD 0.05	2.91	43.5	279.5	4.0	4.5	4.0	4.0
0.38							
Polissya zone							
Broad-leaved	3.89	40.0	284.6	3.6	4.3	4.7	4.0
Fodder	3.80	40.5	279.1	5.0	4.6	4.7	4.8
LSD 0.05	0.35						

Conclusions

Varieties of winter barley intentionally created to produce green bulk, are elastic and provide high green bulk yields and normal seeds productivity in various climatic zones of Ukraine without additional expenses.

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The quality characteristics of new varieties of oats.

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Introduction. In 1995 the acreage of spring oat for grain was about 45.000 ha, which is 11% of the whole acreage of cereals in Latvia, and the total harvesting of grain was 73.300 tons. At present oats were grown as a fodder crop, but more than 10.000 tons per year were used for oat-flaks and other food production. Each year Latvian farmers are growing about 10 spring oats varieties, five of them are Latvian origin.

The oats breeders must develop varieties with high yield potential, short growing period, good lodging and diseases resistance, high protein and fat content of the kernels, but cultivars, which are using for food, request high 1000 kernels and volume weight, low husk percentage, too.

Materials and Methods. Competitive trials of new oats varieties were carried out during 1991-1995 in the State Stende Plant Breeding and Agriculture Research Station. The soil type: sod-podzolic soil, loamy sand. The mineral fertilizer application: N-50kg ha⁻¹, P₂O₅-60kg ha⁻¹, K₂O-60kg ha⁻¹. The precrop was potato. The seed rate: 550 kernels/ m².

Spring oats varieties: 'Mara' ('Astor' / 'Stendes vēlās' / 'Premis')
'Liva' ('Stendes vēlās' / Stende 14908)
'Laima' (Pol 1712 / D-104)
'Arta' ('Hankia' / 'Kurokaura')

These varieties are evaluated compared to 'Selma', which is used as the official standard variety in the oat trials since 1977. The trial plot was 10 m² in four replications. The yield, heading in days, 1000 kernels and volume weigh, husk percentage, protein and fat content of the kernels, resistance to crown rust were estimated and established.

Results and Discussions. The average date of five years trials experiment at Stende were represented in table 1 and 2.

Table 1. The yield and quality of oats varieties in Stende (1991- 1995)

Variety	Yield kg ha ⁻¹	%	Heading in days	TKW g	Volume weight g l ⁻¹
Selma- standard	4260	100	110	37,8	509
Mara	4830	113,4	110	41,6	504
Liva	4060	95,3	114	39,1	490
Laima	4710	110,6	109	40,4	515
Arta	3580	84,0	102	38,4	521

1.Yield. The most yielding varieties were 'Mara' and 'Laima' correspondingly 4830 and 4710 kg ha⁻¹. It was superior to 'Selma' for 113,4 and 110,6%.

2.1000 kernels weight. An important trait for food oat is high 1000 kernel weight would be at least 39 g. The TKW of varieties 'Mara', 'Liva' and 'Laima' corresponds to these requirements. Average TKW of 'Arta' was 38,4 g and it was superior to 'Selma' by 0,6 g.

3. Volume weight. The volume weight of all testing oats varieties correspond to requirements for food productions. Varieties 'Arta' and 'Laima' were the highests volume weight respectiveli 521 and 515 g l⁻¹.

4. Heading in days. The variety 'Arta' was early ripening, the groving period was 6-8 days shorter than 'Selma'. Period of vegitation of 'Mara' and 'Laima' were equal to 'Selma'. The latest variety was 'Liva'.

Table 2. The grain quality of oats varieties in Stende (1991 - 1995)

Variety	Protein content %	Fat content %	Husk %
Selma- standard	10,6	4,1	27,5
Mara	14,1	4,4	25,6
Liva	14,2	4,5	24,6
Laima	11,6	5,4	26,9
Arta	14,9	4,6	21,5

5. Husk percentage. The variety 'Arta' was charactering with the lowest husk percent. It was considerably lower than 'Selma' (by 6%). Varieties 'Mara', 'Liva' and 'Laima' were medium high husk percent.

6. Protein content. Protein content of the kernels for food oat is recomended 11,5% or more. The tested varieties characterized with protein content 11,6-14,9%. It was higher than standard 'Selma'. Average protein content of 'Arta' was 14,95, but the highest protein percent was 15,85 in 1993.

7. Fat content. Average fat content of 'Laima' was 5,4%, which was the highest index from tested varieties and for 1,3% higher than the standard 'Selma'. Fat percent of the other varieties was equel to 'Selma'.

8. Diseases resistance. Varieties 'Mara' and 'Laima' were resistant to crown rust, 'Liva' - medium resistant, 'Arta' and 'Selma' - medium susceptible.

Conclusions. The results of testing displays that 'Mara', 'Liva' and 'Laima' turn to be varieties with high yield, good grain quality and resistance to diseases and have been included in the Recommended growing list in Latvia since 1990, 1991 and 1996 years.

Although 'Arta' was medium grain yielding and low resistance to crown rust, but this variety have especially high quality of grain and short growing period, which is very important in wretched weather conditions. 'Arta' is perspective oats varietiy and has been delivered to the official varieties testing in 1994.

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Section VI: Genetic Resources/Diversity

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Developing a barley core subset in the USDA-ARS National Small Grains Collection. H.E. BOCKELMAN, USDA-ARS-NSGC, P.O. Box 307, Aberdeen, ID 83210, USA.

Abstract. A barley core subset is being developed in the USDA-ARS National Small Grains Collection (NSGC) to assist scientists studying diversity in barley, to assist in focusing requests for new evaluations, and to aid in the management of the still increasing size of the NSGC. In 1994 a preliminary core subset of 2,300 accessions was established representing approximately 9% of the *Hordeum vulgare* ssp. *vulgare* accessions in the NSGC. Accessions were randomly selected based on the log of the total number of accessions from each country of origin. Accessions chosen are from 105 countries and represent landraces, breeding lines, cultivars, and genetic stocks. Complete agronomic, spike, and seed descriptor data are being collected on this core. In addition disease, insect, and quality descriptor data will then be utilized to select the final core subset consisting of about 5-7% of the total collection, but still containing most of the diversity of the total collection.

Characterization through growth cicle and growth pattern, yield and yield components, and grain quality of the barley germplasm in Uruguay. A.J. CASTRO, O. ERNST, E. HOFFMAN and O. BENTANCUR. Est.Exp."Dr. Mario A. Cassinoni"(EEMAC), Facultad de Agronomía, Universidad de la Repùblica, Ruta 3 km.373, Paysandú 60000, Uruguay.

INTRODUCTION. Barley genetic variability in the world is small and has severe constraints by the malting industry, which has been acting as a limiting factor for the breeding programs. Luizzi y Castro (1992) studied the genealogies of the barley cultivars sowed in Uruguay in 1990-91 and showed the limited genetic basis used. This situation is the same in other production areas (Peeters,1988). In order to face this problem, the EEMAC begun in 1991 a research program which looks for a quantification and characterization of the available genetic variability for variables of agronomic and industrial interest, as a support for the breeding work. The main objective of the breeding program is to develop new genotypes with improved malting barley yield potential for Uruguayan conditions, mantaining the present grain quality. The first results allowed grouping varieties according to their initial growth (Hoffman et al.,1995), yield, yield components and heading date (Castro et al.,1995). In this study the available genetic variation is characterized according to its behaviour in two contrasting years.

MATERIALS AND METHODS. Forty one genotypes of different origins (Brasil 16, Argentina 1, USA 6, Canada 1, Southafrica 5, Europe 10, unknown 2) and the 5 more widely sown varieties in Uruguay (used as checks), were grown in the EEMAC in 1992 and 1993. The checks are varieties introduced from Australia (Clipper, Stirling and Est.Quebracho), Brazil (MN 599) and USA (Bowman). The sowing dates (July 9-10th,1992 and July 15th, 1993) and general agronomic conditions were very similar, therefore the differences in yield and quality were assumed to be a consequence of the limiting environmental conditions in 1993 (1.6°C higher mean daily temperature during the heading-physiological maturity period and 172 mm aditional rainfall). The mean yield was 5287 kg/ha in 1992 and 3682 kg/ha in 1993.

Comparisons between pairs of genotypes were made in order to generate a distance matrix based on the distance coefficient "Taxonomic Mean Distance" that allowed grouping using the UPMGA method of the NTSYS clustering procedure. The variables used in the analisys were selected according to the correlations between them and the study of the principal components analisys, using the PRINCOMP procedure of SAS. Variables highly correlated or with low eigenvector values were eliminated, until reaching the 85% of total variation in the first 4 principal components.

RESULTS AND DISCUSSION. Nine groups were formed with mean values presented in Table 1. Group B was subdivided because it represents the 57% of the genotypes analized. Group D than includes MN 599 showed a relative lower yield in the low yielding year (1993). Group A showed a relative grain weight higher and lower yield in that year. This group includes all the australian checks, and it can be considered an estimation of present production situation. These genotypes decrease their yield in bad climatic conditions but could maintain the grain quality. This behaviour is very valuable in economical terms because allows a good comercialitiation every year. Groups A and B include all the materials that had a behaviour similar to the checks in 1993. In 1993 (limiting environmental conditions) no genotype yielded better than the best check while in 1992

(good conditions to express the yield potential) 10 genotypes did better than the best check. None of the 10 genotypes belonged to groups A or B1. Therefore, genotypes of these groups, with tendencies between years similar to the check genotypes, had lower absolute values. This means that this genotypes may not be valubles for improving yield potentials in Uruguay.

Table 1.- Group means of variables included in the joint analysis(*)

Group	Yield	Biomass	Harvest index	grain weight	Spikes /m ²	Grain /esp	Gr. plumpness >2.8	Gr. plumpness >2.5	Tillers fert.	Nº
A	- 4.0	-11.9	4.5	11.3	3.2	-10.7	23.5	5.9	15.5	- 9.9
B	5.5	3.4	2.4	- 1.4	- 2.1	2.6	- 0.8	0.1	- 1.5	- 2.5
C	- 4.9	10.5	-14.1	- 7.0	4.2	12.8	-12.8	- 4.8	22.1	-18.2
D	-12.3	- 5.0	- 8.3	- 1.0	23.9	10.5	- 5.1	0.3	-10.5	33.8
E	2.7	- 2.2	6.6	8.8	66.2	3.5	- 4.6	- 2.4	20.1	47.9
F	18.7	21.0	1.1	13.1	0.5	9.1	30.1	15.0	8.3	- 7.7
G	-15.3	-13.5	- 2.0	-19.6	37.6	-56.1	-35.9	-13.0	9.8	30.5
H	-20.2	- 6.3	-12.5	14.7	-76.0	-15.2	- 2.8	1.8	-13.9	-63.0
I	-21.8	-10.5	-11.8	-20.3	-99.0	43.1	5.6	-11.5	-79.6	- 4.2
B1	10.8	2.1	8.4	6.2	2.3	- 3.3	21.6	4.4	13.3	-12.8
B2	3.9	3.8	0.7	- 3.6	- 3.3	4.2	- 7.1	- 1.2	- 5.7	0.4
B21	4.6	5.5	- 0.6	- 3.4	- 0.5	1.2	- 8.3	- 1.7	0.8	- 2.8
B22	1.2	- 3.5	6.2	- 4.6	-15.1	17.1	- 2.2	0.9	-33.2	14.0
B211	-2.4	- 1.7	- 0.6	- 5.0	- 2.6	6.5	-13.2	- 3.1	7.1	-12.5
B212	10.8	11.8	- 0.6	- 1.9	1.3	- 3.5	- 3.9	0.3	- 4.9	5.8

(*)- $y = (y_{93} * 100) / Y_{93} - (y_{92} * 100) / Y_{92}$ y=observed value Y= mean value

Table 2.- Behaviour in both years of the genotypes that surpassed the best check yield in 1992.

Genotype	Yield (kg/ha)		Harvest index		% of grains of diameter >2.5mm	
	1992	1993	1992	1993	1992	1993
PFC 86109	5791	3829	0.401	0.349	95.3	87.7
MN 610	5700	3823	0.424	0.380	91.1	88.8
Berit	5959	3229	0.442	0.377	88.9	86.8
Sybilla	5650	4076	0.436	0.392	92.0	84.3
Corniche	5638	3723	0.443	0.362	93.0	88.7
Berolina	5979	3957	0.422	0.375	89.3	82.9
Wpg 8412	5766	3861	0.377	0.350	94.5	83.8
Poland	6197	3279	0.412	0.348	89.3	72.3
LCI 176	5848	3528	0.367	0.292	89.0	84.6
UCD 8710399	5831	3189	0.449	0.371	58.1	45.4
Quebracho	5625	3887	0.433	0.426	95.3	95.0
Bowman	5388	4149	0.407	0.381	95.5	97.1

All the genotypes with better yield than checks in 1992 (Table 2) had less than 90% of plump kernels (>2.5mm), the commercial minimum, in 1993. This is a risk factor in the

perspective of unfavorable production conditions. One possible reason for this behaviour is that the heading date of those materials is 5.4 days later than checks. This results in a delay in the grain filling period (fig.1), with very little change in the duration of this period (33.3 and 34.5 days). They were probably under higher temperatures and more water stress in the period when kernel weight and plumpness were defined.

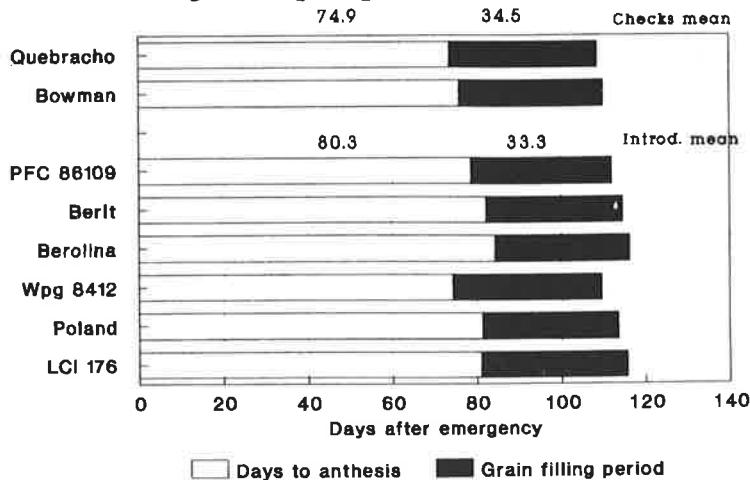


Fig. 1.- Cicles and grain filling periods in 1993 of genotypes with higher yields in 1992 and the best checks.

There are small probabilities of improving the barley yield potential in Uruguay maintaining the grain quality, using introduced varieties. The materials with higher yield potentials had, in general, longer growth cycles and delayed heading dates, which were related with grain filling problems and grain quality. On the other side, the materials with better grain quality under limiting environmental conditions did not exceed the yield of the currently sown varieties. In the long term, local breeding programs working with genotypes of different origins seems to be the most reliable way to improve the present yield level.

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Laboratory methods as an integral part of varietal discrimination for plant variety rights.
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Introduction. A grant of Plant Variety Rights (synonymous with Plant Breeder Rights in Australia, and Plant Varietal Protection in USA) gives the grant owner the exclusive right to produce and market reproductive material of a new variety. For a successful grant, the variety must be new; distinct from others in the marketplace, uniform and stable (commonly abbreviated to DUS); and have an acceptable denomination. While it is relatively easy to demonstrate and satisfy most of these criteria, we find the requirements of distinctness are the most time consuming and expensive component of an application.

Each country may have minor modifications to the basic UPOV (International Union for the Protection of New Varieties of Plants) rules of describing a new variety. An objective description is typically of about 35 simply inherited - phenotypically obvious, and 10 complexly inherited, plant characters; the latter to be evaluated in replicated trials over at least two seasons and possibly multiple sites. The problem for the plant breeder is that the characters involved in DUS testing are not of the type normally associated with routine plant breeding and/or selection criteria. As a consequence, DUS testing is often initiated late in the breeding or early commercialisation of a variety, and, combined with the requirement for multiple year evaluations for quantitative DUS traits, usually leads to a protracted delay in gaining a PVR grant.

Based on earlier work (Cross, 1992), the distinctiveness component of DUS evaluations could easily be simplified to be less time consuming and without loss in discriminating power if a combination of simply inherited - phenotypically obvious plant and seed characters were combined with routine laboratory discrimination using sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) of seed storage proteins. This paper presents a simplified laboratory method describing new barley varieties currently being forwarded for commercialisation in Australia.

Materials and Methods. Chemical composition is based on modifications of Nielson and Johansen (1986) and of Slack et al. (1985) of Doll and Anderson's (1981) original procedure. The modifications were necessary to enable relatively quick hordein extraction and gel preparation procedures, and to optimise polypeptide resolution. Allelic designations at the *Hor1*, *Hor2* and *Hor3* loci and their respective standard cultivars are as described by Shewry et al. (1983) and Johansen and Shewry (1986).

Extraction: whole single grains are extracted at a ratio of 45 mg flour to 1.0 ml of extraction buffer, comprising 50% propan-2-ol in 0.04M Tris/borate (pH 8.6) and 4mM dithiothreitol reducing agent, and held at room temperature for one hour with occasional (typically three times) mixing. Centrifuged for 4 minutes at 14,000 rpm. Hordein was concentrated by first pre-heating 400 µl supernatant at 50°C for 30 minutes and alkylating with 8mM iodoacetamide for 15 minutes at 50 °C, then precipitated by adding 600µl of water and freezing for at least 2 hours. Centrifuged and supernatant discarded, the hordein was redissolved in 750µl of extraction buffer containing 1% SDS, 10% glycerol and 0.1% bromophenol blue.

Electrophoresis: a vertical SDS-polyacrylamide gel system ("Biorad Protean II") comprising stacking and separation phases. Stacking gel:0.8% acrylamide, 0.2% N,N'-methylene bisacrylamide, 1.4% Tris, adjusted to pH6.8 with HCl; degas; 0.1% SDS, 0.08% TEMED, 0.6%

ammonium persulphate. Separation gel: 12% acrylamide, 0.1% N,N'-methylene bisacrylamide, 4.5% Tris, adjusted to pH8.8 with HCl; degas; 0.1% SDS, 0.05% TEMED, 0.5% ammonium persulphate. Upper tray electrode buffer: 0.5% Tris, 0.25% boric acid, 01% SDS, adjusted to pH8.6 with HCl. Lower tray electrode buffer: 5.1% Tris, 0.1% SDS adjusted to pH8.6 with HCl. Run for 17 hours (overnight) at 80V; fix hordein pattern (1 hour) in 10% glacial acetic acid and 50% methanol; wash in water (2 hours) and stain with 200ppm Coomassie Brilliant Blue R in 6% trichloroacetic acid. Gels are then photographed and/or digitised for permanent record.

Results and Discussion. Using the described SDS-PAGE method five barley varieties, new to the Australian market, are shown in figure 1. Polypeptide banding patterns are clear and make for easy discrimination, including allelic description based on pair-wise comparisons to known hordein standards (data not presented). Based on SDS-PAGE alone, the Crop & Food Research varieties "Chieftain" and "Empress" are clearly different and, regardless of their phenotypic similarity or otherwise, this method would enable a relatively easy and quick distinctiveness statement to be prepared.

While the concept of laboratory methods for description of plant varieties is not new (e.g. Linde-Laursen et al., 1982; Nielson and Johansen, 1986), laboratory discrimination has had only minor adoption by the UPOV convention for discrimination of new plant varieties. Currently, UPOV accepts SDS-PAGE results for barley (and wheat and oats: UPOV, 1993) only as supportive information and not as an integral part of the discrimination process. At Crop & Food Research, we have made grant applications where several of the "most closely resembling varieties" are phenotypically very similar for the simply inherited traits, and distinguishable only by quantitative measurement. This involved significant costs in terms of time (2 - 3 years) and labour to satisfy the distinctiveness criterion. Using the laboratory methods described, there are 14 allelic variants at the *Hor1* locus, 19 at *Hor2* and 2 variants at *Hor3* giving a very powerful level of discrimination among these three independent loci. An example of this discriminatory power was discussed by Cross (1992), who described a world collection of 1379 varieties using simply inherited - phenotypically obvious traits similar to those endorsed by UPOV. Using that whole plant and seed data, he discriminated 44% of that collection. However, when these data were combined to laboratory discrimination, including SDS-PAGE, 96% of that collection could be uniquely identified. A similar argument may apply when applied to PVR grant applications.

Despite the power of SDS-PAGE in discriminating varieties being well established, few studies combine both the biochemical treatment and simply inherited - phenotypically obvious whole plant traits in a form suitable for PVR purposes. If more case studies combining the two forms are presented, then some advances could be made in proposing changes in the UPOV convention to a cost effective, direct and simple approach.

Fig. 1. SDS-PAGE of nine current and prospective barley varieties for release in Australia, showing hordein banding patterns and tentative allelic designations.

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Genetic Diversity Of Ethiopian Barley Landraces Assessed By Morphological, Protein And RFLP Markers.

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Morphological, isozyme/hordein and restriction fragment length polymorphism (RFLP) markers were used to estimate genetic variation within and between barley (*Hordeum vulgare*) populations. In addition, the within and between estimates in relation to geographical origin, altitude and agro-ecological zone were assessed. A total of 51 landrace populations (15-20 genotypes per population) was considered for morphological and isozyme studies. All six morphological traits showed polymorphism, and the level of polymorphism varied among the traits. Shanon-Weaver index based on six morphological traits was computed and mean diversity (H') for Ethiopia was 0.71 ± 0.09 and the result was similar to Negassa's (1985) and Engels' (1994) estimates. A clinal pattern of variation was observed in row number and rachilla with altitude, the former also in relation to north-south direction. A decline in mean diversity with increasing elevation was noted (Demissie and Bjørnstad, in press). A total of 15 enzyme (6 enzyme systems) and 2 storage protein encoding loci were analyzed for variability. Of the 17 loci, 7 were polymorphic and 10 monomorphic when the 95% criterion of polymorphism was applied. The mean number of alleles per locus (A) was 1.5, expected heterozygosity (H) 0.134, percentage of polymorphic loci (P) 35.3 and the mean F_{ST} 0.474 (Demissie and Bjørnstad, accepted). The latter is comparable with the result reported for in breeding species (Hamrick and Godt, 1990). Forty-three landrace genotypes each representing one population were assayed for RFLPs with 31 barley genomic and cDNA probes (50 probe x enzyme combinations) distributed over all barley chromosomes. Polymorphism was detected in most probes (86%) with at least one restriction enzyme, however, the level of polymorphism was 62% when the 95% criterion of polymorphism was applied. Of the seven, chromosomes, chromosomes 3 and 6 appeared the most polymorphic whereas chromosomes 1, 4, and 5 long arm seemed considerably less

polymorphic for the probes considered. Genetic similarity estimated between two individual lines ranged from 0.698 ± 0.057 to 0.982 ± 0.024 . The within-population GS of 14 populations ranged from 0.837 ± 0.039 to 0.987 ± 0.033 (Demissie and Bjørnstad, submitted). A comparative study of RFLP profile (28 probes) for barley germplasm from Ethiopia, North America, Europe and Asia showed that the modern cultivars were more diverse than the landrace accessions Bjørnstad et al.). However, it must be pointed out that the probes used in the present study were selected due to high polymorphism in European/American mapping studies which might thus bias the diversity estimates in that direction. The most distinct was, however, one *H. spontaneum* accession compared to the landraces and modern varieties. Levels of DNA diversity were weakly correlated with other markers and so are the other two. Based on the result of these marker analyses, variation was the highest in high altitude areas especially in Arsi/Bale, Shewa, Gonder/Tigray, Welega, Gamu Gofa regions depending on the marker system. Until further studies, mainly of adaptive traits, these areas may be considered priority areas for more germplasm collection and *in situ* conservation.

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Investigation of genetic changes associated with the domestication of barley. R.P. ELLIS¹, E. BAIRD¹, N. BONAR¹, B.P. FORSTER¹, W. POWELL¹, W.T.B. THOMAS¹, R. WAUGH¹, I. AHMAN² and B. KRISTIANSSON², ¹Scottish Crop Research Institute, Dundee, DD2 5DA, U.K. and ²Svalof Weibull AB, S268 81, Svalov, Sweden.

Introduction. Paterson *et al.* (1995) examined the changes associated with the evolution of cereal crops. In barley, maize, millet, rice, sorghum and wheat domestication resulted from changes such as the development of a non-shattering rachis and enlarged fruits. There has been selection for heading date to give adaptation to specific environments which may be outside the "centre of origin". Barley cultivation started in the fertile crescent with a climate characterised by dry steppe, short daylength and winter rainfall conditions. This contrasts strongly with the climate of northern Britain, with long summer days, low temperatures and sufficient, year round, rainfall to support very yields. Paterson *et al.* (1995) were the first to show that the conservation of gene order over species, known for major genes, also applied to quantitative traits. They further suggested that correspondence in gene order reflects the occurrence of similar if not identical genes. Paterson *et al.* (1995) only considered heading date in barley so it is important to explore further characters.

Methods. We used AFLPs segregating in a population of doubled haploids from the cross Lina x HS92 to construct a linkage map. Lina is a tall cultivar bred at Svalof while HS92 is an accession of *Hordeum vulgare* subsp. *spontaneum* (Koch) from Canada Park, Israel. Plants were grown in both heated and unheated glasshouses and observations made on heading date, height, awn length, ear length, esterase, anthocyanin, lemma spiculation, seed shape, gramine and cold tolerance. DNA was extracted and 570 AFLPs were used to construct a genome map with the "Joinmap" programme (Stamm, 1993). QTLs were located on this map by stepwise regression.

Results and Discussion. An outline of the AFLP map is given in Figure 1 in which AFLPs are assigned to arbitrary groups (not all these groupings have been as yet identified with particular barley chromosomes). Markers are included at approximately 10cM intervals or where an association with a QTL was detected. The number of markers associated with each trait and the proportion of the total variance accounted for is given in Table 1.

The taxonomy of *Hordeum vulgare* L. has been revised repeatedly as knowledge of the morphology and distribution of the species has improved. The definitive characters of the species are annual habit, large auricles, glumes of the central spikelets flattened and central spikelets at least 3 mm wide (von Bothmer *et al.* 1991). The subspecies *spontaneum* (C. Koch), brittle, and *vulgare*, tough, are distinguished by the ease of disarticulation of the rachis. Assays of milling energy (Ellis *et al.*, 1993) also show that ssp. *spontaneum* has a much tougher lemma and awn.

Barley has been one of the most intensively studied higher plant species and so has one of the best mapped genomes of the important cereals. Historically many attributes have been observed and linkage maps compiled on morphological characteristics (Sogaard and Wettstein-Knowles, 1987). More recently maps have been published based on markers derived from DNA, such as RFLP (Graner *et al.* 1991; Heun *et al.* 1992), and AFLP (Thomas *et al.* in preparation). These maps are characteristically denser than those based on plant morphology and have greatly facilitated mapping of quantitative traits (QTL). Thomas *et al.* (1995) have reported linkage between a number of QTLs for characters of economic significance with RFLP and RAPD markers. Our research shows that AFLP are of great use in studying characters that have been important in the evolution of barley as a crop plant. Thus by studying the segregation of these traits in a wide cross we can localise major effects to specific regions of the genome. This will provide much needed information on the consequences of domestication in relation to accessing genetic variability in a barley breeding programme.

Table 1. Association of traits and AFLP markers

Trait	Number/markers	%Variation
Anthocyanin	10	53
Awn length	4	45
Ear length	8	62
Freezing tolerance	3	26
Gramine content	6	50
Heading date unvernalised	5	40
Heading date vernalised	10	74
Height	4	65
Spicules	10	85
Seed shape	1	9

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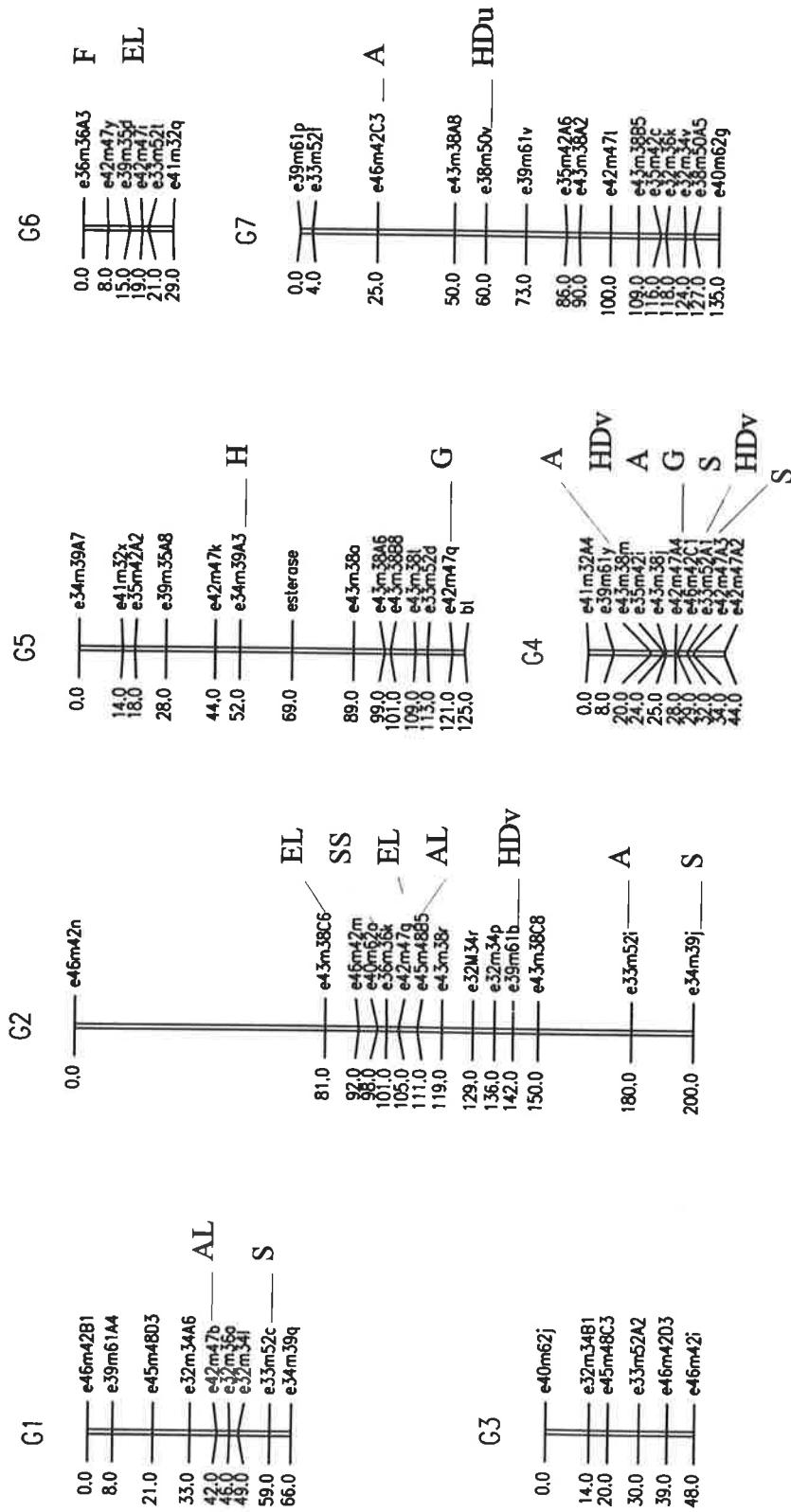


Figure 1. Map of the barley genome based on AFLPs in Lina x HS92. QTL associations with markers are indicated by; A=anthocyanin, AL=awn length, EL=ear length, F=freezing tolerance, G=gramine content, HDu=heading date unvernalisied, HDv=heading date vernalisied, H=height, S=spicules, SS=seed shape.

Use of Recurrent Introgressive Population Enrichment (RIPE) for utilization of plant genetic resources in a barley breeding program. D.E. FALK, Crop Science Department, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Introduction. Gene banks contain extensive genetic resources of the major crop species, yet most plant breeders generally confine their programs to a relatively minute portion of the germplasm available to them. Breeders are reluctant to dilute their highly developed elite stocks with unimproved ('raw') germplasm and progress continues to be made in most crop species. Most breeders do, however, recognize the risks inherent in the constricted germplasm base they work with. The narrowness of the genetic base of modern North American cultivars is a common concern plant breeders. Pedigrees of North American six-row malting barley are estimated to contain contributions from only 11 ancestral land races (Eslick and Hockett, 1974). Martin et al. (1991) used coefficients of parentage based on pedigree information as an index of genetic diversity among North American spring barley cultivars. They determined that seven ancestors contributed 52% of the six-rowed germplasm pool, with the cultivar Lion, originally introduced as a source of smooth awns, occurring in about 93% of the pedigrees, and the Manchuria-type cultivars dominating the pool. Despite the severely constricted breeding base, satisfactory genetic improvement appears to be continuing in most crop species. There are two other factors which should be considered in assessing the present status of rate of genetic gain and its future prospects. First, the easy gains typically occur during the early cycles of selection when the number of potentially segregating loci and allelic variability are at their maximum. In virtually all crops, the number of breeding cycles based on modern genetic principles (post 1900) is low. Even in barley which has a long breeding history, extrapolations from the study of Eslick and Hockett (1974) indicate that no more than eight cycles of selection have been completed. Another commonly cited reason to broaden the germplasm base is to decrease the potential vulnerability of crops to severe biotic and abiotic stresses. It is highly unlikely that the few immediate progenitors of our modern cultivars embodied all the best developmental pathways and adaptive strategies for enhanced economic productivity, especially considering that these progenitors were originally grown simply because they were better adapted than the other chance introductions. These original cultivars were not selected for ability to perform under modern, intensive cultural practices.

Even though there are good reasons to diversify the breeding base, progress continues in most crops, and breeders must develop cultivars that meet the standards of today's highly competitive market; they can not afford to work with germplasm that, even temporarily, dilutes agronomic performance or quality. Modern breeders would like to diversify the gene pool, but to do so would decrease, at least in the short term, the productivity of the resulting material. The net result is that germplasm banks are "seed morgues", rather than seed banks. Diversification of a crop breeding base can efficiently be

achieved through introgression of new germplasm into elite lines.

The RIPE system. A system has been designed to combine introduction of new germplasm and preservation of past progress in barley; this system is called RIPE - Recurrent Introgressive Population Enrichment (Kannenberg and Falk, 1995). It is based on male sterile facilitated recurrent selection with all introductions having some trait of proven merit. The RIPE system employs genetic male sterility, specifically msg6 which is tightly linked in coupling phase to the sex1 locus (Falk, et al., 1981). Because of the tight linkage to msg6, the plants produced from shrunken seed will be male-sterile, i.e., they will be sex1 msg6/sex1 msg6. Crossing is done by hand, but because emasculation is not required, large numbers of crosses can be produced with relative ease. Around 500-700 crosses are normally produced in a 2-3 week crossing period each year.

There are four sequential steps in the RIPE system leading to incorporation of new introductions into the E level (Fig. 1). Selected lines at each level are crossed with male steriles from the E level to increase the amount of E germplasm through progressive introgression. Thus, the Base level is 50% E, the Intermediate level is 75% E, the High level is 87.5% E and, at 93.25% E, selected lines are incorporated into the E level. As lines come into the E level, they would average about 7% new genetic material to maintain the evolutionary capacity of the E level without significantly diluting the existing, high performing, highly adapted E germplasm.

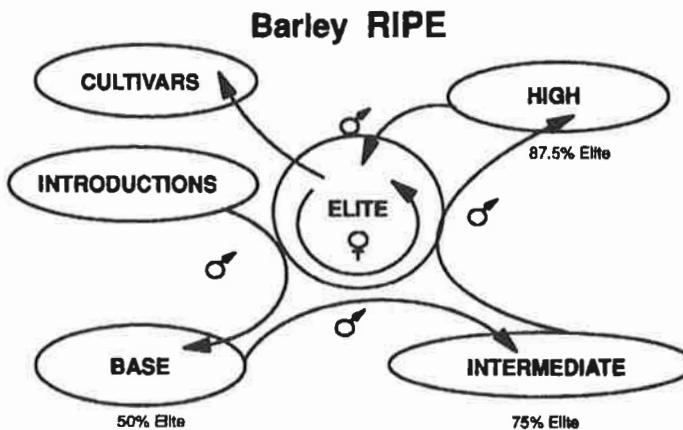


Figure 1. Schematic of the Recurrent Introgressive Population Enrichment (RIPE) breeding system for barley.

The E population is maintained through a fairly standard, intensive recurrent selection procedure which requires two years per cycle. The highest yielding F_4 lines each cycle are selected to be used as male parents for the next cycle. Shrunken seed from E x E F_2 populations having the highest bulk performance are used as females in each cycle. Crosses are made in the autumn in indoor growth facilities and F_1 plants selfed over the winter. The F_2

generation is grown in the field in Canada. Plump F_3 seeds are sent to California in the autumn for further increase and some agronomic selection. Individual F_3 plants are harvested separately to give F_3 -derived F_4 lines for yield evaluation as unreplicated plots in Canada in the second summer. Selected lines are used as parents in the next cycle of the system. Thus, five generations are produced in two years, culminating with a yield trial and selection of the next cycle parents. The same basic procedure is used at the B, I, and H levels in the RIPE system, with selected lines from each respective level used as males crossed onto male steriles derived from the E level in each cycle. An introduction will require eight years, i.e., two years at each of the B, I, H, and E levels, before any of its genome might be introgressed into the E level. Some effort is made to maintain diversity by selecting the best line derived from each male parent in the preceding crossing cycle.

At the E level, recurrent selection will continue to shape the germplasm into unique and agronomically superior genotypes. The RIPE system is designed to allow ample opportunity for introgression of new germplasm with agronomic potential and should do so without dilution of the potential for producing high-performing, well-adapted cultivars with improved disease resistance, agronomic characteristics and quality.

Conclusion The introgression of new germplasm to broaden the breeding base has the potential to add new genes and gene complexes that can raise the genetic ceiling on improvement and reduce genetic vulnerability to widespread biotic and abiotic stresses. Further, previously unexploited germplasm may lead to new developmental pathways and ecological adaptations that are important to meet the needs of changing agronomic practices. New germplasm, however, will not be used by breeders unless it is at least equal in agronomic performance to current breeding sources. RIPE for barley has the potential to significantly diversify the breeding base while still allowing the development of commercially competitive lines and cultivars. Open-ended breeding systems based on introgression of new germplasm, such as the RIPE, are necessary to ensure continued, long-term progress in crop improvement.

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On-Farm Evaluation of Farmer Varieties (Landraces) and Breeding Lines in Ethiopia. Hailu Gebre and Joop van Lier, Institute of Agricultural Research, P.O. Box 2003, Addis Ababa, Ethiopia.

Introduction. Barley is an important grain in Ethiopia with about one million tons of annual production. It is produced mostly for food and for home-made drinks. The straw is used as fodder for livestock. Barley has diverse ecologies, being grown from 1800 to about 3400 m altitude in different seasons and production systems. At the highest altitudes barley is the only crop that can be grown.

A large number of farmer varieties (landraces) are used to suit specific conditions and needs, such as adaptation to a growing season (eg. short rains, long rains), system of production (eg. early harvest), end-uses (eg. type of food or drink), and level of soil fertility. Farmers often differentiate varieties by names that describe their appearance (grain color, row type, hulled or hull-less condition), end-use, or maturity date.

In 1994 a number of major farmer varieties were collected and evaluated along with breeding lines on farmers' fields. The materials were tested without and with a moderate amount of fertilizer. Farmers' fields were used to test material under a more realistic environment than the experimental stations and to involve farmers in selection.

Materials and Methods. The materials included 19 farmer varieties, 5 improved varieties and a local check (Table 1 and 2). They were grouped into two sets based on maturity of the farmer varieties and were tested at 32 sites. A RCB design with three replications was used with the fertilized (41 kg N and 46 kg P2O5/ha) trial planted next to the unfertilized one. Seed was hand-sown in rows at a rate of 85 kg/ha, using plots of 3m² of which 2m² was harvested.

Results. Grain yield data for 22 sites were analyzed. Yields varied across locations (Table 3 and 4). Low yields were obtained at Angolela and Faji due to waterlogging, poor seedbed at Degem, and low moisture at Kabbe. Cultivars were significantly different at 18 locations. The local check performed satisfactorily at most locations. It was outyielded only at 6 sites. In the late set Luyita (2839 kg/ha) and Semereta (2838 kg/ha) were superior at Hosaina, HB-42 (2247 kg/ha) at Gayint, ARDU 12-60B (2818 kg/ha), HB-42 (2469 kg/ha), Semereta (2427 kg/ha) and 3336-20 (2401 kg/ha) at Dinscho. ARDU 12-60B and 3336-20 are pure line selection of Arussi landraces, whereas HB-42 is a local breeding line.

In the early set Somie (2383 kg/ha) was better at Adet, Semereta (2105 kg/ha) and Aybo Gebs (1798 kg/ha) at Degem, and Shasho (4578 kg/ha) and Somie (4545 kg/ha) at Shambu. Somie and Semereta are farmer varieties in Adet and Degem areas, respectively.

There was a positive response to fertilizer in all sites except Gassera and Sinana, indicating the poor soil fertility in most barley growing areas. Fertilizer increased grain yield from 587 to 2413 kg/ha in the late set (Table 3) and from 605 to 4183 kg/ha in the early set (Table 4). Fertilizer also increased plant height by about 20 cm, hastened crop maturity by about 12 days, and promoted incidence of scald and leaf blotch

diseases. The three improved varieties (HB-120, HB-42 and Beka) had the lowest scald scores.

Interaction between cultivars and fertilizer was only significant at Holetta, Altufa and Gassera. Fertilizer decreased the yield of the local check at Altufa and Gassera. Some cultivars showed a tendency to lodge after application of fertilizer.

Conclusions. The trials showed that farmer varieties have good yield potential, but are adapted to specific environments. Pure line selections from some local germplasm have shown superior yields, indicating the availability of good components. Next to the selection of pure lines, the IAR breeding program is focussing on mass selection by selecting superior sources in different farmer varieties and on improving deficiencies in the local germplasm (disease and lodging susceptibility) through hybridization.

Table 1. Source of seed and some characteristics of test entries in the late set.

Variety	Region	Row type	grain color	Special features
Baleme	west Shewa	Ir	W	Grown on poor soils
Magie	south Gonder	6/Ir	B/W	Grown in a mixture with wheat
Semereta	north-west Shewa	2	B	For early harvest systems
Awra Gebis	north Gonder	6/Ir	B/W	Grown in a mixture with wheat
Muga	Arsi	2/Ir	B	For late harvest systems
Senef Kolo	west Shewa	2	W	Grain suited for roasting
Luyita	east Gojam	2	B	For medium harvest systems
Dimbulo	east Gojam	6/2	W	For late harvest systems
Mirtzer	Arsi	6/2	W/B	For late harvest systems
3336-20	improved variety	2	W	Food Barley
HB-42	improved variety	6	W	Food Barley
ARDU 12-60B	improved variety	6	W	Food Barley
HB-120	improved variety	6	W	Malting Barley
Beka	improved variety	2	W	Malting Barley

Table 2. Source of seed and some characteristics of test entries in the early set.

Variety	Region	Row type	grain color	Special features
Semereta	north-west Shewa	2	B	For early harvest systems
Tebel	south Gonder	Ir	B/W	For early harvest systems
Belga	north Gonder	6/2/Ir	B/W	For early harvest systems
Shasho	Bale	Ir	W	For main season
Aruso Bale	Bale	2/Ir	B/W	For main and short seasons
Demiyyie	north Shewa	6/Ir	B	For short season and burntsoil
Somie	east Gojam	Ir	W	intermediate harvest systems
Ehilzer	Wello	Ir	B/W	For low rainfall environments
Kessele	north Shewa	6/Ir	B/W	For short season
Aybo Gebis	Wello	6	W	Needs good drainage
HB-120	improved variety	2	W	Malting Barley
Beka	improved variety	2	W	Malting Barley

Table 3. Yield (kg/ha) of test varieties and local checks in the late set.

Region	Location	All varieties			Local Check		
		F0	F1	Diff.	F0	F1	Diff.
west Shewa	Holetta	1592	3329	1737	2241	3662	1421
	Altufa	3315	5038	1723	4643	4288	-355
north Shewa	Angolela	467	1097	630	607	1241	634
	Faji	701	1405	704	740	1218	478
south Shewa	Hosaina	947	3360	2413	1000	3512	2512
south Gonder	Gayint	1440	2027	587	1301	2359	1058
Bale	Dinsho	1567	2784	1217	1568	2175	607
	Gassera	1381	1557	176	2472	2970	498
Borena	Bore	1187	3174	1987	1222	3500	2278

Table 4. Yield (kg/ha) of test varieties and local checks in the early set.

Region	Location	All varieties			Local Check		
		F0	F1	Diff.	F0	F1	Diff.
Tigray	Aynalem	812	1699	887	1705	2957	1252
	Atsbi	1517	2395	878	1995	2568	573
	Ganta Afeshum	595	3533	2940	972	4697	3725
	Korem	1818	4183	2369	2507	6045	3538
Wello	Kabbe	585	875	290	758	870	112
	Kutaber	1232	1762	530	1794	2623	829
Gojam	Adet	1387	2430	1043	1830	1803	-27
south Gonder	Farta	1120	1674	554	1634	2677	1043
west Shewa	Holetta	2198	3560	1362	2346	3270	924
	Degem	801	1406	605	1256	1602	346
Bale	Sinana	1903	1872	313	3240	2772	-468
	Gassea	2186	2558	372	3603	2443	-1160
Wellega	Shambu	3322	4032	710	2444	3756	1312

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Wild barley for breeding in dry areas and for self-regenerating pasture barley

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Introduction. Wild barley *Hordeum vulgare* ssp. *spontaneum* or its natural outcrosses with ssp. *vulgare* (ssp. *agriocirthon*) is well adapted to drought and heat, conditions prevailing in the Mediterranean countries of North Africa and West Asia. Wild barley is also found in Central Asia up to China. Its survival is based on its adaptation to harsh conditions, but also on seed dispersal mechanisms, seed dormancy, shrunken seeds, etc (Hadjichristodoulou, 1995). It occupies mainly the edges of the cultivated fields, because overgrazing has eliminated wild barley from the continuously grazed natural pastures.

Wild barley is very easy to use in breeding, because it crosses readily, even naturally, with cultivated barley. However, its uses in applied breeding work has been very limited till now. Experimental evidence is presented from Cyprus and other countries which shows the high potential of wild barley as valuable source of genes in improving the productivity of fertile cropped lands and marginal or pasture areas in the dry regions.

Materials and Methods. The wild barley material ssp. *spontaneum* and ssp. *agriocirthon*, was selected from the central plain of Cyprus, an area of 250m altitude and average annual precipitation of 300 mm. Single-spike progenies (over 10,000) were evaluated at Athalassa, near Nicosia, in nurseries and the best genotypes were used in the crossing programme with the highest yielding grain producing varieties or the leafy hay varieties (Hadjichristodoulou, 1992(a), 1992(b), 1993, 1995).

In the F₂-F₄, individual plant selection was exercised in two directions for different uses. The best tough-rachis genotypes of each cross were selected for the programme aiming at improving grain yield, and the brittle - rachis genotypes were allowed to drop the seed on the soil. After regeneration for 2-3 seasons, by visual selection, the best crosses were bulked to form the population or variety Mia Milia. Also the best lines of ssp. *agriocirthon* were bulked to form the variety Akhera.

The material selected for grain was evaluated in the variety trials, at several locations and seasons (Hadjichristodoulou, 1993). The brittle-rachis material, namely the varieties Mia Milia and Akhera, were sown in a marginal land at Athalassa and were treated as permanent self-regenerating pasture without reseeding (Hadjichristodoulou, 1995).

Results and Discussion. A. Grain yield. It was easy to fix the tough rachis gene by selecting for 2-3 generations in the segregating populations of crosses with wild barley. The best selections from 23 crosses, tested in seven trials during 1989-92, outyielded their ssp. *vulgare* parents and the improved check variety Kantara by 13-22% (Hadjichristodoulou, 1993). In recent trials, the highest yielding lines from seven crosses tested at eight locations during 1992-1995, outyielded the commercially grown variety, Athenais, by 1.7% to 8.5% (Table 1).

Table 1. Grain yield (kg/ha) of selections from crosses between *Hordeum vulgare* spp. *vulgare* and wild barley (ssp. *spontaneum* or ssp. *agriocrithon*).

Variety/Cross No.	Year of testing and number of locations			Weighted mean (8 loc)
	1992/93 (3 loc)	1993/94 (2 loc)	1994/95 (3 loc)	
Athenais (control)	5827	4525	4624	5050
CYB84-3846	6005	4205	4889	5137
CYB84-3861*	6211	4781	5213	5479
CYB85-4065	5990	5161	5093	5447
CYB85-3950*	6184	5182	4989	5485
CYB85-4078	6040	5146	4881	5382
CYB85-4083-5D	5755	5162	5205	5401
CYB-4083-1D	5507	5476	5070	5335
SX	134	349	193	

* Crosses with wild barley ssp. *spontaneum*. All other crosses were made with ssp. *agriocrithon*.

It is interesting to note that these selections were consistently superior to Athenais. Brown et al. (1988) selected lines with grain yield and grain size approaching or equal, and sometimes significantly higher than those of Clipper and more recent commercial cultivars.

Compared to Athenais, the selections from wild barley crosses were earlier (1-5 days), produced more spike-bearing tillers per m² (up to 32%), more grains per spike (up to 53%) and had higher volume weight of the grain (up to 9.5%), but their 1000-grain weight was lower 27.8g-34.5g, compared to 34.6g of Athenais. Indications for transgressive segregation were obtained for grain yield, straw yield, total biological yield, harvest index and volume weight. For example the volume weight of Athenais was 59.3 kg/hl and of the selections from the wild barley crosses 55.3-68.0 kg/hl. Transgressive segregation for yield and other traits was reported by Frey et al. (1984) in backcrosses, though in our study, this was shown in single crosses, with both ssp. *spontaneum* and ssp. *agriocrithon*.

B. Pasture Barley. Shallow, marginal land at Athalassa near Nicosia, sown in 1986-89 with the varieties Mia Milia (mixture of 117 crosses) and Akhera (mixture of 2400 genotypes of ssp. *agriocrithon*), regenerates each year until now, 1996, with no need to reseed. Grazing is controlled, starting when the plants reach tillering stage, repeatedly, depending on the rainfall. The dry matter removed depends on the total amount and distribution of annual rainfall, 2.8 - 8-7 t/ha. (Hadjichristodoulou, 1995). The animals were removed from the pasture around 40 days before maturity, to allow for seed production. After maturity, dry pasture was grazed. Because of the brittle rachis and shrunken type genes, all seeds fall into the soil before full maturity, and therefore they are protected from the grazing animals. The success of the continuous regeneration of the pasture, depends also on the high level of seed dormancy of the wild barley.

Man-guided evolution of barley was directed towards tough rachis grain producing genotypes. Now by reverse evolution, towards brittle rachis gene, a new crop and new farming system is developed, the self-regenerating pasture barley. In the West Asia and North Africa countries, there is no pasture species available for improving pastures. Pasture barley varieties, adapted to the harsh conditions, provide a unique opportunity to improve marginal lands and natural pasture lands.

It is evident from this work that there are no major problems in using wild barley germplasm in breeding for dryland conditions and for new uses, such as pasture improvement. Breeders must be encouraged to use this valuable genetic resource.

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RFLP Polymorphism among European accessions of the Barley Core Collection. B.G. HATZ, A. JAHOOR and G. FISCHBECK, Lehrstuhl für Pflanzenbau und Pflanzenzüchtung, Technische Universität München, D-85350 Freising-Weihenstephan, Germany

Introduction

For conservation of genetic resources by development of core collections as well as for breeding it is necessary to know about the genetic variability in the germplasm of the relevant species. A core collection should be representative. It was shown, that core collections assembled with evaluation data and cluster analysis better represent the germplasm collection than collections only based on passport data and random selection (Diwan et al., 1995). Therefore the European accessions of the Barley Core Collection were screened for their actual genetic diversity by using RFLPs.

Materials and Methods

Selection of barley lines: 217 different *H. vulgare* accessions (122 2-rowed spring, 10 6-rowed spring, 31 2-rowed winter and 54 6-rowed winter), representing the primary genpool, were included in the RFLP analysis. They were selected to reflect the genetic diversity in the European germplasm pool and represent a range of barley cultivars released between 1900 and 1990. For polymorphism comparisons, a set of 32 wild *Hordeum* species was screened, representing the tertiary genpool of the genus *Hordeum*. The seeds of the accessions were obtained from different genbanks in Europe and the USA and from the breeding companies.

Selection of the probes: For detection of restriction fragments 44 barley clones were used, derived from mapping experiments with inter- and intraspecific crosses (Graner et al., 1991). The RFLP-probes were chosen to provide a good coverage of the barley genome with at least 5 probes per chromosome, good signal strength and clearly distinguishable fragments.

RFLP analysis: Isolation of genomic DNA, separate restriction enzyme digestion with *Bam*HI, *Eco*RI and *Hind*III, agarose gel electrophoresis, alkaline southern blotting to nylon membranes and hybridization methods were all performed as described in detail by Jahoor et al., 1991. Two check varieties and a λ -*Hind*III molecular standard were included on each gel.

Statistical analysis: Genetic similarities (GS) between each pair of lines were calculated according to the formula suggested by Nei and Li (1979).

For measuring the different degrees of diversity of the primary versa the tertiary genpool a CEC-Index was calculated by scoring each polymorphism between two lines with 1. The ratio between the polymorphic comparisons to all comparisons made was the CEC-Index. The average of all CEC-Indexes was used as diversity value.

Results and Discussion

RFLP polymorphism among European barley cultivars: 43 DNA clones detected polymorphisms among the barley lines with at least one of the restriction enzymes used. One RFLP-probe MWG599 was monomorphic for all enzymes. A total of 99 clone-enzyme combinations (CEC) with 319 distinct RFLP bands were used for calculation of GS. The GS based on the RFLP data between all possible pairs of lines ranged from 0.43 to 0.99 (Table 1). The GS within the 2-rowed spring barley was the smallest with 0.59 and greatest with 0.99. The average within the 2-rowed spring cultivars was 0.79. The smallest GS value within the 6-rowed winter lines was 0.43, the greatest GS was (0.99). The average within the 6-rowed winter barleys was 0.76. The GS within the 2-rowed winter lines was smallest with 0.49, greatest with 0.93, the average within the 2-rowed winter lines was 0.72. On average, the GS among the spring barleys was greater than among the winter cultivars. GS estimates between lines of different breeding groups were on average smaller than those within groups. The smallest GS

estimate was found for line combinations between 6-rowed winter and 2-rowed spring barley (0.62), the greatest estimate (0.70) was found for the combination 2-rowed winter with 2-rowed spring barley.

Table 1: Genetic similarity (GS) estimates within and between breeding groups of European barley cultivars

Breeding Group	N ^a	Genetic Similarity		
		Mean	Min	Max
within spring 2r	7381	0,79	0,59	0,99
within spring 6r	45	0,78	0,57	0,90
within winter 2r	465	0,72	0,49	0,93
within winter 6r	1431	0,76	0,43	0,99
spring 2r x winter 6r	6588	0,62	0,44	0,83
spring 2r x winter 2r	3782	0,70	0,46	0,87
winter 2r x winter 6r	1674	0,66	0,43	0,98

^a N, number of pairwise line comparisons within each category

Diversity values of *H. vulgare* and wild *Hordeum* ssp.: As expected the wild *Hordeum* species showed much more variability than the cultivated lines of ssp. *vulgare*. In cultivated barley a diversity value of 0.343 was calculated, whereas for the wild species a diversity value of 0.901 was found. This considerable difference is also demonstrated by the number of fragments found per CEC (2.5 fragments/CEC for the cultivars, 11.5 fragments/CEC for the *Hordeum* ssp.).

Principal component and cluster analysis: Both principal component and cluster analysis were used to reveal the association between the accessions. PcoA provided a faithful representation of relationships among major groups by separating the spring cultivars from the winter barley lines by the first principal coordinate, which accounted for 53% of the total variation. Unexpected arrangement of few cultivars was observed, strengthen the suspicion of mistaken genbank samples. The dendrogram generated by cluster analysis was compared to the pedigree relatedness of the cultivars, which in majority was in good agreement with the origin and expected pedigree relationships of the lines.

In a second step cluster analysis was performed using the RFLP probes for each chromosome separately.

Parental contribution to progeny genomes: 33 CECs out of the 99CECs with known single copy banding patterns were assumed to be alleles of a single locus and were referred to as genotypes. This data was used to examine the inheritance of parental alleles in the progeny generations. It was expected that the number of each parent's alleles in a cultivar would be of similar size, assuming no selection pressure. In the majority of the observed crosses the progenies received between 0.3 and 0.7 of their genetic information from one parent, which is likely due to intense selection for recombinant genotypes. Surprisingly most crosses contained alleles, which were not found in one of the parents, on the average 2 loci (6.2%) per examined progeny. It was possible to show that these differing alleles were passed on to the next

generation. In cases of too many deviating alleles (eg. 36%) the idea of wrong seed samples of the progeny or false pedigree information need to be assumed. This results indicate that RFLP profiles can be used as verification for pedigree records. Besides the parental contribution, the tracking of chromosomal loci in a defined pedigree was possible to some extent. As example the pedigree of Maris Otter, an English 2-rowed winter barley is illustrated. Twelve of the 33 loci (36%) found in Maris Otter had alleles, traceable to one or the other of its immediate parent, Pioneer or Proctor. Pioneer had 12 informative loci (36%) with alleles that could be traced to its parents, Spratt Archer or Tschermaks 2 row. Likewise, Proctor had 7 loci (21%) with alleles that could be traced to its parents, Kenia and Plumage Archer. The tracing of alleles in following generations was not very successful, since the used 33 single-copy RFLP markers distinguished a maximum of only 5 alleles at a locus. Therefore tracking a particular ancestor allele was made ambiguously difficult after only one generation, at best the allele could be followed through 3 generations. This is in agreement with similar studies made on soybean (Lorenzen et al., 1995). The limitations of single copy RFLP probes in detecting not more than 5 different alleles will not be solved by just using low copy RFLP probes. Normally multiple banding patterns of low copy probes describe more than one locus on the same or another chromosome. Nevertheless without verified correlations between the detected fragments and the map locations all interpretations on inheritance schemes remain uncertain.

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Spatial distribution of reactions against powdery mildew in two subpopulations of *Hordeum spontaneum* from Israel

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Introduction

Collections of *Hordeum spontaneum* have shown to contain high percentages of mildew resistant accessions in mildew tests carried out in Europe (Fischbeck et al. 1976) as well as in the United States (Moseman et al. 1983). With the present investigation an attempt has been made to reveal the structure of genetic diversity for mildew resistance in two dense stands of *Hordeum spontaneum* against adapted mildew cultures (from Israel) and unadapted mildew cultures (from Europe).

Materials and Methods

Dense stands of *Hordeum spontaneum* at two sites (Lahav and Lakhish), located about 20 km apart from each other in the Judäan foothills in Israel have been sampled in squares of 140 x 140 m by harvesting single heads at 5 m distances in both directions.

Single head progenies from each of the 2 x 784 positions have been subjected to separate infection trials with two sets of 5 mildew cultures, each of which representing a wide range of differences in virulence within the respective mildew populations.

Mildew reactions were analysed with the detached leaf technique (Jahoor and Fischbeck, 1987) applied in the primary leaf stage. The readings were classified resistant (infection types 0,I,II) or susceptible (infection types III, IV).

Results and Discussion

The data summarized in table I indicate a somewhat higher level of resistant reaction types in the sample from Lahav as compared to Lakhish with both sets of mildew cultures. A major difference is indicated with the high percentages of accessions resistant to all European cultures as compared to the very low percentages with resistance to all of the endemic mildew cultures. No significant differences are apparent in the frequencies of resistant reactions against 1-4 European cultures, while the level of resistances against endemic cultures consistently increases with a decreasing number of cultures that remain avirulent. In the end only 50-60 % of the accessions have been susceptible to the complete set of endemic cultures as compared to about 30% complete susceptibility against the set of European cultures.

The potential of $2^5 = 32$ reaction patterns has been almost completely realized within each of the two subpopulations and with both sets of mildew cultures, indicating a high ranking level of genetic diversity in *H. spontaneum* populations in Israel (Nevo et al 1986) also for mildew

resistance. It is interesting to note that close to 90% of the observed reaction patterns occurred in frequencies below 5 % in both populations with no significant differences between the source of inoculum that has been used.

The spatial distribution of reaction types with both set of mildew cultures is characterised by a mosaic of cluster effects (Table I). Rather high levels of resistance versus susceptibility and vice versa are found to be stretched over larger parts of the sampled area, but not much agreement is noted between the pictures obtained with the different sets of mildew cultures. Furthermore equal reaction patterns maintained over more than two immediately neighbouring positions in either direction with one set of mildew cultures only exceptionally equals in its reaction pattern also against the other set of mildew cultures.

Since mildew attack of *H. spontaneum* stands in the Judäan foothills usually remains at rather low levels, the spatial distribution of reaction patterns probably reflects preexisting genetic diversity more than response to selection and may be explained on the basis of multilocal differentiation between polymorphic major gene loci, which became established by founder effects and have been continuously dissolved by segregations following the low rate of outcrossing (Turner et al. 1982) between the founding genotypes. In such a way and together with other types of protection (Fischbeck et al. 1976) a high level of genetic diversity for mildew resistance may have developed even without selection pressure that is able to provide a system of population buffering (Segal et al. 1982) against a multitude of virulence combinations.

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Table I: Mildew reactions in two subpopulations of *H. spontaneum* (Lahav = Lv; Lakhish = Ls) against two sets of five mildew cultures, originating from Europe and from Israel

Resistance (%) against with mildew cultures	from Europe		from Israel	
number of cultures	Lh	Ls	Lh	Ls
5	29,8	19,7	1,2	0,3
4	11,4	10,0	3,7	0,8
3	12,3	14,0	12,1	4,1
2	9,2	11,3	11,4	8,9
1	10,9	11,9	24,1	22,3
0	26,5	33,1	47,4	63,6
number of reaction patterns observed				
	31	32	31	30
frequency (%) within the subpopulation				
< 1	11	12	17	19
1-2	10	7	7	4
2-5	8	9	3	4
5-10	-	2	3	1
10-20	-	1	1	1
> 20	2	2	1	1

Patterns of genetic variation among four esterase loci in barley landraces from the Fertile Crescent, A.A. JARADAT and H.I. MALKAWI, International Plant Genetic Resources Institute, WANA Regional Office, Aleppo, Syria and Department of Biology, Yarmouk University, Irbid, Jordan

Introduction

Esterases (EC 3.1.1.-), among other isozyme systems, have been extensively utilized in studying the genetic structure of wild (Jaradat, 1992) and cultivated barley (Kahler and Allard, 1981; Konishi, 1995). Esterases were among the most differentiating loci between populations of *Hordeum spontaneum* from the Fertile Crescent, as indicated by their average degree of gene diversity (Nevo et al., 1986). Moreover, substantial within-population variation, and the localization of certain esterase loci to particular environments, have been found in populations of *Hordeum spontaneum* (Brown et al., 1978) and varieties of *Hordeum vulgare* (Konishi, 1995). This paper presents patterns of genetic variation, based on four esterase loci, in 269 landrace genotypes from five countries in the Fertile Crescent.

Materials and Methods

Number of landraces genotypes and country of origin of the *Hordeum vulgare* accessions used in this study are presented in Table 1. Each accession was assayed for four esterase enzyme loci following Kahler and Allard (1981). The enzymes assayed are coded for by genes of the four esterase loci (Est-1, Est-2, Est-4 and Est-5). The alleles were labelled alphabetically in order of decreasing mobilities of their allozymes, as suggested by Nevo et al. (1986). Allelic frequencies were calculated for each esterase locus and country of origin (Kahler and Allard, 1981). Gene diversity and number of effective alleles were estimated for each loci and country of origin. Finally, the four esterase were reduced to a diallelic state, and then the pattern of multilocus structure in the whole set of landrace genotypes was determined by discrete log-linear multivariate analysis following the method described by Zhang et al. (1990).

Results and Discussion

Number of accessions, gene diversity, number of effective alleles for each locus and country of origin are presented in Table 1. Also, frequencies of 2, 3 and 4 esterase loci significantly associated are presented for each country of origin. Gene diversities varied considerably among esterase loci within and among countries of origin. Est-1 ranked first, followed, in decreasing order, by Est-4, Est-2 and Est-5. These confirm those obtained by Konishi (1995) for 340 accessions of barley landraces from West Asia including Turkey. However, these results contradict those obtained for *Hordeum spontaneum* from the Fertile Crescent region, where gene diversity decreased as follows: Est-2>Est-4> Est-5>Est-1 (Brown et al., 1987; Nevo et al., 1986; Jaradat, 1992).

Frequencies for 2, 3 or 4 esterase loci significantly associated are presented in Table 1. On average, this frequency dropped from 51.42 for 2-loci association to 22.86 for 3-loci, and to only 4.98 for all four esterase loci being significantly associated. Accessions from each of Iran, Jordan and Turkey have the highest frequencies for 2-loci associations, and Syria has the least. The 3-loci associations were reasonably similar among countries, except for

Turkey (29.2). Syrian accessions had the lowest frequency for a 4-loci association as compared with the remaining four countries.

Table 1. Number of accessions (NA), gene diversity (GA), number of effective alleles (NEA), frequencies for significantly associated 2-, 3-, and 4-loci for 4 esterase loci in landrace barley accessions from 5 countries in the Fertile Crescent.

Country	NA	Locus	GD	NEA	No. Esterase loci sig. associated		
					2	3	4
					Freq. (%)		
Iran	48	Est-1	0.75	4.26			
		Est-2	0.40	4.51			
		Est-4	0.58	1.88			
		Est-5	0.21	3.36			
		Mean	0.485b*	3.5a	56.2	21.1	6.3
Iraq	36	Est-1	0.54	4.25			
		Est-2	0.58	1.62			
		Est-4	0.42	1.92			
		Est-5	0.25	1.25			
		Mean	0.448c	2.26b	43.8	19.8	4.0
Jordan	50	Est-1	0.69	3.24			
		Est-2	0.55	2.56			
		Est-4	0.49	1.58			
		Est-5	0.29	1.32			
		Mean	0.505a	2.18b	62.1	25.2	5.2
Syria	45	Est-1	0.63	2.08			
		Est-2	0.38	1.20			
		Est-4	0.46	2.08			
		Est-5	0.20	1.00			
		Mean	0.418d	1.59d	39.2	19.0	2.1
Turkey	90	Est-1	0.79	1.85			
		Est-2	0.44	3.42			
		Est-4	0.52	1.84			
		Est-5	0.19	1.02			
		Mean	0.485b	2.03c	55.8	29.2	7.3

*, Means, within each column, followed by the same letter do not differ significantly (Tukey=0.05)

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Wild Oats in Jordan. G. KANAN and A.A. JARADAT, Muta'a University, Karak, Jordan and International Plant Genetic Resources Institute

Abstract

Seven wild oat species (*Avena barbata*, *A. clauda*, *A. eriantha*, *A. hirtula*, *A. longiglumis*, *A. sterilis* and *A. wiestii*) were collected from three different agroecological regions in Jordan. Evaluation of 9 morphological and developmental characters revealed major intra- and inter-specific variation, both within and among agroecological regions. *Avena sterilis* was the most widespread species covering areas below (-270 m) and above (>1300 m) sea level. *A. clauda* and *A. eriantha* were restricted to the highlands of Jordan, while the remaining species were mostly distributed in both the Jordan valley and the steppe regions. All seven species were highly variable for number of spikelets per panicle, date of panicle emergence and seed density. Diploid species (*A. wiestii* and *A. hirtula*) had the longest panicles, largest number of whorls per panicle, tallest mature plants and largest number of leaves per plant. The tetraploid species, *A. barabata*, was intermediate for most traits, however, it was the latest in panicle emergence. The hexaploid species *A. sterilis*, had the lowest values for most traits, however, it had the largest glumes and awns. On the other hand, it had fewer number of spikelets per panicle and shorter plants when compared with the diploide and tetraploid species.

Materials and methods

Mature seeds were collected from individual plants (or panicles, when necessary) from 67 locations in three ecogeographical region of Jordan (Jordan Valley, Mediterranean highlands and the Steppe). A minimum of 15 seed were collected per plant or panicle. Germination was induced by dehulling, piercing the seed coat with a needle, and placing five seed on two discs of filter paper wetted with a 10-ppm solution of gibberellic acid in 9-cm Petri plates. After germination seedlings were transplanted into plastic containers containing a 3:1 clay-loam soil:peatmoss mixture. Eleven morphological and developmental traits (Table 1) were recorded on each plant at physiological maturity. The variety Porter (*A. sativa*) was used as a check. Also, the whole collection was subjected to a taxonomic study using a key to *Avena* species (Ladizinsky, 1985) and the ploidy level of each accession was confirmed through karyotyping. Data were used to carry out a comparative study on wild *Avena* species in Jordan.

Results and discussion

Species of wild oats: The karyotyping and the taxonomic study (Ladizinsky, 1985) showed that seven *Avena* species are found in Jordan (Table 1). Five of these species were diploides, one was a tetraploid and one was a hexaploid. The following is a brief discription of each of these species. *Avena clauda* ($2n=14$), was found in a restricted region in the highlands of Jordan. *Avena eriantha* ($2n=14$) has a very restricted distribution in the highlands of Jordan. *Avena longiglumes* ($2n=14$) has a wide range of distribution in Jordan, however, it is mainly found in the Jordan valley. *Avena hirtula* ($2n=14$) is mostly distributed in the highlands. *Avena wiestii* ($2n=14$) has a wide distribution in Jordan, ranging from the highlands to the Steppe. *Avena barbata* ($2n=28$) forms massive stands in the Jordan valley, the highlands and in wadi catchments in the Steppe. *Avena sterilis* ($2n=42$) has the widest distribution of all *Avena* species in Jordan.

Variation for quantitative traits among Avena species in Jordan:

Analysis of variance for quantitative and developmental traits (Table 1) indicate highly significant differences among species. Panicle length: The diploid species *A. longiglumis* has the longest panicles (20.2 cm), however, it did not differ significantly from the diploid *A. wiestii*. The diploid *A. hirtula* and the tetraploid *A. barbata* did not differ significantly from each other. However, these two species were intermediate between the former diploids and the hexaploid species (*A. sterilis* and the check *A. sativa*). Number of whorles per panicle: The hexaploid species had the lowest number of whorles (5.5); they differed significantly from diploids and tetraploid species. The diploid species *A. longiglumis* had the highest number of whorles (7.1). Number of spikelets per plant: *A. wiestii* produced the largest number of spikelets per plant (23.0). It differed significantly from *A. longiglumis*, *A. barbata* and *A. hirtula*. The later ones also differed significantly from the wild and cultivated hexaploids. Glume length: The wild hexaploid species had the longest glumes (3.13 cm), while *A. sativa* had the shortest (2.1 cm); the remaining species were intermediate; differences between these three groups were highly significant. Awn length: The same trend was observed for this trait as for glume length. Date of panicle emergence: *A. sativa* and *A. hirtula* were the earliest (21 days), *A. barbata* was the latest (47.5 days), while the remaining species were intermediate. Number of tillers per plant: No major differences were observed among species for this trait. However, *A. sativa* had the largest number (4.5) and *A. longiglumis* had the lowest number of tillers per plant (2.9). Number of leaves per plant: No major differences among species were observed for total number of leaves produced per plant, however, *A. longiglumis* produced the highest number (12.2) and *A. hirtula* produced the lowest (8.85). Mature plant height: *A. wiestii* produced the tallest plants (122 cm), followed by *A. longiglumis* (100 cm). The shortest plants were produced by *A. barbata* (73 cm), while plants of the remaining species were intermediate in plant height.

Comparisons among ploidy level:

Diploid species were the highest for panicle length, number of whorles, number of spikelets and mature plant height (Table 2). They were followed by the tetraploid species, then by the hexaploid ones. Another trend was observed for each of glume length, awn length, number of tillers per plant and number of leaves per plant were hexaploids were the highest, followed by the diploids and finally by the tetraploid. On the other hand, the tetraploid species *A. barbata* was the latest for panicle emergence, while the diploid species were the earliest; the hexaploids were intermediate. This study showed that most *Avena* species are widely distributed in Jordan and their distributional ranges coincide with what have been reported in the literature (e.g., Bor, 1968; Ladizinsky, 1970; Feinbrun-Dothan, 1986). However, the only subspecies which was not found in Jordan is *Avena sterilis* subsp. *ludoviciana*.

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Table 1. Mean separation (Tukey=0.05) among six wild *Avena* species and a cultivar for 9 quantitative traits

Character	<i>lo</i>	<i>we</i>	<i>hi</i>	<i>ba</i>	<i>st</i>	<i>sa</i>
Panicle length	20.2a	20.1a	16.4b	15.8b	12.2c	9.5d
No. of whorles	7.12a	6.8a	6.6a	6.5a	5.5b	5.1b
No. of spikelets	17.5b	23.0a	12.8b	15.8b	7.1c	8.3c
Glume length	2.7b	2.4b	2.6b	2.4b	3.1a	2.1c
Awn length	3.9b	3.6b	3.8b	3.7b	5.3a	2.0c
Date of panicle emergence	41.1a	38.0a	20.2b	47.5a	37.5a	20.8b
No of tillers	2.9a	4.1a	3.1a	3.0a	3.9a	4.5a
Plant height	100b	122a	80d	74d	83c	75d
No of leaves	12.2a	11.4a	8.9a	10.7a	11.0a	10.0a

* Means, within each row, followed by the same letter do not significantly differ ($P<0.05$); *lo*: *A. longiglumis*, *wi*: *A. wiesii*, *hi*: *A. hirtula*, *ba*: *A. barbata* *st*: *A. sterilis*, and *sa*: *A. sativa*.

Table 2. Mean separation (Tukey=0.05) among three ploidy levels of six wild *Avena* species and a cultivar for 9 quantitative traits.

Character	Diploid	Tetraploid	Hexaploid
Panicle length	19.13a	15.85b	12.11c
No. of whorles	6.84a	6.54a	5.17
No. of spikelets	18.73a	15.78a	7.20b
Glume length	2.50b	2.40b	3.08a
Awn length	3.18b	3.77b	5.16a
Date of panicle emergence	31.56b	47.52a	36.50b
No of tillers	3.54ab	3.00b	3.97a
Plant height	100a	82bc	73c
No of leaves	10.9a	10.75a	11.00a

Germplasm collection and evaluation of barley in Egypt. M.A. KHALIFA,
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Introduction. Genetic variability, segregating populations, land races, old varieties, wild types and germplasm collections, are the raw-material of the certain of new cultivars, high in yielding capacity, well adapted to certain stress, resistant to many crop production adveristes mainly diseases and insects. Cereal scientists can obtain valuable gene poll from germplasm collection for desirable characteristics especially in the scope of crop quality or plant resistant to specific disease or insect infestation and environmental stresses (Heat, drought & salinity). Many institutions and scientists presents great activity on the problem; the highest interest was denoted on how to collect germplasm, the way to preserve it, how to evaluate and use it, and how to store and exchange information about it.

It is greatly hopped according to the data accomplished from the present project of disease reaction, soil analysis and characterization to have useful plant material for barley improvement.

Materials & Methods. Preliminary trips had been made to collect information about the sites which could be included in the field trips. A survey of the places which had soil stress or in which old land varieties and wild types of the crop under investigation and their related species may exist were obtained. Also, information on sites in which pathogenic fungi and viruses are prevailing were recorded.

Several trips were undertaken through the period of 1979-1988 to collect barley, germplasm accessions. Some plant characteristics were recorded during collection. Soil samples were also obtained from the collecting sites. Furthermore, notes on pest infestation were recorded. Collecting sites included: Lower Egypt, Upper Egypt, Red Sea, Suez Canal and Mersy-Matreh provinces. Collection emphasis was scoped on the healthy productive plants.

Grain samples of barley accessions were sent to U.S.D.A. scientists of the project; for testing the reaction against infection with leaf rust and powdery mildew pathogens. The reaction were taken on a 0-9 scale; with 0-3 equal highly resistant to resistant, 4-6 equal moderately resistant and 7-9 equal to susceptible to very susceptible. The cultures were 47-19 of *Puccinia hordei* (leaf rust) and CR₃ of *Erysiphe graminis hordei* (powdery mildew). Those two cultures are rather virulent. The reactions were read on the first leaf for leaf rust and on the second and third leaves for powdery mildew. Following inoculation the plants were maintained in a light and temperature controlled room. The temperature was between 18-21°C with lights from coal white fluorescent tubes twelve hours per day. Some fertilizer was added to the water to assure vigorous plant growth.

Accessions of barley were sown at Assiut for seed increase and observations. A set of the available commercial varieties were sown for comparison. The usual cultural practices and pest control were adopted. The following methods of soil analysis were used: 1- Mechanical analysis: Soil texture was characterized by using a boyoucos hydrometer, (Day, 1965). 2- Soil PH: Measured in a 1:2.5 soil water suspension by a glass electrode, (Jackson, 1958). 3- Total soluble salts: Measured by conductivity of the saturated soil extract in millimhos/cm, (U.S.S.L.S., 1954). 4- Cation exchange

Table (1): Some characteristics of barley accessions recorded during collection from all regions and the main soil types.

Plant and spike characteristics			Main soil types	Disease and insect records	
1)	Six-and two-rowed, deficiens, and irregulare.		1) Clay 2) Loamy 3) Silty 4) Alkaline 5) Sandy 6) Saline sandy	1)	Loose and covered smuts (present)
2)	Husked and huskles kernels			2)	Leaf rust
3)	Land races, old varieties and wild types			3)	Powdery mildew, leaf stripe, Net blotch (present but rare).
				4)	Aphids (present)

Table (2): Reaction of barley accessions collected from all sites; after screening; against 0-9 scale to leaf rust (LR) and powdery mildew (PM) pathogens*.

Dise- ase	HR-R						MR 4-6		S-VS 7-9		Accessions tested	
	0	1	2	3	Total	%	Total	%	Total	%	Total	%
LR	15	35	21	7	78	56.5	18	13.0	42	30.5	138	100
PM	1	29	3	28	61	27.5	52	23.4	109	49.1	222	100

*HR-R : Highly resistant to resistant.

MR : Moderately resistant.

S-VS : Susceptible to very susceptible.

capacity (CEC) and exchangeable sodium: Determined according to Jackson (1958).
5- Exchangeable sodium percentage (ESP %) calculated as follows:

$$\text{ESP \%} = \frac{\text{Exchangeable Na}}{\text{CEC}} \times 100$$

Preliminary evaluation was carried out after seed increase and screening. Final evaluation of the barley accessions were carried out for two successive seasons; i.e. 1988/89 and 1990/1991.

Results & Discussion. Several trips were conducted to collect land races, old varieties, and wild types of barley. Collecting sites included Lower Egypt, Upper Egypt, Red Sea, Suez Canal and Mersy-Matroph provinces. Collection emphasis was scoped on the healthy productive plants particularly from sites had a type or another of soil stress. The total number of accessions collected; after screening; were 540, and 154 of barley and wild barley; respectively.

Reaction of barley accessions collected from all sites after screening to leaf rust and powdery mildew pathogens revealed that of barley accessions were highly resistant to resistant to leaf rust and powdery mildew pathogens (56.5 and 27.5 %), respectively

The analysis of soil samples obtained from the collecting sites of all regions showed that 145 samples were clay, 49 were loamy clay and 115 varied from sandy-loam and loamy sand to sand. Salinity in 66 samples varied from moderate to high or very high. Estimation of ESP values showed that 15 locations were of ESP values more than 15 %; out of these, 5 samples were saline sodic.

Evaluation of barley accessions revealed that 32 accessions were significantly earlier than the early check. Moreover; 2, 3, 9 and 4 accessions significantly exceeded the higher check cultivars for plant height, yield per plant, harvest index and seed weight, respectively. Also, 209 and 33 accessions exceeded the higher check cultivars for number of spikes per plant and yield per drill, respectively. It was detected, in general, that accessions outyielded the best checks were superior in number of spikes per plant and seed index.

Barley accessions collected from the very high saline sites showed that many were earlier than any of the checks. Furthermore, some accessions outyielded any of the checks in plant yield or yield per drill. Others of those barley accessions were better in seed index than the checks.

There were 25 or 29.8 % and 4 or 12.1 % of barley accessions equal or exceeded the checks in yield per drill from these highly to moderately resistant to leaf rust (0-5) and powdery mildew (0-2), respectively.

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Barley genetic resources and breeders. J. LEKEŠ, CEREALIA, SNP 3989, CZ-76701
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In my previous six papers presented at the IBGS (1963-1991) I tried to recommend breeders to use genetic barley resources suitable for breeding programmes, to point out main problems of their investigations and to seek possibilities of their more effective utilization in breeding programmes.

Based on analyses of genetic resources, the most important varieties and evaluation of breeding barley during 150 years the varieties were included according to the period of their breeding in V groups (Table 1). It is apparent that the elements decisive for cultivar productivity have changed considerably: (i) gradual shortening stem length by 15 to 20 cm on average at each stage; a total reduction is 23 cm during 150 years, (ii) increase in the genetic yield potential which reaches 156 % in present varieties, (iii) increase in spike productivity. Three-year results obtained for the groups of varieties in comparative trials carried out at three different locations (climatic and soil conditions) show a clear tendency (Fig. 1); the quality and stability of the traits increase in accordance with the developmental groups, i.e. with the degree of breeding and potential yield performance of the varieties (a survey according to the methods by Vargas et al. 1983). More than 200,000 accessions are registered in the world, however, most of best-known varieties were developed without their participation. They were obtained by either the selection from important varieties or their mutual hybridization. Therefore, breeders need to know genetic values and breeding utility of at least the most important accessions.

Two international programmes should have improved this state: 1) The programme coordinated by Vavilov Institute in Petersburg in East-European countries (plus Mongolia) which resulted in elaborating unified classifiers and knowledge exchange. 2) The European Barley Database within the IBPGR Programme, FAO, coordinated by the Institute of Genetics in Gatersleben (East Germany) is on the level of passport-data registration for 55,369 accessions covering 44 barley collections (without the biggest one at VIR Petersburg). Despite the effort of cooperating countries the present results which gather primarily phenotypic expressions and the variability in morphological traits are not sufficient for successful implementation of breeding programmes.

To improve the utilization of genetic resources the collections of chosen donors have to be specified as follows:

- collections of donors with wide agroecological plasticity and positive response to specific growing conditions, in particular with high spike productivity and tillering, resistance to lodging, and grain quality for different end use (malt production or feeding),
- specific collections for agroecologically defined regions, e.g. donors of drought resistance winterhardiness, earliness, etc.,
- collections that are aiming at the selection of donors with a special trait, e.g. race-specific resistance to some diseases with the most effective utilization of nutrients, etc.

It is desirable to involve breeders in these activities because they have most knowledge of a donor character and as well as they are interested in them with regard to lowering costs and increasing effectiveness of breeding programmes. Such intentions of studying genetic resources (though there has already been some effort to realize them in individual cases) can be achieved at only well organized division of work of respective institutions in these programmes.

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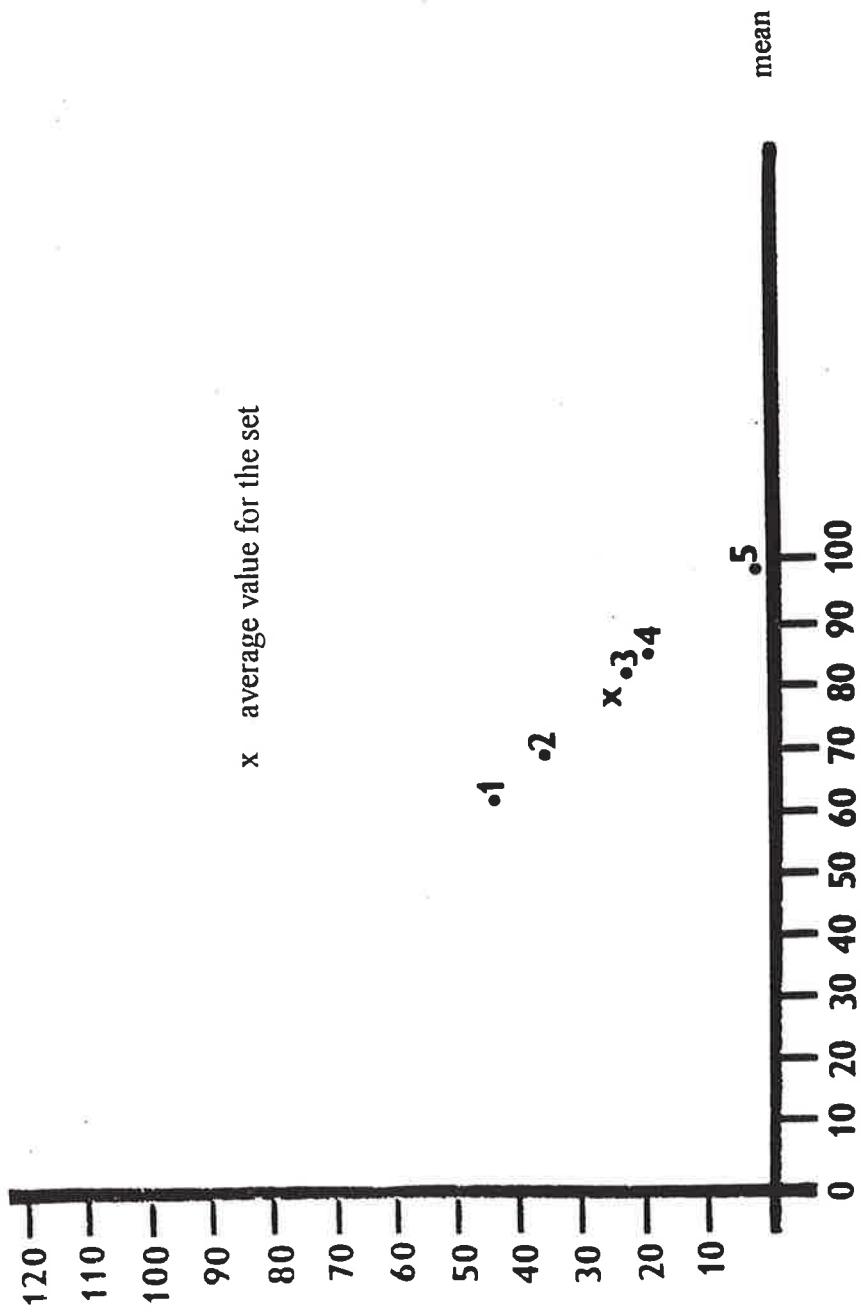
I. The most important varieties and donors of spring barley
produced in the course of purposeful selection

Groups of varieties	Year of breeding	Origin of variety
I. Varieties produced by individual selection from local varieties - primary donors in commercial		
Proskowetz Hana Pedigree (CS*)	1884	individual selection from local Hana varieties
Ackermans Bavaria (D)	1908	individual selection from local South Bavarian var.
Chevalier (GB)	1829-1904	individual selection from local South English var.
Viner Vyatsky (SU)	1915	individual selection from North Russian local var.
Opavsky-Kneifl (Vollkorn-gerste) (CS)	1926	individual selection from local Hana varieties
II. Varieties produced by simple hybridization - generally on the basis of the Hana barley.		
Kenia (DK)	1931	Abed Binder x Gull
Valticky (CS)	1939	South Moravian local varieties x Opavsky-Kneifl
Heines Haisa (DDR)	1940	Hanna x Ackermans Isaria
Ackermans Donaria (D)	1941	Ackermans Isaria x Opavsky-Kneifl
Proctor (GB)	1952	Kenia x Plumage Archer
III. Varieties produced by hybridization on the basis of groups I, II		
Firlbecks Union (D)	1956	Weihenst. Mehltolerant C.P. x Ack. Donaria x Firlbecks III
Deba Abed (DK)	1966	Abed Denso x Weihenstephaner II
Aramir (NL)	1974	Volla x Emir
IV. Diamant variety and varieties of Diamant type improved to have the better grain fulness		
Diamant (CS)	1966	X-ray mutation of the Valticky variety
Trumpf (DDR)	1972	Diamant x Alsa x Abyssinian x Union
Koral (CS)	1978	Hana x [(Carlsberg II x F. Union) x (Alsa x Celechovicky x Immune 25)]
V. Short-stemmed varieties complemented to include important genes of resistance to the diseases		
KM 1192 (CS)	1974	Abed Denso x X-ray mutation of the Kneifl barley
Zenit (CS)	1985	KM 1402/74 x Karat
Victor (CS)	1994	Rg Valticky - dwarf form resistant to powdery mildew x long-stemmed form

*) International abbreviation of the country of origin is given in parentheses, with each variety, genetic resource, or donor.

coefficient of variation

1. GRAPHICAL REPRESENTATION OF THE QUALITY INDEX IN THE
DIFFERENT GROUPS OF VARIETIES



Barley and oat data in *GrainGenes*. D.E. Matthews¹, M.E. Sorrells¹, J.M. Wong² and O.D. Anderson²

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Introduction. *GrainGenes* is a genome database for Triticeae, oats, and sugarcane, being developed as part of the United States Department of Agriculture, National Agricultural Library's Plant Genome Program, initiated by Jerome P. Miksche and currently directed by Henry L. Shands. It is one of approximately a dozen publicly accessible plant genome databases in existence [1], most of them members of the same program. Other such databases of particular interest to barley and oat scientists include *RiceGenes*, *MaizeDB*, *SorghumDB* and *PathoGenes* [1].

The *GrainGenes* project comprises three distinct information services, all available by anonymous Internet access: the *GrainGenes Database*, *GrainGenes Gopher*, and *GrainGenes Webserver*. These services differ in user interface, data format, and the particular datasets they contain, but all are focused on the same kinds of information: genome maps, DNA clones and sequences, classical genes, genetic stocks, and genotypes and traits of cultivars and breeding lines. Related information about pathology and taxonomy is also included, as well as references and an address book of scientists working on these crop species.

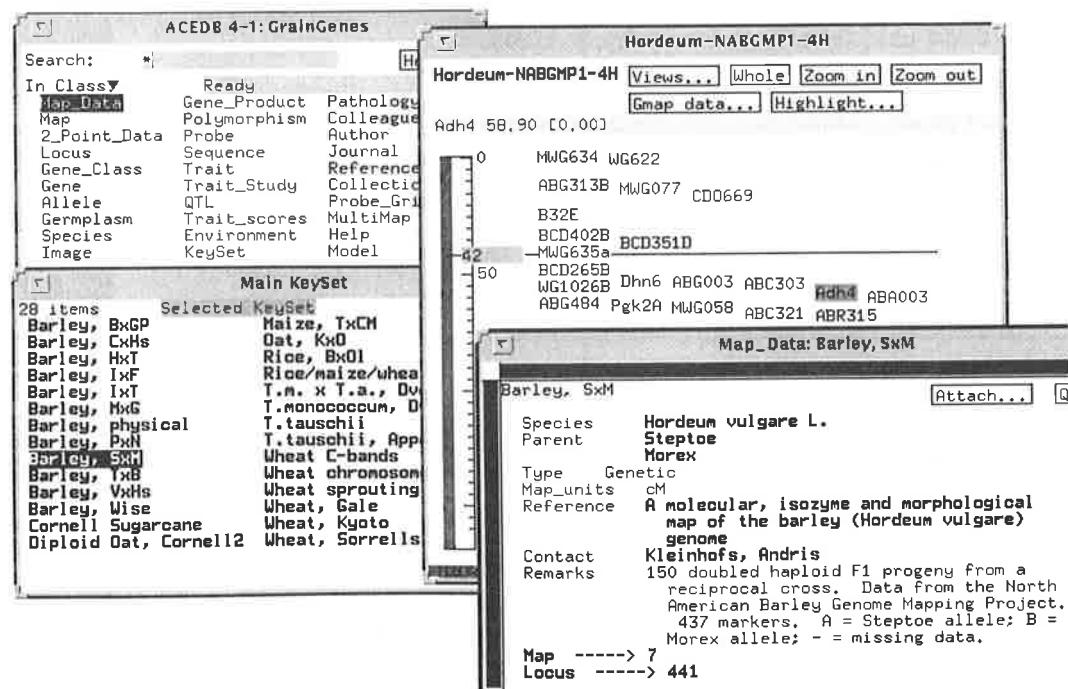


Fig. 1. The ACEDB user interface. The main window (upper left) lists the data classes. Clicking the mouse on a class produces a list of records in the Keyset window. Clicking on the name of a record shows the record in a text window (lower right) or a graphical display such as the genetic map. Boldface words in the text window, and most words in the map display, are links to other records: clicking on them causes the corresponding records to be displayed, each in a separate window.

GrainGenes Database. The *GrainGenes Database* contains highly structured, highly inter-linked data, and is the best format both for performing sophisticated queries and for locating information by “browsing” from record to record. It uses the Unix database management software ACEDB, and can be downloaded in its native form for running on a local Unix machine [2], or accessed via the Internet through a gateway to the World Wide Web [3]. Using ACEDB directly provides a hypertexted, multi-windowed user interface (Fig. 1); the WWW gateway emulates this except in a single window. A partial list of barley and oat data available in the *GrainGenes Database* includes:

- barley maps (Steptoe x Morex, Harrington x TR306, Proctor x Nudinka, Igri x Franka, Vada x *H. spontaneum*, Vogelsanger Gold x Alf)
- oat maps (Kanota x Ogle, *A. atlantica* x *A. hirtula*, *A. strigosa* x *A. wiestii*)
- higher resolution maps, and molecular markers for genes (*Mla*, crown rust resistance)
- names and locations of barley genes, and gene maps for chromosomes 2, 4 and 5
- catalogue of barley powdery mildew resistance genes and stocks
- Triticeae consensus map, groups 1 - 3
- estimates of the sizes of mapped bands for the oat Kanota x Ogle map
- RFLP data for 80 oat cultivars and 170 *A. sterilis* accessions
- Genbank sequence records for *Hordeum* and *Avena*
- addresses of 170 collections of *Hordeum* and *Avena* spp.
- colleague address information from NABGMP and the *Oat Newsletter*

GrainGenes Gopher. This service is primarily for distributing information in a flexible format without requiring a particular data structure, to make things as easy as possible for data contributors. The basic medium is directories (menus) of plain text files. Large files, including the entire contents of the *GrainGenes Database*, can be indexed for fast searching via simple full-text queries. In addition, files in various binary formats (word-processor, spreadsheet) can be made available for downloading by those who have appropriate software to use them, and images can be viewed directly. Examples of data found in the *Gopher* but not in the *Database* are the NABGMP Steptoe x Morex trait dataset [4] and the *Cereal Rust Bulletin* [5].

GrainGenes Webserver. The *Webserver* [6] contains links to the *Database* and *Gopher* services described above, so everything can be reached from here. The unique feature of the World Wide Web is its ability to present documents formatted optimally for human reading, including such luxuries as *italic fonts*, imbedded graphics and hypertext links. GrainGenes examples include the *Barley Genetics Newsletter* [7] and the article “Anchor probes for comparative mapping of grass species” [8]. This server also contains links to information of interest provided by other Internet information servers around the world.

In Progress. Datasets currently being added to the *GrainGenes Database* include barley maps (Proctor x Nudinka updated with AFLP markers, and Vogelsanger Gold x Tystofte Prentice), cloned barley genes, and the oat gene catalogue. WWW documents being prepared are descriptions of nearly 400 Barley Genetic Stocks, and an update of the NABGMP Steptoe x Morex trait dataset. We are also developing a mirror site in France for all the GrainGenes Internet services, to improve connection speeds for European users, and a Macintosh version of the native ACEDB *Database* for users without Unix machines.

Grains Mailgroup. A fourth GrainGenes service, the *grains mailgroup*, is a bulletin board for communication among scientists working on the GrainGenes crops, on any

matter of common interest. Electronic mail messages sent to the address grains@green genes.cit.cornell.edu are automatically re-posted to all members of the group, currently about 200 [9]. To be added to the group (or to be removed, or to update your address), contact Dave Matthews, matthews@green genes.cit.cornell.edu.

Acknowledgments. Although GrainGenes obtains some data indirectly via the literature, most of it is submitted directly, either by the scientists who obtained it or by experts who have evaluated and compiled it from the literature. We owe these data donors a great debt of appreciation for their efforts in assembling and checking these datasets, adding all necessary contextual information, and assisting us in interpreting them, converting to database format, and validating. Wherever possible these data sources are acknowledged on every database record to which they have contributed.

Here is an attempt at a complete list of those who have provided or promised barley or oat data (with sincere apologies in advance to anyone we've omitted): Tom Blake, Memo Briceno, James Brown, Arla Bush, Richard DeScenzo, Brian Forster, Jerry Franckowiak, Henriette Giese, Andreas Graner, Pat Hayes, Ann-Charlotte Heiberg, Manfred Heun, Mark Hughes, Morten Hulden, Jens Jensen, Helms Joergensen, Ken Kephart, Andy Kleinhofs, Dave Kudrna, Gottfried Kuenzel, Peter Langridge, David Laurie, Mike Lee, Udda Lundqvist, Diane Mather, Louise O'Donoughue, Laura Oberthur, Greg Penner, Soren Rasmussen, Mark Sorrells, Nick Tinker, Allen Van Deynze, Penny von Wettstein-Knowles, JoAnn Walldren, Roger Wise. Thanks to you all, and we hope this list will be much longer by the next International Oat Congress / International Barley Genetics Symposium.

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DISTRIBUTION OF β -AMYLASE ALLELES IN CULTIVATED BARLEY FOR THE EURO-ASIA REGIONS (THE FORMER U.S.S.R.). V.P. Netsvetaev, Belselect Co., Seed Station, Oktyabrsky Vil., Belgorod Reg., 309090, Russia.

Introduction. β -amylase isozymes of seed are controlled by the Bmy 1 locus on the long arm of chromosome 4 (Nielsen et al., 1983; Netsvetaev, 1993). Three alleles (Bmy 1Br, Bmy 1Ar, Bmy 1A1 from varieties Birk, Aramir, and Algerian, respectively) were found in cultivated barley (Nielsen and Johansen, 1986; Netsvetaev 1992). The nature of these β -amylase isoenzyme differences is not known. However, absence of the f915 b fragment under PCR procedures, using primer P6 (Netsvetaev et al., 1995), was associated with the electrophoretically fast β -amylase isoenzyme (=BMY 1Ar). Bmy 1Br and Bmy 1Ar alleles may differ in a deficiency similar to f915, if the f915 factor involves the Bmy 1 locus. Such allele peculiarities may influence the enzymic activity of their allele products - the β -amylase variants.

Materials and Methods. 179 spring barley varieties certificated for the former USSR territory in 1929-1990 were classified by β -amylase isoenzyme types (Netsvetaev, 1992). Electrophoretic separation of β -amylase enzymes was carried out in polyacrylamide gel with tris-glycine buffer (pH 8.3) as described previously (Netsvetaev, 1993). A-amylase activity of malt in F₁ Maja X Viner heterogeneous and inbred lines (PBI, Odessa, 1994) was determined by using DDR standard procedure (1982). Other analysis were done according by Sozinov et al. (1977). Variants of the hordein encoded by Hrd A, Hrd B, Hrd F loci were analyzed by electrophoresis in starch gel at pH 3.1 (Pomortsev et al., 1985). The frequency distributions of these three β -amylase alleles were estimated for various geographic regions.

Results and Discussion. Seeds of lines possessing the Bmy 1Br allele had a greater amylolytic activity than seeds of lines with the alternative Bmy 1Ar allele (Table 1). Consequently, the fast β -amylase isoenzyme (BMY 1Ar) was characterized by lower enzymic activity and slow isozyme (BMY 1Br) had higher amylolytic activity. The β -amylase alleles probably differed in the deficiency determining the functional part of the enzyme molecule. Hordein polypeptides (Hrd-loci) influenced the level of amylolytic activity and other quantitative traits as well as a β -amylase isozymes (Table 1). Hordeins A, C, D, E (or C - according to Shewry et al., 1981) do not have disulphide bounds in molecular structure of the polypeptides in comparison with B-, F-, G-hordeins. Consequently, differences in amylolytic activity of lines having alternative Hrd-alleles may be associated with another hordein factor (=Hrd G) which is tightly linked to the Hrd A locus and has disulphide bounds. These data agree with earlier results (Netsvetaev et al., 1984), when recombinant plants for the Hrd A - Hrd G segment were analyzed.

The geographic dependence of the Bmy 1 allele frequency distribution for Euro-Asia territory in spring barley varieties was expected if the association of the Bmy 1 alleles with grain yield and enzymatic properties of isozymes in these climatic conditions of seedling growth are taken into consideration. Allelic frequency of Bmy 1 factors in a geographic region was dependent on latitude. The frequency of the Bmy 1Ar allele in the Northern region exceeded 50%. About 72% of the varieties in the southern region had the Bmy 1Br allele, while the frequencies of Bmy 1Ar and the Bmy 1A1 were 26 and 2%, respectively. The allelic distribution did not change from West to East. The allelic frequencies at the Bmy 1 locus varied with the temperature and moisture

conditions of a province. For example, the correlations between Amy 1Ar frequency and sum of effective temperatures ($>10^{\circ}\text{C}$) as well as precipitation were $r=0.657\pm0.157$ and $r=+0.550\pm0.174$, respectively. the coefficient of multiple correlation including these two climatic parameters was 0.745. Thus, these factors determine over 55 % of the whole variability in the allelic distribution of the Bmy 1Ar for 25 geographic provinces of the former USSR. Belgorod (H) is disposed in the intermediate area between North and South regions in the European part of Russia. Apparently, alleles of Bmy 1 locus in intermediate regions will be characterized non stability to adaptive and/or select value in dependence of year environments. For example, the Bmy 1Ar allele among varieties of Belgorod region (=H) is estimated at frequency value about 40, 5±6, 2%. The Bmy 1A1 allele is not found here. As shown in Table 1, the Bmy 1Ar factor stimulated greater grain yield in 1995 contrasting to 1994 near Belgorod. Odessa (S) is disposed in South region of Ukraine and the frequency of the Bmy 1Ar allele in certificated spring varieties was formed by 14.3±6.9%. The Bmy 1Br was found in 85.7±13.2% of varieties. Apparently, genotypes with the Bmy 1Br allele in South regions determine better adaptive and/or agronomic value for steppe environments than a spring barley with alternative alleles.

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Table 1. Alleles of Bmy 1 locus and level of quantitative traits in barley

Source [#]	Character	Alleles of Bmy 1			
		Ar	Br	Ar - Br	
		n	$\bar{x} \pm s.e.$	n	$\bar{x} \pm s.e.$
1	α -amylase (SKB units)				
	Hrd A2 B19 hrd G	22	654 \pm 15	17	711 \pm 11
	Hrd A12 B19 Hrd G	16	648 \pm 20	19	669 \pm 23
2	Grain weight per plant (g)				
	Hrd A2 B19 hrd G	22	3.2 \pm 0.3	17	3.8 \pm 0.2
	Hrd A12 B19 Hrd G	16	3.6 \pm 0.2	19	2.8 \pm 0.2
2	No. of grains per plant				
	Hrd A2 B19 hrd G	22	101 \pm 8	17	129 \pm 7
	Hrd A12 B19 Hrd G	16	103 \pm 7	19	91 \pm 7
3	α -amylase (con. un)	37	180 \pm 8	50	204 \pm 7
3	Shoot in 5-days seedling (%)	16	0.7 \pm 0.2	8	1.5 \pm 0.3
4	Protein content (%)	26	17.1 \pm 0.2	27	16.4 \pm 0.2
4	Extract (%)	26	72.7 \pm 0.3	27	74 \pm 0.3
5	Grain yield (t/ha)	17	7.26 \pm 0.36	6	7.34 \pm 0.95
6	Grain yield (t/ha)	43	4.09 \pm 0.12	51	3.69 \pm 0.10
6	3-day germination (%)	43	99.23 \pm 0.10	51	99.51 \pm 0.07

[#]1 - E._{oo} Maja X Viner, 1986; 2 - F._{oo} Maja X Viner, 1987; 3 - PBI, Odessa, 1990; 4 - PBI, Odessa, 1994; 5 - PBI, Belgorod, 1994; 6 *p < 0.05; **p < 0.01

Genetic diversity in barley landrace populations from Sardinia, Italy.

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Introduction. Barley (*Hordeum vulgare* L.) is the second most widely-cultivated cereal in Sardinia island, Italy. In this island the cultivation of modern varieties is limited and many farmers grow local populations of the six-row landrace "S'orgiu sardu" (Attene *et al.*, in press). The aim of this paper is to investigate genetic diversity among and within the 12 local populations by isozyme and morphological and phenological traits.

Materials and Methods. Twelve populations collected in Sardinia in 1990 were studied. Each population was represent by 20 randomly chosen lines (two cycles of single head progeny). Each line of the Sardinian material was examined for four morphological traits: row type, (two-row or six-row); aleurone colour (blue or white); spike density (dense or lax) and antocyanic glume nerve (presence or absence). The same materials were also evaluated for growth habit (winter/spring). Allelic variation were analyzed at three loci for esterase (Est1, Est2 and Est4), one for aspartate aminotransferase (Aat 3) and two for phosphogluconate dehydrogenase (Pgd1 and Pgd2). In this report, Nei's diversity (H) and populations differentiation (Dst) statistics, which are related to Simpson's measure of ecological diversity, are used to summarized the genetic, and the discrete phenotypic data. Let P_{ji} denote the frequency of the i th allele or character-state, at the j th locus or character. Then the average diversity over all r loci for any population is: $H=1-\sum\sum p_{ji}^2/r$. In measuring the extent of genetic or phenotypic differentiation, either at a single locus, or averaged over several loci, the total diversity (H_T) is first computed. When based on several loci, the value for the total diversity is the average of the H_T values for the loci. From this, the proportion of this diversity which is expressed between population (Gst) is estimated as $Dst/H_T=1-\bar{H}/H_T$. In the case of a single locus, \bar{H} is the average over all population of the within population diversity at the locus.

Results and discussion. The average values for the less frequent class is 0.4 for absence of antocyanic glume, 0.133 for white aleurone, 0.05 for winter type, 0.013 for dense spike and 0.004 for two row type. Significant correlation ($r=0.67$; $P\leq 0.018$) between the population frequencies of the white aleurone and the rainfall of the experimental station nearest to the collection sites was found. In particular this class is less frequent in southern populations. The frequency of the winter type is 0.05 considering all the materials; in four population of South Sardinia is absent. Also for winter type a significant correlation ($r=0.72$, $P\leq 0.009$) with rainfall was found. The overall average diversity (\bar{H}) was 0.14 varying from 0.02 to 0.24. The 84% of the total

variation ($H_T=0.17$) was within population. All the materials were homogeneous to carry the same alleles at loci Aat2 Aat3 Est2 and Pg2. For those enzymes the alleles detected were those most common in the European barley germplasm (Konishi, 1995). Allelic variation was found at esterase loci Est1 and Est4 which are tightly linked and are located near the terminal of the long arm of chromosome 3 (Konishi and Matsuura, 1987). In the Sardinian barley 6 different esterase genotypes were found: Ca-Su, Al-At, Pr-Su, Ca-Nz, Al-Su and Ca-At. The last three were very rare (0.004, one line only) and the frequency of the type Pr-Su was 0.033. The most common genotypes were the type Ca-Su (0.7) and the type Al-At (0.26). Also for isozyme most of the variation was located within populations (84%). High correlations between the population frequencies of the esterase genotypes Ca-Su and Al-At and the rainfall of the experimental stations nearest to the collection sites were found. The genotype Ca-Su is more frequent in the populations collected in the sites with higher rainfall ($r=0.91$; $P\leq 0.0001$) in contrast with the genotype Al-At that is more frequent in the dry sites ($r=-0.93$; $P\leq 0.0001$). The genotype Pr-Su was detected in half the populations studied in particular from the South. Also for this genotype a negative correlation ($r=-0.63$; $P\leq 0.029$) with rainfall was found to be significant. With very few exception the Sardinian barley is characterized by a lax six-row spike and is in general spring type. The presence of three dense six-row and one two row could be originated from old landraces nowadays extinct or from commercial varieties or also by mutation. All the dense and two-row types found differ from the other Sardinian materials for several features. In fact in comparison with the other strains studied they are late in heading time and present a short stature (Papa, *et al.*, in preparation); they are also usually characterized by features that are rare in the others materials, three of them are winter type and two of them present two of the unique isozyme genotypes. Considering that the mutation rate of enzymes loci in barley should be lower than 3.6×10^{-6} and lower than 8.85×10^{-7} for morphological traits (Kahler *et al.*, 1984) the probability that the mutation occurred only in rare genotypes is very low. Moreover their late flowering and in particular their short stature suggest that they derived from modern varieties indicating that at least in some cases, gene flow from commercial varieties into Sardinian landrace populations occurred. Gene flow among plant populations can take place in two ways: pollen dispersal to a different population, successful fertilization of an ovule by this pollen and finally establishment of the resulting seed within the site or also by dispersal of seed, and the successful establishment of dispersed seed within a new population. In the case of Sardinian barley gene flow most probably was caused by seed dispersal. In fact the low outcrossing rate in barley associated to different heading time between these materials and the others suggest that outcrossing is highly improbable although a specific study should be conduct to asses the gene flow between commercial varieties and Sardinian landrace populations. Considering all the

populations the partitioning of diversity between and within population was the same for both type of markers: $Gst = 0.164$ for the morpho-phenological traits and $Gst = 0.161$ for isozymes. A recent work on the same materials (Papa, et al., 1996) based on RAPD analysis has shown similar results: the within populations diversity was 90% of the total diversity. Both studies suggest that all of the populations studied belong to the same landrace. This confirm the hypothesis of Attene et al., (in press) based on farmers information. The frequencies of the isoenzymes genotypes among populations was varying non randomly, in particular the genotypes Ca-Su was more frequent in the mesic environments and the genotypes Al-At in the more xeric ones. The isozyme genotype Pr-Su was found only in the populations collected from South Sardinia. The results presented in this paper indicate a genetic differentiation between the population collected in the two different areas as also suggest by agronomical (Papa, et al., in preparation) and RAPD data (Papa, et al., 1996) and also suggest that this differentiation is probably related to different adaptive value rather than different germplasm origin or genetic drift. These results indicate the presence of a wide genetic variability within the populations studied and a clear differentiation between the populations collected in North Sardinia from those collected in the South related to differential adaptation to environmental characteristics and in particular mesic vs. xeric conditions.

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Genetic diversity in wild and cultivated barley (*Hordeum vulgare* L.) revealed by RAPD analysis

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Introduction. In order to preserve and exploit genetic resources of crop plants a systematic assessment of the available genetic variability is necessary. In this study, Random Amplified Polymorphic DNA (RAPD) analysis was used to estimate genetic diversity in wild and cultivated barley *Hordeum vulgare* L..

Materials and Methods.

Plant material. Plant materials used in this study consisted of 31 accessions obtained from IPK, Gatersleben, Germany, ICARDA, Aleppo, Syria, IMGV, Perugia Italy:

11 landrace accessions: 3 lines (Q/10, SNG/17 and Sinis O/27) extracted from three populations of the Sardinian landrace *S'orgiu sardu*; (Italy1, Italy2 and Italy3 respectively in Figure 1); 1 line (Zambaka) extracted from the Syrian landrace Arabic Aswad (Syria1); 3 populations collected in Egypt (ICB115950, ICB118510 and ICB116547; Egypt1, Egypt2 and Egypt3); 2 populations from Nepal (HOR11002 and HOR11001; Nepal1 and Nepal2) and 2 populations from Ethiopia (HOR7718 and HOR10443; Ethiopia1 and Ethiopia2).

8 varieties: 2 two-row spring varieties (Formula and Palmella Blue), 2 two-row winter varieties (Kelibia and Pipkin), 2 six-row spring varieties (Sunbar and Rihane) and 2 six-row winter varieties (Glennan and Vetulio).

12 populations of wild barley: 4 from Israel (HS10, HS76, HS41 and HS35; HsISR1, HsISR2, HsISR3 and HsISR4), 4 from Iran (HOR2686, HOR2689, HOR2681 and HOR3295; HsIRN1, HsIRN2, HsIRN3 and HsIRN4) and 4 from Turkmenistan (HOR2699, HOR2701, HOR2707 and HOR4862; HsTRK1, HsTRK2, HsTRK3 and HsTRK4).

DNA isolation and RAPD analysis. One seed per accession was planted and seedlings tissues were harvested for DNA extraction. DNA was isolated according to Edwards *et al.* (1991). The PCR (polymerase chain reaction) conditions were defined according to Barcaccia (1994). In total 18 random sequence 10-mer primers were used in this study. These primers were selected in preliminary experiments for the total number of reproducible DNA bands generated using barley templates without any preliminary selection among primers for the number of polymorphic bands detected (Huff *et al.*, 1993). The data matrix was obtained evaluating for each line the presence or absence of unique and shared DNA fragments.

Data analysis. The Shannon's diversity index was used to quantify the level of genomic polymorphism detected

$$H_0 = - \sum p_i \ln p_i$$

where p_i is the frequency of the phenotype i ; H_0 can be calculated and compared for different populations. The average diversity over the n different populations $H_{pop} = (1/n) \sum H_0$, and the diversity calculated from the phenotypic frequencies p in all the populations considered together $H_T = -\sum p \ln p$.

Estimates of similarity between populations were based on the probability that an amplified fragments from one accession will be also present in another: $D_{AB} = 2 \times n. \text{ of shared fragments} / (n. \text{ of fragments}_A + n. \text{ of fragments}_B)$. On this basis a cluster analysis was performed.

Results and Discussion. Nineteen primers generated polymorphic profiles and one was monomorphic. A total of 177 bands were scored of which 78 (44%) were reproducibly polymorphic. The number of products generated by each primer varied from 4 to 18, with an average of 9.8; the number of polymorphic fragments for each primer was ranging from 0 to 10 with an average of 4.3. Thirteen unique RAPD phenotypes were also identified, 3 from cultivated and 10 from wild barley.

The diversity calculated for all the accession considered together (H_T) was 0.43. The index of phenotypic diversity over the wild and cultivated barley (H_{pop}) was 0.09. The diversity index (H_0) for wild and cultivated barley were respectively 0.42 and 0.35. Considering the three sets of materials utilized in this study the diversity index (H_0) was 0.42 for wild barley, 0.34 for landraces and 0.25 for varieties. This study demonstrated that at the molecular level wild barley is more polymorphic than cultivated barley and that the diversity of landraces is intermediate between the level of barley varieties and the level of wild barley. Similar results were also found by Brown & Munday (1982) and Saghai Maroof et. al. (1995). These results highlight the importance of wild and landraces germplasm as source of genetic diversity in barley which should be preserved and utilized.

The resulting dendrogram (Figure 1) largely matches existing knowledge on genetic differentiation among barley germplasm. All the cultivated materials were clustered together with the exception of accessions from Nepal and from Ethiopia, which are considered secondary centers of diversity for cultivated barley. This study suggest that RAPD analysis may be useful in assessing the genetic diversity of barley germplasm.

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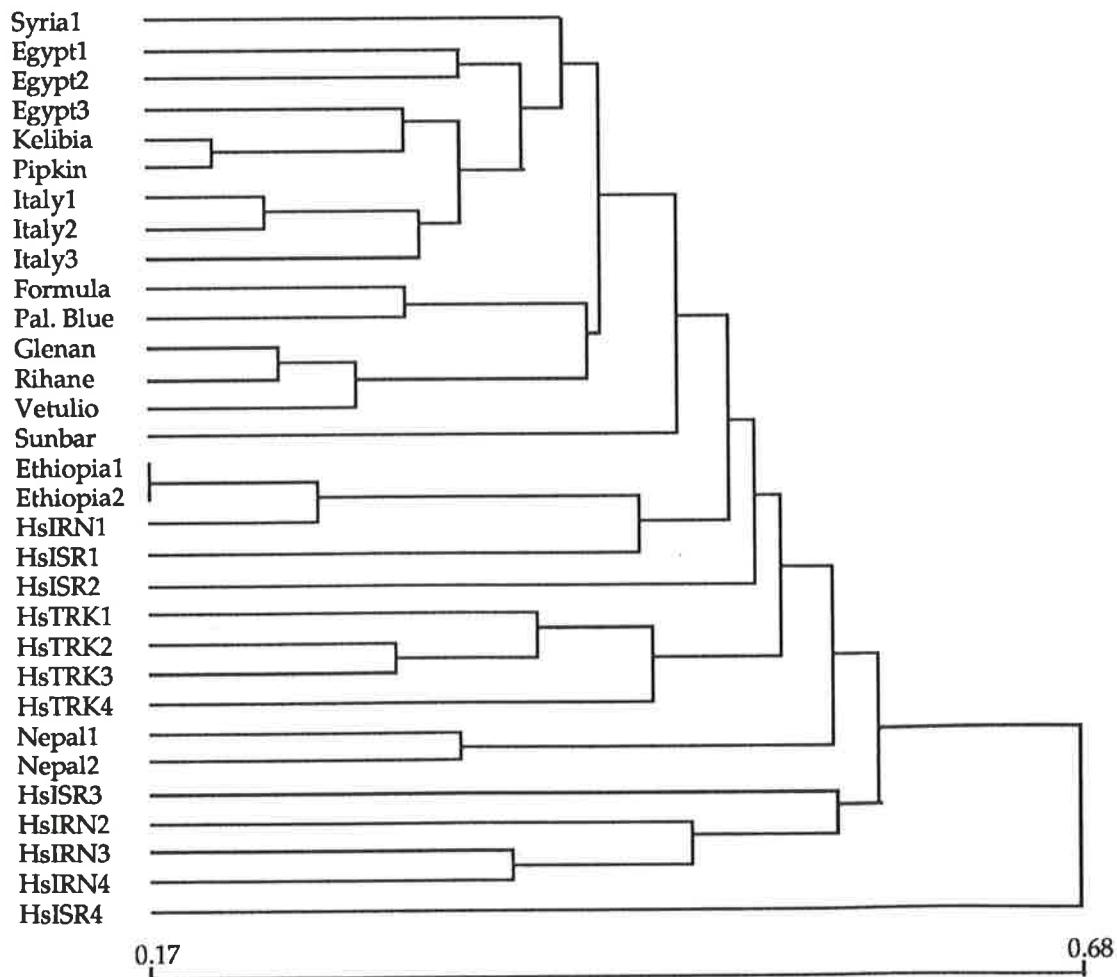
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Figure 1. Dendrogram of the 31 wild and cultivated barley accessions based on average linkage cluster analysis.



Quality trait evaluation of oat and barley germplasm from the USDA-ARS

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Introduction. A systematic evaluation of entries in the USDA-ARS National Small Grains Collection (NSGC), deposited in the USDA, Agricultural Research Service National Small Grain Germplasm Facility at Aberdeen, ID has been a mission of the ARS for many years. Entries in the collection, which number 23,247 for cultivated barley and 10,295 for cultivated oat, are evaluated for agronomic and pest resistance traits as well as certain quality traits. Data are entered in the Germplasm Resources Information Network (GRIN) database where they are accessible by interested small grains workers. This study involves the evaluation of oat for protein, oil, and β -glucan contents and barley for protein and β -glucan. This report describes progress to date. It is expected that at least three more years will be required to complete these analyses on the oat and barley collections.

Oat groats are generally considered to be the grain highest in protein and oil, although their concentrations range widely depending on genotype and growing environment (1,2). Both oat and barley have higher β -glucan contents than other cereal grains (3). High β -glucan is considered to be a desirable attribute for human consumption because of its effect in lowering the cholesterol levels of hypercholesterolemic individuals (4). Also, high β -glucan gives these grains a low glycemic index, making them useful dietary components for diabetics (5). Conversely, high β -glucan is undesirable for malting barley (6) and for certain animal feeds, especially young poultry (7). High protein is considered desirable for human and animal feed, but is not currently a high priority for plant breeders. Low protein levels are demanded for malting barleys (8). Oat millers prefer low oil content, although high oil lines might be desirable for certain animal feeds due to their higher energy content.

Experimental. Entries in the collections are grown periodically at Aberdeen, ID and Maricopa, AZ. Samples of oat entries are dehulled, and about 7 g of groats sent to Madison for analysis. About 14 g of each barley entry are submitted for analysis. In Madison, the samples are first analyzed with a Tecator Food and Feed Analyzer, Model 1255, which analyzes whole grain samples by near infrared transmittance. These scans are used to calculate protein and oil contents. The instrument is calibrated for protein against values obtained by combustion in a nitrogen analyzer (Leco model 428). A N to protein conversion factor of 6.25 was used. Each data set of about 1000 samples is calibrated. For oil, the instrument is calibrated against NMR oil values obtained from Fred Kolb, Univ. of Illinois. A single calibration is used for all samples. For β -glucan analysis, the samples are dried overnight at 65°C and ground in a Retsch ZM-1 Centrifugal Mill (Brinkman) to pass a 0.5 mm screen. Subsamples (50 mg) of the ground grain are extracted in boiling 0.075 M H₂SO₄ and analyzed by a flow injection analysis procedure, measuring the fluorescence from the reaction with Cellufluor (9). Due to the large number of entries, duplicate analyses of only single extractions were performed, although unusually high or low values were confirmed. A check (Morex barley) was inserted for every 12 samples.

Results. Among 6770 barley NSGC and other elite entries screened, protein ranged from 6.9 to 25.0% with a mean of 14.8 (Table 1). The samples were skewed slightly towards the high end (Fig. 1). Barley β -glucan ranged from 2.4 to 8.9%, with a mean of 4.8%. The protein of 7518 oat NSGC and other elite entries screened averaged 21.1% (groat basis), considerably higher than for barley (Table 1, Fig. 1). The range was 11.9 to 32.0%. Oat β -glucan was slightly lower than barley, ranging from 2.3 to 8.5% with an average of 4.6%. Descriptive statistics for each species are shown in Table 1. Data on oat oil content will also be presented.

Protein and β -glucan were positively correlated for barley ($R=0.18$, $P>0.01$) and negatively correlated for oat ($R=-0.14$, $P>0.01$). These coefficients, although significant, were small and all combinations of high and low protein and β -glucan were available within the entries of both species.

Discussion. Complete data for all entries analyzed are available on the GRIN database. The range in β -glucan and protein found in the germplasm analyzed so far indicate considerable variability in both species. The range and distribution of β -glucan levels was similar for both species, whereas oat had considerably higher protein than barley. Adapted genotypes of oat (10) and barley (3) show considerably narrower ranges of values. The β -glucan content of certain hulless, waxy barleys has been reported to exceed 10%, significantly higher than the range of entries screened to date (11). Seed samples of entries in the NSGC are available to plant breeders who may wish to use them as parents in breeding for enhanced quality.

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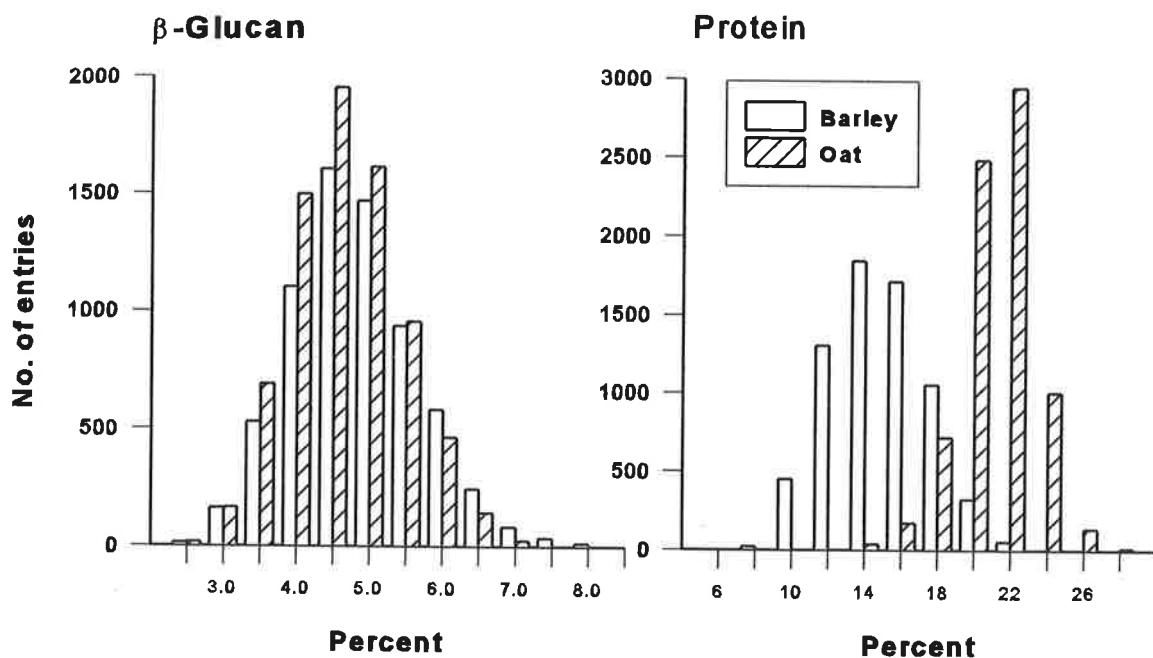


Fig. 1. β -Glucan and protein contents of barley and oat entries from the National Small Grains Collection

Table 1. Descriptive statistics of barley and oat analyses for percent protein and β -glucan.

Statistic	Barley		Oat	
	Protein	β -Glucan	Protein	β -Glucan
Mean	14.8	4.80	21.1	4.64
Median	14.8	4.74	21.2	4.60
Standard Deviation	2.61	0.87	1.95	0.76
Kurtosis	-0.41	0.38	1.04	0.01
Skewness	0.16	0.43	-0.30	0.27
Range	18.1	6.49	20.1	6.17
Minimum	6.9	2.43	11.9	2.28
Maximum	25.0	8.92	32.0	8.45
Count	6770	6770	7518	7518

UTILIZATION OF INTRODUCED GERMPLASM IN MALTING BARLEY BREEDING. ¹N. Pržulj, ¹Momčilović Vojislava, ²D. Knežević, ¹Institute of Field and Vegetable Crops Novi Sad, Maksima Gorkog 30, 21000 Novi Sad, Yugoslavia

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Introduction. In Yugoslavia, as well as Europe, only two-rowed barley is used for beer production, and it is only the form that the paper deals with. Up until the late fifties two-rowed genotypes from of Russia, Austria, and Hungary were the ones that dominated in the malting barley production in Yugoslavia. The old Yugoslav winter malting barley (WMB) and spring malting barley (SMB) varieties were suitable for the low level of production practices that were then in use. The winter varieties had high and stable yields (3-3,5 t/ha) as well as a low protein and a satisfactory extract content (around 11% and 80%, respectively), but were also characterized by tall stature (around 110 cm) and low resistance to lodging. The SMB varieties had lower yields (2-2,5 t/ha) and good technological characteristics of malt. In the period between 1957 and 1962, the intensive agrotechnics, i.e. high N doses and a later sowing, already introduced into the wheat production, began to be applied in the barley production as well. The varieties then on offer were not adapted to the new agricultural practices, and the new circumstances resulted in extremely poor agronomic and technological characteristics. This was the reason for the introduction of malting barley varieties from western and central Europe, which were adapted to intensive agricultural practice. Selected for different environmental conditions, the introduced genotypes could, however, provide no basis for a stable malting barley production. In 1963, consequently a domestic barley breeding program was established in Yugoslavia. The objective of this paper is to present the genetic base and pedigrees of Yugoslav WMB and SMB.

Materials and Methods. In terms of her climatic and edaphic characteristics, Yugoslavia is a very heterogeneous region, with mountainous areas where spring barley is grown only, and plains and hilly regions for winter barley growing. Barley breeding in Yugoslavia is done by the Institute of Field and Vegetable Crops Novi Sad, the Small Grains Center Kragujevac and the Center of Agricultural and Technological Research Zaječar, which are located in different climate regions. The extent of foreign variety utilization in Yugoslav hybridization programs can be taken to be a measure of their adaptability to the new agroecological growing conditions, since varieties chosen for crosses were those having the best performances. The number of varieties bred from a particular cross can be considered a measure of their specific combining abilities, whereas that of varieties bred from all the crosses of a particular genotype would present a measure of its general combining ability. Since 1995 Novi Sad has released 13 WMB and 16 SMB, Kragujevac 1 WMB and 10 SMB and Zaječar 4 WMB. Similarities between the 44 WMB and SMB varieties were determined by SDS-PAGE electrophoresis.

Results and Discussion. The first crosses were done mostly between the French, Swedish and Dutch winter six-rowed and two-rowed varieties having the highest performances in Yugoslav growing conditions. Ager is the variety with the highest percentage (29,7%) of germplasm in the Yugoslav WMB varietal offer; 94% of the WMB have it included in their genetic base (From Tab. 1). Germplasm of the line Fr 33 is represented in the WMB

genepool with 29,5%, and is found in 65% of the varieties. The actual participation of Fr 33 exceeds 50% in most of the WMB varieties. The third place is taken by Emir, whose presence amounts to 20% of germplasm in 88% of the WMB varieties. Germplasm of Union can be found in the genetic base of 29% of the winter varieties, contributing a total of 11% to their genotypes.

The foreign genotype with the greatest participation in the Yugoslav SMB varieties is Union, with a germplasm contribution of 21,5%. It is part of the genetic base of 52% of the SMB varieties (From Tab. 1). In the complex crosses Union always came as the last or one of the last parents and due to this has a large germplasm percentage in the progeny. Ceres (6-rowed) has been included in 59% of SMB varieties, with the total germplasm contribution of 7,2%. The Dutch variety Emir can be found in 48% of the spring varieties and has the total germplasm contribution of 12,2%. Gerda and Ceres (2r) are in the genetic base of 30% and 26% of the Yugoslav SMB varieties, respectively. The SMB varieties also have a significant portion of English genotypes' germplasm: 7,7% from CGS 44-74, 5,8% from Georgie and 3,9% from Sundance.

Union is the variety used the most in the malting barley breeding program, comprising 17,18% of the total genetic background of Yugoslav WMB and SMB varieties taken together (Table 1). It is part of the genetic base of 43% of the varieties and was used more for spring than winter barley breeding. Emir was successfully used in breeding both WMB and SMB and is found in the genetic base of most (63,6%) of the varieties. The French genotypes Ager and Fr 33 are included in 50% and 25% of the varieties, respectively. Fr 33 comprises more than 50% of the genepool in 25% of Yugoslav malting barley varieties. Germplasm of Gerda and Ceres (2r) are in the genotype of 20% and 18% of the varieties, respectively (mostly the spring ones). Other varieties widely used in hybridization, such as CGS 44-74, Ceres (6r), Georgie, Sundance, Piruette and etc, were also included in the spring barley breeding program.

The most utilized genotypes in the Yugoslav malting barley breeding program originated from Germany, and they represent 31,95% genetic background of the Yugoslav malting barley varieties. Following these are the French ones with 26,84%, albeit their representatives were only two - Ager and Fr 33. The six Dutch genotypes shared 20,68%, the three English 10,22% and four Swedish 6,58% of the Yugoslav malting barley genepool. Participation of varieties from other countries is considerably lower. According to a variation found in a hordein banding patterns, 44 Yugoslav varieties were classified into five group. The genetic background had strong influence on the determination of similarities between the barley varieties according to hordein composition. Varieties with similar pedigrees and common ancestors showed similar hordein composition. The results obtained by SDS-PAGE are similar to those mentioned by Rainen et al. (1992).

Genetic variability is one of the most important prerequisites for successful breeding of each species (Borojević, 1983). Genotypes from different agroecological conditions of growing, as well as exotic germplasm (Vetelainen et al., 1995), enlarge genetic variability and possibilities for the selection of lines with desirable performances. The introduced genotypes from Europe, America and Asia were the foundations stones of Yugoslav malting barley breeding program (Pržulj et al., 1995a). Barley breeding in other countries has also been based on introduced genotypes. (Riggs et al., 1981; Jedel and Helm, 1994a,b). The breeding should maximize the use of highly adaptive genotypes with a good general combining ability, as was the case with the variety Union. Good crosses, should not

Tab.1 Percentage of foreign germplasm in the Yugoslav winter (w) and spring (s) malting barley varieties developed in Novi Sad, Kragujevac and Zaječar

Varieties	Novi Sad			Kragujevac			Zaječar			Total		
	w	s	Total	w	s	Total	w	s	Total	w	s	Total
Union	-	11.50	11.50	-	1.14	1.14	4.54	-	4.54	4.54	12.64	17.18
Emir	6.89	7.19	14.08	-	-	-	1.28	-	1.28	8.17	7.19	15.36
Ager	6.89	1.49	8.38	-	-	-	5.25	-	5.25	12.14	1.49	13.63
Fr 33	12.07	1.14	13.21	-	-	-	-	-	-	12.07	1.14	13.21
Gerda	-	-	-	0.57	6.25	6.82	-	-	-	0.57	6.25	6.82
Ceres (2r)	-	-	-	0.14	5.11	5.25	-	-	-	0.14	5.11	5.25
CGS 44-74	-	-	-	-	4.54	4.54	-	-	-	-	4.54	4.54
Ceres (6r)	-	4.24	4.24	-	-	-	-	-	-	-	4.24	4.24
Georgie	-	-	-	-	3.41	3.41	-	-	-	-	3.41	3.41
Sundance	-	2.27	2.27	-	-	-	-	-	-	-	2.27	2.27
Pirouette	-	-	-	-	2.27	2.27	-	-	-	-	2.27	2.27
Spartan	-	1.99	1.99	-	-	-	-	-	-	-	1.99	1.99
Gazelle	-	1.92	1.92	-	-	-	-	-	-	-	1.92	1.92
Sonja	1.14	-	1.14	-	-	-	0.28	-	0.28	1.42	-	1.42
Herta	-	1.42	1.42	-	-	-	-	-	-	-	1.42	1.42
Magnif. 102	-	1.14	1.14	-	-	-	-	-	-	-	1.14	1.14
Freja	-	0.91	0.91	-	-	-	-	-	-	-	0.91	0.91
Menuet	-	0.57	0.57	-	-	-	-	-	-	-	0.57	0.57
Dura	-	-	-	0.57	-	0.57	-	-	-	0.57	-	0.57
Una	-	-	-	0.57	-	0.57	-	-	-	0.57	-	0.57
Aramir	-	0.28	0.28	-	-	-	-	-	-	-	0.28	0.28
MG 58239	-	-	-	0.28	-	0.28	-	-	-	0.28	-	0.28
Maksi. goli	0.14	-	0.14	-	-	-	-	-	-	0.14	-	0.14
Engl. India	-	0.14	0.14	-	-	-	-	-	-	-	0.14	0.14
Euglen Dea	-	-	-	0.14	-	0.14	-	-	-	0.14	-	0.14
OSK 101-19	0.07	-	0.07	-	-	-	-	-	-	0.07	-	0.07
OAC-21	0.07	-	0.07	-	-	-	-	-	-	0.07	-	0.07
Akka	-	0.01	0.01	-	-	-	-	-	-	-	0.01	0.01

be overexploited, because the selected lines would be genetically too similar. In order for us to fully exploit the available germplasm, winter varieties should be used in the spring malting barley breeding programs and vice versa. Genotypes of spring habit with high yields and good biological adaptability, tolerant to lower temperatures, should be used in the breeding of WMB just as WMB genotypes should be used in SMB breeding (Pržulj et al., 1995b).

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Latvian barley genetic resources: conservation and characterization.

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Introduction. Regarding the Convention on Biological Diversity each country is responsible for conservation of their own plant genetic resources (PGR).

Before 1991, as in most soviet republics of the former USSR, there were no special PGR activities in Latvia. For the purpose of conservation of the Latvian PGR the collection of the N. Vavilov All-Union Institute (VIR) in St. Petersburg has been used. In Latvia there were only working collections for breeding, researching and education purposes at that time. Barley was kept in collections of Priekuli and Stende Plant Breeding Stations, of Latvian Agriculture University, and the Institute of Biology.

Since 1993 the Working Board for Agricultural Plant Genetic Resources was organized by the Latvian Society of Geneticists and Breeders. However there is no all-embracing national PGR programme in Latvia, PGR activities are supported now by the Latvian Council of Sciences. In the frame of the project for PGR of agricultural plants a network of institutions dealing with PGR is established (Rashal, 1994). All the institutions keeping the barley collections are included in the network. On the first stage the main objectives of the agricultural plants PGR project are considered to be establishing an extensive inventory of the origin and conditions of PGR existing in the Latvian collections and creating a computerized Latvian PGR data base.

Current situation with barley genetic resources conservation. A data base of Latvian PGR is created at the Plant Genetics Laboratory of the Institute of Biology (Rashal, 1995). The data base is designed in Microsoft FoxPro for MS-DOS 2.6 which provides high speed of data processing and standard data exchange format (dbf-file format). A special application with user friendly interface is also created. The application allows the users to modify easily, append and delete data, make queries, export data into text files etc.

The Latvian PGR data base contains data of more than 8000 accessions from 78 species stored in most of PGR collections in Latvia. About 3000 accessions have Latvian origin, among them cereals (173), forage (1526), potato (153), fruit and berries (952), and others. There are 1548 barley accessions in the Latvian PGR data base, 123 of them are of Latvian origin. All these accessions are kept until now in the working collections mentioned above, because at the moment there is not really a gene bank with long term storage facilities in Latvia. Such gene bank will be created in the nearest future in collaboration with Nordic Gene Bank (Alnarp, Sweden) due to Nordic-Baltic co-operation, supported by the Nordic Council of Ministers.

At the present only passport data are included in database (name of accession, country of origin, breeding institution, names of breeders, years of realization and registration, pedigrees, donor etc.). Evaluation and observation data will be added in the following steps.

Characterization of the Latvian barley genetic resources. All barley accessions of the Latvian origin belong to spring barley. Among them 18 are commercial varieties and others are advanced breeding lines, dihaploid lines and mutants.

Origin of the Latvian varieties. No Latvian landraces have been surveyed until now, but some of them are included in the pedigrees of three commercial varieties of Latvian origin. Analysis of pedigrees showed that practically all Latvian varieties were based on selections from Moravian and Sweden landraces. These landraces are two out of four which produced superior progenies in the first cycle of crossbreeding in Europe in the beginning of this century (Fischbeck, 1992). Among other two, Bavarian landraces were included as progenitors only in two varieties, but there are no Latvian barley varieties with England landraces in pedigree. In most pedigrees of the Latvian varieties Danish variety 'Maja' appeared which was developed in the second cycle of crossbreeding by using varieties originating from Moravia and Sweden. This variety was exclusively well adopted to Latvian environment and was grown commercially in Latvia for about 40 years. Among other important progenitors some early Finland varieties could be noted.

Resistance to powdery mildew. Powdery mildew caused by *Erysiphe graminis* f.sp. *hordei* is one of the most widely spread barley diseases in Latvia. 16 barley varieties released in different years in Latvian plant breeding stations as

Table 1

Powdery mildew resistance genes in barley varieties of Latvian origin

Variety	Year of registration	Detected resistance genes
Vairogs	1930	none
Kombainieris	1955	none
Priekulu 1	1959	none
Priekulu 60	1972*	none
Stendes	1972*	none
Abava	1980	Mlg
Ilga	1983*	u1u2
Agra	1984*	Mla9
Imula	1990	Mla9, MIk
Linga	1990	u1u2
Balga	1993	u1u2
Klinta	1993	MILa
Rasa	1993	Mla7, Mlg
Ruja	1993	Mlg
Gate	1995*	Mla7, Mlg
Sencis	1995*	Mla13, MI(Ru3),
MIg, MILa		

* Year of release

well as 79 advanced breeding lines were analyzed. The investigation was carried out in the Risø National Laboratory (Roskilde, Denmark) by analysis of resistance spectrum to 22 powdery mildew isolates (Tueryapina et all., 1996). The results were analyzed in conformity with the gene-for-gene relationships. The resistance genes of the analyzed genotypes were identified by comparing their reaction spectrums with those of differentials possessing the known genes. As far as there has been no special programme for disease resistant barley breeding in Latvia until now, the use of resistance genes in varieties has been uncontrolled and the selection of resistance genes was indirect.

The results of analysis of these 16 varieties are given in Table 1. For a long time Latvian barley varieties had no specific mildew resistance genes. Those appeared firstly only in the 1980-s. Among genes recognized in the tested barley varieties only *Mla13* could be classified as effective against the Latvian barley powdery mildew population. Genes *Mlg*, *Mla7* and *Mla9* are not fully effective. Unidentified resistance from line KM 1192, designated as *u1u2* (Jensen et al., 1992) was used in some varieties released after 1990. This resistance is of medium efficiency in Latvia now. The analysis of the advanced breeding lines showed them to have a wider set of resistance genes. In addition to those mentioned above they posses genes *Mla1*, *Mla3*, *Mla6*, *Mla12*, *Mla14*, and also some unknown resistance genes. *Mla1* and *Mla3* are also effective under Latvian conditions.

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Genetic diversity in barley. D.C. RASMUSSON, R.L. PHILLIPS and K.B. HELLEWELL, Agronomy and Plant Genetics, University of Minnesota, 411 Borlaug Hall, 1991 Buford Circle, St. Paul, MN, 55108 USA.

Introduction. The genetic gap between elite gene pools and unimproved germplasm is growing larger with each breeding cycle (Rasmusson, 1991). As the genetic gap has widened in recent decades, introgression of genetic diversity from unimproved stocks has become difficult and has most often been limited to genes for pest resistance. At the same time, the gene base in elite gene pools is narrowing. This narrowing will continue because new varieties are obtained from relatively few crosses or closely related series of crosses between improved parents with evermore favorable gene combinations (Gilmour, 1995).

The purposes of this paper are to describe genetic gains made in a narrow barley gene pool, to highlight the importance of genetic diversity, and to suggest that mechanisms may exist to provide a continuing source of genetic diversity in elite breeding populations.

Materials and Methods. Germplasm contributing to recent University of Minnesota barley varieties traces almost exclusively to post-1960 introductions from North Dakota and Brandon, Manitoba. These two centers, which emphasized six-row malting barley breeding, to some degree shared a common gene pool. This germplasm ultimately led to development and release in Minnesota of Manker in 1974 and Morex in 1978 (Figure 1). As indicated in the figure, the Manker by Morex cross gave rise to Robust. In the same crossing block, a full sister of Morex, M28, also was crossed to Manker giving rise to M72-146. Crossing Robust with M72-146, and later their progeny to Robust, led to Excel. Three-quarters of Stander germplasm (released in 1993) came from inside the Morex x Manker lineage.

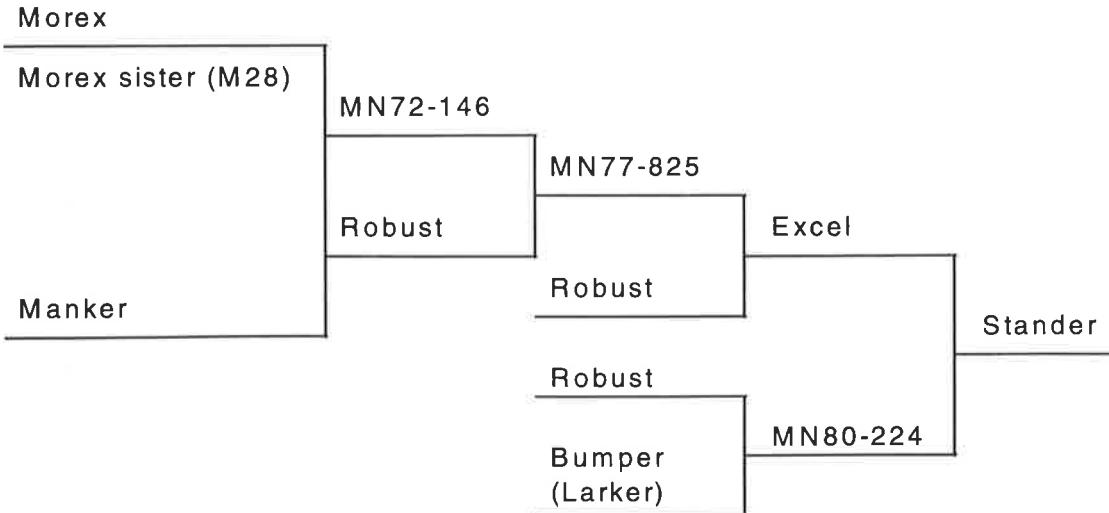


Figure 1. Pedigree of Minnesota malting barley cultivars (1972-1993)

Data from a regional nursery were utilized to assess performance for five traits of four cultivars--Larker, Morex, Robust, and Excel. Accordingly, the data are a measure of regional rather than local adaptation. Malting quality was evaluated using grain from selected locations throughout the region.

Results and Discussion. The barley cultivars released over the period 1978 to 1993 provided significant improvement for two agronomic and three malting quality traits (Table 1). Morex represented a breakthrough increase in malt extract of 2.2% and a 25% increase in alpha amylase compared to Larker, the cultivar Morex replaced. Robust exceeded Morex in grain yield by 10%, and had improved lodging resistance (Table 1). Excel exceeded its parent, Robust, in grain yield, malt extract and alpha amylase; and was lower in grain protein. The interesting point is that Excel is essentially a "Robust progeny" based on the pedigree relationships (Figure 1); even so, sizeable gains were made for several essential traits. Stander, the newest cultivar in the pedigree tree is similar to Excel for most traits but superior in resistance to lodging (data not shown).

Table 1. Mean performance of four cultivars for five traits in regional trials.

Trait	No. trials	Larker (1961) ¹	Morex (1978)	Robust (1983)	Excel (1990)	Increase (decrease) (%)
Extract (%)	31	76.4	78.6	78.6	79.2	3.7
Alpha-amylase (20° units)	31	34.8	43.4	33.2	42.3	21.6
Protein (%)	31	14.6	14.2	13.9	13.1	(10.3)
Grain yield (Mg ha ⁻¹)	57	3.8	3.8	4.2	4.5	18.4
Lodging (%)	11	--	31	16	17	(45)

¹ Cultivar release date.

We conclude that the genetic gains in Morex and Robust are typical of what is occurring in breeding programs in general, i.e., modest levels of genetic diversity based on pedigree information permit sizeable genetic gains. The gains in grain yield, malt extract, alpha amylase, and grain protein achieved with Excel and Stander are not easily explained. Based on the pedigree tree (Figure 1), Excel is essentially a progeny of Robust. Making a number of assumptions, the coefficient of parentage between Robust and M77-825 (the parents of Excel) is estimated to be 0.87. Stander is also of special interest because its resistance to lodging is superior to any of its parental genotypes including Bumper (Figure 1).

Concern about the genetic gap and the diminishing diversity in elite gene pools should lead to an examination of the concept that all of the genetic variability that is useful in selection existed in the original parents. Another hypothesis is that the genome is dynamic and that new genotypic and phenotypic variation arises each generation. A dynamic genome would account for the results obtained in the University of Illinois long-term selection experiment with oil and protein in maize (Dudley and Lambert, 1992) and

in breeding experiments with maize (Hallauer, 1981). In each case, genetic diversity exceeded expectation in later generations.

We hypothesize that original variation is augmented by *de novo* variation and that these combined sources of variation can lead to epistatic relationships that are substantially more important than previously recognized (Moreno, 1994). Support for the notion of elevated epistasis can be found in the literature. Doebley et al. (1995) analyzed two quantitative trait loci (QTL) controlling differences in plant and inflorescence architecture between maize and teosinte. When brought together in one genotype, the two QTLs substantially transformed both plant and inflorescence architecture. In studying pairs of QTLs in soybean, Lark et al. (1995) found that an allele at one locus that explained little or no height variation by itself had a large effect on height via interaction with other loci. Geiger (1988) concluded that long-term selection accumulates coadapted gene arrangements which can be disrupted in subsequent generations.

Conclusion. The barley example shows that good progress was made for several traits within a narrow germplasm. Based on this result and evidence from long-term selection experiments that variation is rarely exhausted, we suggest that the genome is dynamic and that new variation arises each generation. We further hypothesize that variation produced by *de novo* genetic processes, coupled with elevated epistasis, make a valuable contribution to selection progress.

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Pattern Recognition of Barley Seeds Using Fourier Descriptors and Neural Networks

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Recent advances in technology, coupled with the falling price of computers, has made machine vision a rapidly growing field, especially in areas in which minimal product handling and objectivity is desirable. This project focuses on the use of a machine vision system which takes the place of the eye of a human inspector. A pattern recognition algorithm takes the place of the human brain in making decisions based on the features captured by the automated vision system. The system is used in the variety separation of three types of barley seeds - CDC Guardian, Harrington, and TR118. It is also used in the grading of the quality of the barley.

Pattern Recognition

Pattern recognition is done by taking certain features from the subject and using them to classify the subject into the desired group. The machine vision system is responsible for acquiring the features, therefore the features must be visible to a camera. They should also be invariant to shape transformations such as scaling, changes in the origin point, translation, and rotation. In this work, Zahn and Roskies (ZR) Fourier descriptors act as the shape features.

This technique measures how different the shape of an object is from a circle. The descriptors are in the form of harmonic amplitudes and phase angles, and are easily derived from polygonal shapes.

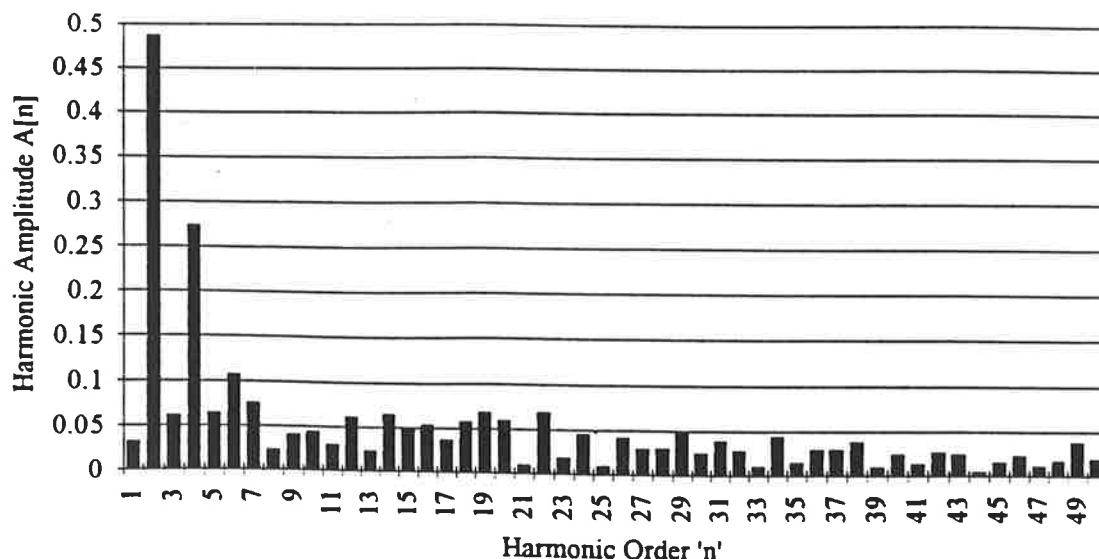


Figure 1: Typical ZR Fourier descriptor spectrum for barley (TR118).

To perform the actual pattern recognition, a neural network was used. This is a computer algorithm that is modeled after the human brain. The neuron is a mathematical model which takes in the inputs (in the form of features), multiplies them by a weighting factor, and adds them together. A neural network is simply a collection of neurons operating in parallel. The network performs pattern recognition by comparing its output obtained by a set of features to a desired output. The weights are then changed until the desired output is achieved.

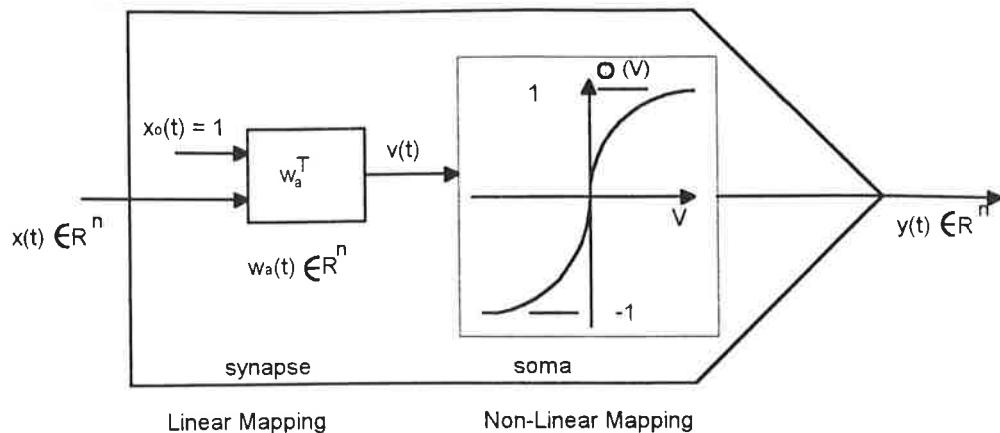


Figure 2: Single neuron structure.

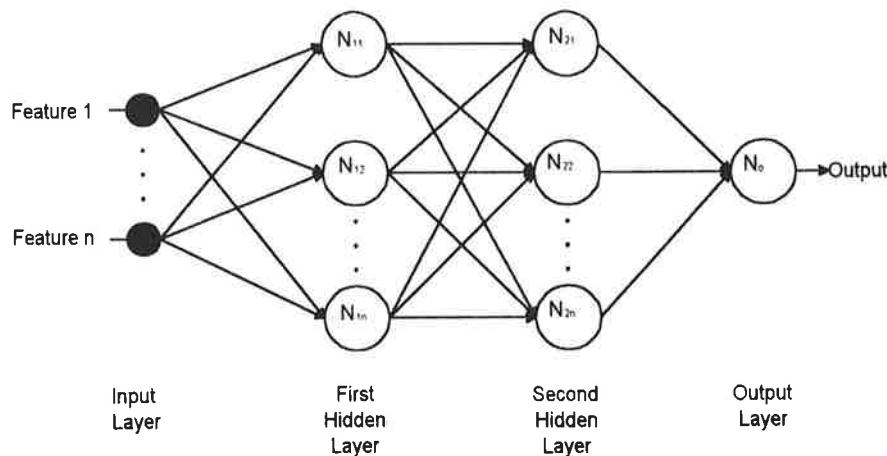


Figure 3: Neural network structure.

Machine Vision System

The system used to acquire the features of the barley is comprised of a Javelin video camera, a Data Translation Quickcapture image acquisition board, a WILD Heerburgg Stereomicroscope, and a Macintosh IIfx computer. Barley seeds were placed on the illuminating base plate of the microscope ten samples at a time. The ZR Fourier descriptors from the seed contours were acquired using image analysis software written by Maurice Romaniuk.

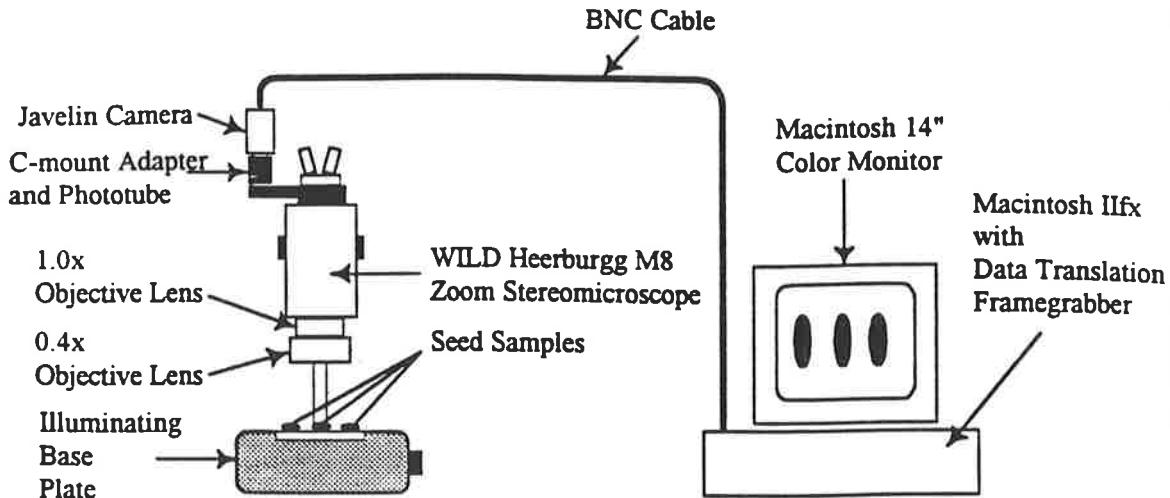


Figure 4: Machine vision setup for seed analysis.

Results

The first experiment done involved the discrimination of 3 varieties of barley. The network structure that produced the best recognition accuracy was one containing 20 inputs, 25 neurons in the two hidden layers, and a single output neuron. Eighty samples from each class was used in testing. A total recognition accuracy of 80.4% was achieved.

The second experiment involved using the machine vision system to perform a contour grading on the seed samples. "Good" contours were defined as having uniform shapes with little or no imperfections while "poor" contours are nonuniform with many imperfections. Six hundred seeds of TR118 barley was used in testing. A neural structure containing 50 inputs, 25 neurons in the two hidden layers, and a single output was used for recognition, with an accuracy of 87.0%.

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Diversity in Himalayan Hullless Barley. KRISHNA P. SHARMA,
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Introduction: Nepal, a country with diversified topography, has numerous microclimates and agro-ecological environments having varying production capabilities. Agro-environments vary greatly over short distances at all directions. Topographical diversification is further enriched by crop, cropping system and ethnicity diversification. Diversification of crops and ethnicity is more complex at higher altitudes compared to lower altitudes. Hullless barley (Hordeum vulgare L.) is one of the crops traditionally grown since centuries at hill, mountain and Himalayan regions of Nepal. This crop is grown for multipurpose uses depending upon ethnicity covering altitudes ranging from 800m to 4500m above sea level.

Hullless barley has been found to be cultivated in all types of production environments from the East to the West of Nepal. Adaptational diversity of this crop at diversified agro-ecological environments suggests its genetic diversity. Barley probably developed to be a distinct identity in the form of six row hullless barley in the Himalayan regions (Vavilov, 1925). Takahashi et. al., 1983 suggested Nepal as the probable centre of diversity of hullless barley. Konishi (1986) evaluated covered and hullless barley from eastern Nepal and reported large diversity. Hullless barley cultivated throughout Nepal covering all agro-ecological zones has not been collected and diversity studied. Therefore, this study was conducted to evaluate comparative diversity of cultivated hullless barley from all over Nepal.

Materials and Methods: Twenty eight hill and mountain districts of Nepal were surveyed and 400 samples of hullless barley were collected from farmers fields during 1990-1991. These samples were grown during 1991-92 cropping season at mid-hill (1300m) near Kathmandu. Field was fertilized with 30:30:20 Kg NPK /ha. Data were recorded on spike row type, spike length, awn type, ear density, grains/spike and grain colour. Diversity was studied based on the morphological differences of spike using the procedure of Takeo Konishi (1986) and collections were classified into botanical varieties such as: Trifurcatum (T), Himalayanse (H), Coelasta (C), Revelatum (R), Philemense (P), Nudinippionicum (n), Nudimontanum (N) and Boteanum (B).

Germplasm not covered by this classification were put as unidentified (UI) symbolized as 2= short awned; 3= 6 row elevated; 4= 2 hooded + 4 awnless rows; 5= 6 rowed dense ear blue grain; 6= 2 elevated hooded + 4 awnless rows; 7= 2 elevated hooded + 4 awned rows; 8= 2 awned + 4 awnless rows; 9= 2 hooded + 4 elevated hooded rows; 10= 4 elevated hooded + 2 awned rows; 11= 2 hooded + 4 awned rows; 12= 4 short + 2 medium awned rows; 13 = 4 hooded + 2 awned rows; 14= 2 hooded + 4 awnless in bozu; 15= 2 elevated hooded + 4 awnless in bozu.

Results and Discussion: Study of germplasm collected from various agro-ecological pockets of Nepal indicated large diversity of hullless barley (Table 1). Degree of diversity and occurrence of

different botanical types in farmers field varied from one region to another. Mid eastern and western regions are found rich in *Trifurcatum* whereas *Coelasta* is dominant at far western region. Himalayanse occurred more frequently all over Nepal than *Revelatum* which was more frequent at eastern compared to the western region. *Philemense*, *Nudimontanum*, *Nudinippionicum* and *Boeteanum* occurred less frequently. In addition to eight botanical types identified, ten more types were found that could not be identified into any existing botanical classification.

Table 1. Botanical diversity of hullless barley at different hill and mountain district of Nepal as evidenced from germplasm grown at mid-hill during 1991-92.

Dist.	No	Botanical varieties and percent occurrence								
		Acc.	T	H	C	R	P	n	N	B
Ilm	17	14	34	36	14	-	-	1	-	2/4/5/8
Thm	4	-	42	20	38	-	-	-	-	-
Tlg	5	-	-	-	-	-	-	-	-	8
Sks	20	92	6	-	<1	-	-	<1	-	4/6/7/8/11
Bjp	20	65	11	10	8	6	-	-	-	2/6/8
Ktg	15	64	12	13	11	-	<1	-	-	4/5/6/7/8/11
Sol	40	35	25	13	19	<1	7	-	-	2/4 - 12
Dok	3	43	24	5	24	-	-	5	-	4/5/6/7
Rmc	23	59	6	10	17	6	-	<1	-	2/4/5/6/7/8/11
Rsu	9	7	28	48	5	-	-	11	-	2/4/5/7
Grk	19	21	49	27	2	-	-	-	-	2/5/6/11/12
Mng	13	10	41	38	9	-	3	-	-	-
Mgd	18	74	3	23	-	-	-	-	-	2/8/9/11
Blg	23	37	19	38	5	-	-	-	<1	2/6/7/8/11
Rlp	16	59	5	36	-	-	-	-	-	2
Rkm	16	54	25	21	-	-	-	-	-	2/13
Jrk	16	43	16	41	-	-	-	-	-	2/13
Dlp	8	-	48	45	-	-	-	8	-	2/5
Jml	11	-	25	72	<1	-	3	-	-	2
Mug	9	13	9	68	1	-	-	10	-	2
Bjr	13	65	10	22	3	-	-	-	-	2/4/8/11
Bjg	20	-	59	38	3	-	-	-	-	2/5
Btd	25	2	12	85	<1	-	-	-	-	2
Drc	10	19	29	50	1	-	-	-	-	2/8
Dot	17	-	-	98	<1	-	-	-	-	2
Dlh	19	-	10	43	47	-	-	-	-	2

Ilm= Ilam; Thm= Tehrathum; Tlg= Taplejung; Sks= Sankhuwasabha; Bjp= Bhojpur; Ktg= Khotang; Sol= Solukhumbu; Dok= Dolakha; Rmc= Ramechap; Rsu= Rasuwa; Grk= Gorkha; Mng= Manang; Mgd= Myagdi; Blg= Baglung; Rlp= Rolpa; Rkm= Rukum; Jrk= Jajarkot; Dlp= Dolpa; Jml= Jumla; Mug= Muju; Bjr= Bajura; Btd= Baitadi; Drc= Darchula; Dot= Doti; Dlh= Dadeldhura.

Of the 400 accessions, majority of them exhibited mixture of two to four botanical types (Table 2). Botanical diversity among and within accessions was larger at mid eastern region. Some accessions from Solukhumbu and Ramechhap districts contained 7-9 different types indicating within accession variation at eastern region was greater than among accessions. Hullless barley

cultivated at far-western regions exhibited less diversity within collection. Results of this study supports the earlier reports (Nakao, 1956; Witcomb, 1975; and Witcomb and Gilani, 1979) that genetic diversity of cultivated Himalayan hullless barley is preserved in the population without artificial selection. Results further supports Nepal as probable centre of diversity of this crop.

Table 2. Comparative presence of different botanical types within accession of hullless barley of Nepal.

Dist.	Acc.	No. of botanical types								
		1	2	3	4	5	6	7	9	
Ilm	17	2	2	7	5	1	-	-	-	
Thm	4	-	-	4	-	-	-	-	-	
Tlg	1	1	-	-	-	-	-	-	-	
Sks	20	-	5	6	5	3	-	1		
Bjp	20	2	3	12	2	1	-	-		
Ktg	15	1	1	7	3	3	-	-		
Sol	37	1	3	5	13	8	5	2		
Dok	3	-	-	1	-	1	1	-		
Rmc	23	2	3	3	3	5	5	1	1	
Rsu	9	1	1	3	2	1	1	-		
Grk	16	5	2	7	1	1	-	-		
Mng	13	2	6	4	1	-	-	-		
Mgd	18	2	7	8	1	-	-	-		
Blg	23	3	2	11	5	2	-	-		
Rlp	16	9	7	-	-	-	-	-		
Rkm	17	-	2	12	2	1	-	-		
Jrk	16	-	2	12	1	1	-	-		
Dlp	8	2	2	3	1	-	-	-		
Jml	11	2	3	3	3	-	-	-		
Mug	9	-	4	3	2	-	-	-		
Bjr	13	-	4	5	2	2	-	-		
Bjg	20	-	3	13	4	-	-	-		
Btd	25	-	18	6	1	-	-	-		
Drc	10	-	-	1	7	2	-	-		
Dot	17	-	17	-	-	-	-	-		
Dlh	19	3	1	14	1	-	-	-		

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RFLP DIVERSITY OF BARLEY IN RUSSIA AND ITS RELATIONSHIPS WITH EUROPEAN CULTIVARS.

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INTRODUCTION

Russia occupies considerable parts of Europe and Asia with many different agroecological regions. Taking into consideration the long cultivation time a high level of barley genetic diversity in Russia may be proposed. Formerly the Russian barley breeding pool was based largely on local landraces. In the last decades additional barley germplasm from different countries, especially from Europe and Canada, have been used for producing modern cultivars. The most representative germplasm collection of Russian barley, which includes some thousand accessions gathered during our century in different regions of Russia and neighbouring countries, is preserved in the VIR. Landraces represent a large part of this collection and may be valuable starting material for barley improvement. However, the insufficiency of the pedigree information about landraces makes difficult their registration and their inclusion into breeding programs. At present, the usefulness of molecular markers such as RFLPs for evaluation of the genetic structure of barley germplasm has been shown (Graner et al., 1994). These markers are inherited independently from the phenotype and, if representative for the entire genome, can provide a comprehensive survey of the genetic variation present in a sample of cultivars. Furthermore, in the Munich Mapping Program more than 400 RFLPs have been mapped in barley, covering the whole genome. The objectives of this study were to (i) assess RFLP patterns for identification of Russian barley accessions and (ii) estimate the genetic relationship both between Russian and some of the European cultivars and landraces based on the RFLP patterns.

MATERIALS AND METHODS

Two groups of accessions were used in this study. The first group contained 82 cultivars and landraces, which were selected from the VIR germplasm collection to represent the wide geographic diversity present in Russia and other countries of the former USSR. There were 39 two-rowed and 43 six-rowed, 49 accessions were landraces or cultivars derived by selection from landraces. The second group contained 11 well known European spring and winter cultivars from different germplasm groups. RFLP analysis methods have been described previously (Graner et al., 1990). In total, 41 previously mapped, largely single-copy clones of barley DNA from the Munich Mapping Program with 5-6 probes per chromosome were used. 68 clone-enzyme (*Hind*III and *Eco*RI) combinations with 335 polymorphic fragments were used for analysis. The program NTSYS-pc v. 1.8 (Rohlf, 1993) was used to generate the distance matrix (Nei, 1972) and to perform the UPGMA clustering.

RESULTS AND DISCUSSION

As revealed by cluster analysis (Fig. 1) 93 accessions - besides the distant landrace 10628 which originated from Tadzhikistan - separated into two major clusters. Cluster A largely

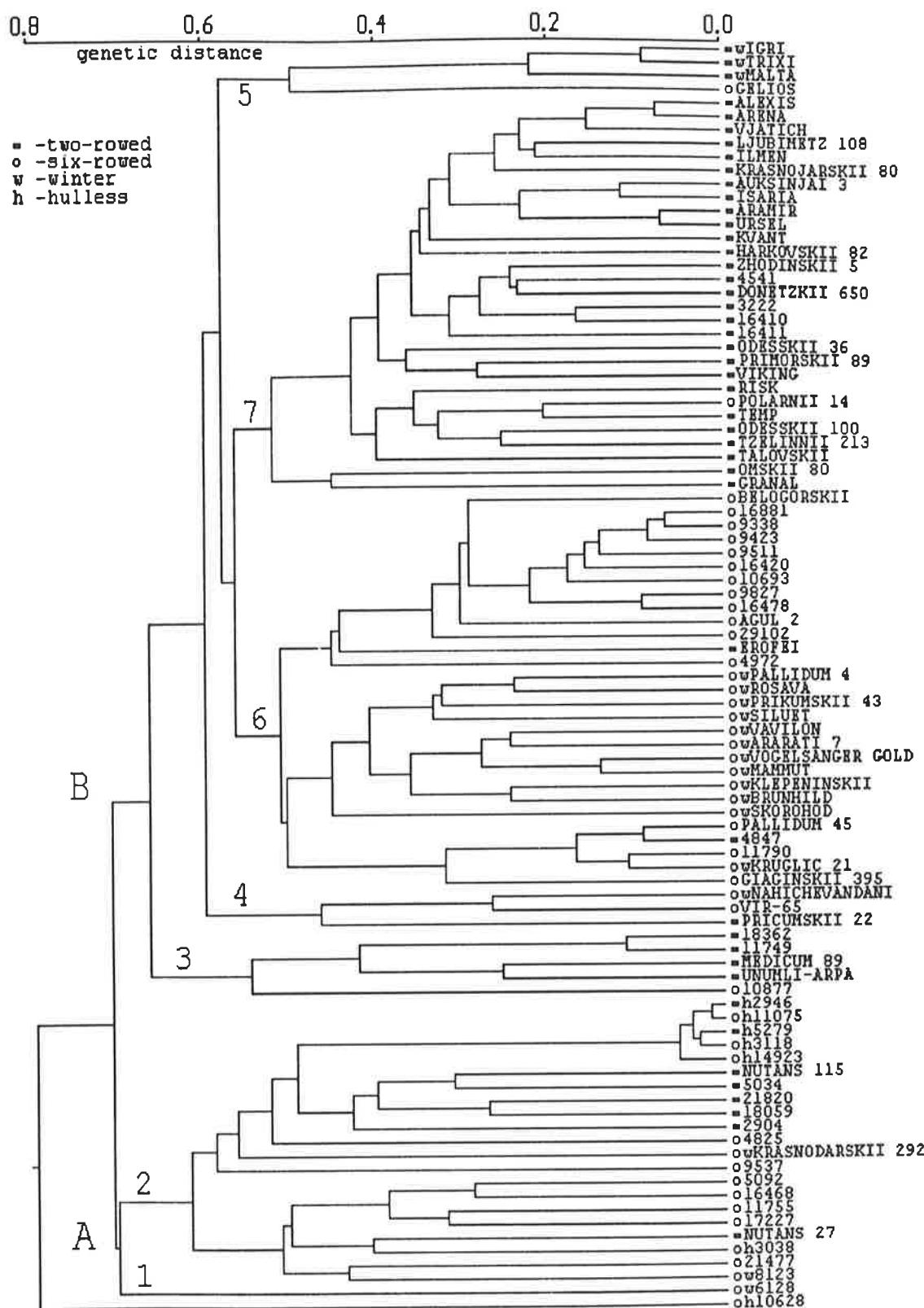


Figure 1: Dendrogram constructed from RFLP-based genetic distances of 93 Russian and European barley accessions.

comprises landraces originating from Central Asia, Siberia and Caucasian regions. This cluster includes both two-rowed and six-rowed accessions and also all of the hulless forms. Besides the distant landrace 6128 which originated from Turkmenistan there are two major sub-clusters: one (1) geographically linked with Central Asia and another (2) is more widespread. Cluster B is more complex. Sub-clusters 6 and 7 are most spacious and divided largely on the base of spike morphology. Sub-cluster 7 consists of two-rowed both European spring cultivars (Alexis, Arena, Isaria, Aramir and Ursel) and spring cultivars and landraces from different regions of Russia. The pedigree information confirms, that some of the West European, East European and Canadian cultivars (Trumpf, Ingrid, Isaria, Emir, Masurka, Chenad, Diamant, Gatway, Keystone and others) were used in breeding of these Russian cultivars. Subcluster 6 consists mainly of six-rowed accessions. It includes a group of spring landraces related to cultivars Belogorskii, Agul2 and Erofei. These were derived from Russian cultivars that carry the cultivar Keystone in their pedigree. The next group includes both European winter cultivars Vogelsanger Gold, Mammut, Brunhild and related to them some of the Russian winter and spring cultivars and landraces. Subcluster 5 comprises the European two-rowed winter cultivars Igri, Trixi and Malta, which have been bred from different germplasm. This group includes the Russian cultivar Gelios, which is related to cultivar Emir. Finally, subclusters 3 and 4 include landraces and some of the cultivars derived by selection from landraces.

Thus, results of RFLP analysis confirm the existence of a high level of genetic diversity present in Russian barley. Together with the West European cultivars, the majority of Russian cultivars form a large, though heterogenous germplasm group (cluster B). An additional, unique germplasm group could be identified in this study (cluster A). This cluster includes a group of landraces originating from diverse regions of Asia and may be connected to the Central Asian center of barley diversity (Trofimovskaya, 1972). Due to its distinctness it might represent a valuable source to increase the variability of the European barley germplasm.

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A diversity analysis of a sample of the Uruguayan National Barley Collection at the phenotypic and molecular level. C. TOJO, F. CAPDEVILLE, S. GERMAN, S. PEREYRA, A. BERRETTA, C. SYLVEIRA and M. BALBI. INIA La Estanzuela, cc 39173, Colonia, 70000, Uruguay.

Introduction. A sample of 244 accessions of the Uruguayan National Barley (*Hordeum vulgare*) Collection was characterized in order to obtain information about the available variability of the germplasm to be used in breeding programs. Descriptive analysis (means and ranks), bivariate analysis (rank correlations) and multivariate analysis (cluster) (Balbi et al., 1994; Castro et al., 1994) were used to determine associations between traits and to characterize the variability.

In this paper we focused on the comparison of two kinds of descriptors: phenotypic traits and analysis of RAPD loci frequencies on a sample of 39 accessions.

Materials and Methods. Thirty nine accessions of the collection were characterized (Table 1).

Table 1. Evaluated genotypes and their origin.

Origin	Genotypes
Argentine:	Maltería 150, Bonita, Magnif 102, Ana
Australia:	Clipper, E. Quebracho, Stirling, Prior, Weeah
Brazil:	Agraria 1, MN 599, MN 636, PFC 86125
Canada:	Bonanza, Argyle
France:	Beka, Volga
Germany:	Triumph, B.Volla, Union, Alexis, Aphrodite, Defra
Holland:	Prisma, Vada
Sweden:	Ariel, Sybilla
Uruguay:	Cervecería La Estanzuela, LEC 2034, Cervecería 71A, FNC I 22, FNC 6 1, CLE 144, CLE 157, CLE 167
USA:	Bowman and LCI 259 (N. Dakota), Morex and Robust (Minnesota)

Phenotypic traits scored at INIA La Estanzuela, Uruguay, were: growth habit, days from emergence to heading, plant height, peduncle length, lodging, leaf rust (caused by *Puccinia hordei*), net blotch (caused by *Drechslera teres*) and spot blotch (caused by *Bipolaris sorokiniana*) infection, yield, yield components, harvest index, grading, grain protein content, kernel shape and husk wrinkling. Characterization of the genomic diversity at the molecular level was done using DNA amplification techniques at Biotechnology Unit (INIA Las Brujas). DNA extracted by modified CTAB method from bulk seedlings of each accession was amplified using arbitrary

sequence oligonucleotides (10mer primers) (Williams et al., 1990).

Cluster analysis (UPGMA) of genotypes was based on estimations of similarity from agronomic traits (Average Taxonomic Distance, Crisci, 1983) and RAPD data matrixes, using Jaccard coefficient for molecular dataset. RAPD loci frequencies were calculated for each group previously defined based on the phenotypic traits cluster analysis and statistically compared.

Results and discussion. Six groups of genotypes were determined from the cluster analysis based on phenotypical characteristics (Figure 1a, gr1-gr6). Five of six 6-row genotypes were clustered in group 6, with the maximum taxonomic distance from the others. Group 3 comprised mostly Australian genotypes and group 4 included only European materials. Two-row Uruguayan genotypes are scattered in groups 1, 2 and 3, indicating that they are phenotypically diverse in our conditions.

Grouping based on RAPD data (Figure 1b) showed that Uruguayan - Uru- accessions had the narrowest genomic diversity, meanwhile German -Ger- and Brazilian -Bra- accessions had widespread distribution in the dendrogram, suggesting a larger genetic base for these genotypes. In many cases genotypes that were grouped had common ancestors.

Some genotypes from the same breeding program or sharing some common ancestor were both phenotypically and genetically similar, as the case of Argyle-Bonanza from Canada, CLE 167-CLE 144 from Uruguay and others. However, analysis based on phenotypic and RAPD information were not completely congruent. Some phenotypically different accessions were genetically similar, as FNCI 22 and CLE 167 while other phenotypically similar genotypes were different at the genomic level: FNC I 22 and Agraria. These results are in agreement with Cross et al., (1992) who found that morphological-agronomical descriptors do not necessarily agree with genomic analysis in estimations of variation when accessions of very different origins are examined.

Groups based on phenotypic traits also had significantly different mean band frequency per primer, except for OPC06 (Figure 2). This suggests that the phenotypic grouping also had a genomic basis.

Results indicate that genomic analysis may be an important complement to phenotypic analysis to determine diversity among barley genotypes.

Acknowledgements. Partial support from the "Mesa Nacional de Entidades de Cebada Cervecería" is gratefully acknowledged.

Figure 1a.

similarity between accessions based on 19 agronomic traits (distance coeff.)

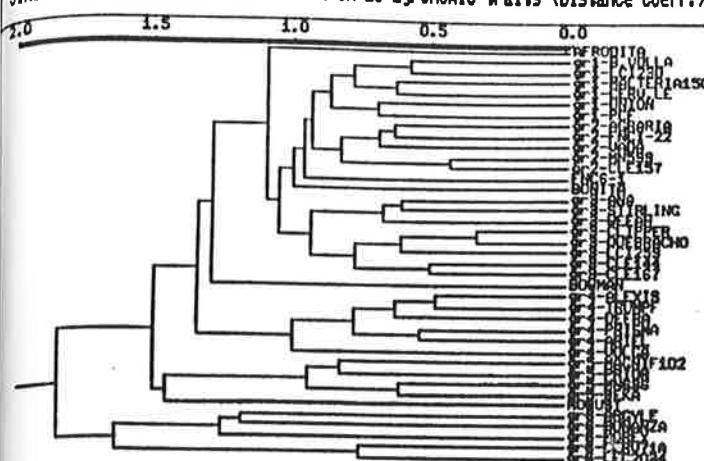


Figure 1b.

RAPD-based similarity between accessions (Jaccard's coefficient)

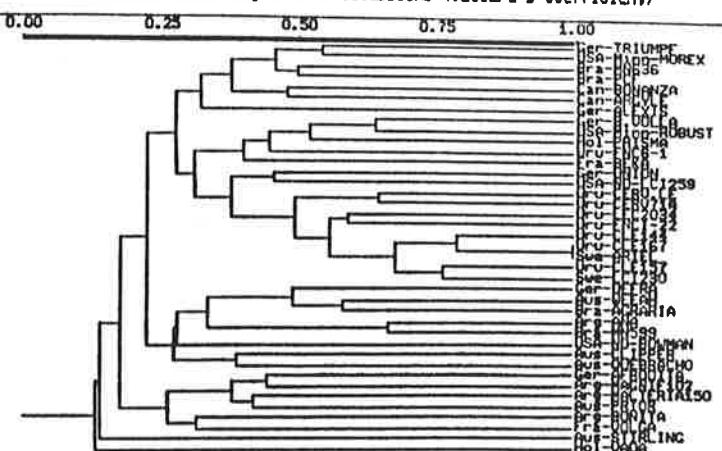
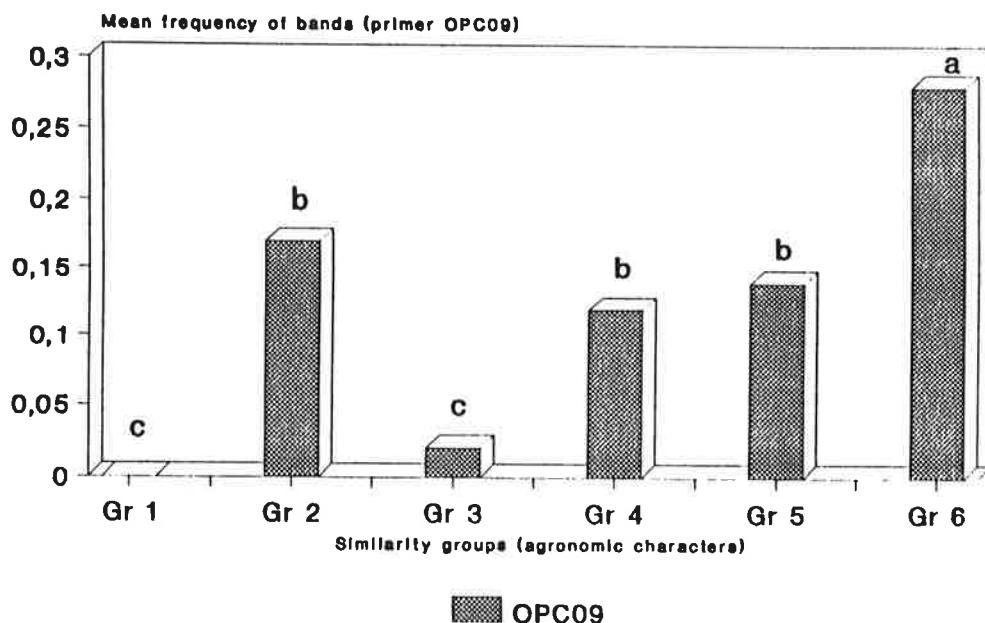


Figure 2. Comparison of RAPD-frequencies between 6 previously defined groups



Means which are not significantly different are followed by the same letter.

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Williams J., Kubelik A, Livak A., Rafalski A. and Tingey S. 1990. In Nucleic Acids Res. 18: 6531-6535.

Enhancement and Characterization of Hulless Oat Germplasm. D.M. WESENBERG¹, D.E. BURRUP¹, D.M. PETERSON², H.G. MARSHALL³, AND H.E. BOCKELMAN¹, USDA-ARS, National Small Grains Germplasm Research Facility, P.O. Box 307, Aberdeen, ID 83210, USA¹; USDA-ARS, Cereal Crops Research Unit, 501 N. Walnut St., Madison, WI 53705, USA²; and Marshall Farm, 1422 E College Avenue, Bellefonte, PA 16823, USA³.

INTRODUCTION. A renewed or expanded interest in hulless oats in North America, recently spearheaded by Dr. Harold Marshall and Dr. Vern Burrows, has lead to the development of a number of new hulless oat varieties in recent years. Factors of importance relative to hulless oat improvement include yield, expression of the hulless character, lodging resistance, shattering resistance, reduced height, "itch factor", germ damage, handling characteristics, and disease resistance. Potential benefits of hulless oats include increased feed value, reduced shipping and storage costs, value added products, and specialty food markets.

MATERIALS and METHODS. The hulless oat enhancement program at Aberdeen, Idaho traces to crosses with JJP 330A made by the senior author as a graduate student at the University of Wisconsin-Madison. The parent JJP 330A was a selection made by Joseph J. Pavek from a cross of CI 3030/CI 2641. A selection from 'Lodi'/JJP 330A, 69Ab1011, was crossed with 'Corbit' at Aberdeen, Idaho resulting in the development of selection 79Ab3811. The selection 86Ab1616, recently approved for release by the University of Idaho Foundation Seed Stocks Committee, was developed at Aberdeen from a cross of 79Ab3811/S7884. The parent S7884 was developed by the University of Saskatchewan at Saskatoon, Saskatchewan and kindly shared by Brian Rossnagel. The selection 86Ab1616 has been evaluated in replicated trials in southern Idaho since 1988. It has also been tested in the Cooperative Naked Oat Test coordinated by H.G. Marshall and more recently it has been tested in the regional Uniform Northwestern States Oat Nursery. Another soon to be released hulless oat selection, 88Ab3073, originated at Aberdeen from a cross of 'Pennlo'/PI 447276. The parent Pennlo was developed by ARS personnel at Pennsylvania State University, University Park, Pennsylvania. The parent PI 447276, equivalent to 'Yung 492', originated in Inner Mongolia and was introduced to the U.S. from the Peoples Republic of China in 1980. The cross of Pennlo/PI 447276 was made by ARS personnel at Pennsylvania State University. Selections were made at Aberdeen, Idaho from early generation seed shared with ARS personnel at the Aberdeen Research and Extension Center. The selection 88Ab3073 has been tested in replicated trials in Idaho since 1990, with testing subsequently expanded to dryland or nonirrigated sites in 1992. It is currently being tested along with 86Ab1616 over a wide range of environments in both the Uniform Northwestern States Oat Nursery and the Cooperative Naked Oat Test.

The program at Aberdeen has benefited from germplasm exchanges and close collaboration with a number of programs concerned with hulless oat improvement, including Marshall Farm, Bellefonte, Pennsylvania; Agriculture and Agri-Food Canada, Ottawa, Ontario; Agriculture and Agri-Food Canada, Winnipeg, Manitoba; Coker's Pedigreed Seed Company, Hartsville, South Carolina; University of Illinois, Urbana, Illinois; North Dakota State University, Fargo, North Dakota; Ohio State University, Wooster, Ohio; Northwest Plant Breeding Co., Pullman, Washington; University of Idaho Tetonia Research and Extension Center, Tetonia, Idaho; Resource Seeds, Inc., Gilroy & Zamora, California, and G.G.C. Western Agro Group Ltd., Kelsey, Alberta.

In addition to the hulless oat enhancement program, the USDA-ARS National Small Grains Collection (NSGC) at Aberdeen maintains 215 hulless oat accessions. The first hulless oat selection in the NSGC was CI 16, which was obtained about 1895. The most recent accession, 'Rhiannon' (PI 592088), entered the NSGC in 1995. Eighteen countries have contributed hulless oat germplasm to the NSGC, with the greatest number of accessions originating in Germany, the United States, China, the United Kingdom, Canada, and Poland.

RESULTS and DISCUSSION. Hulless oat varieties or selections vary significantly in the expression of the hulless trait, ranging from 60% or lower to occasionally 100% at Aberdeen. Additionally, unspecified environmental factors also influence expression of the trait as evidenced by year to year variation in expression of the trait, especially in varieties with intermediate levels of trait expression. The variety 'Hill' has the best record at Aberdeen for expression of the hulless traits. (See Table 1.)

Table 1. Environmental influence on the expression of the hulless trait.

Entry	% Hulless (Cleaned)						Average 1993-95
	1990	1991	1992	1993	1994	1995	
AC Baton*	93	97.8	99.3	96.9	98.3	95.4	96.9
AC Belmont	--	----	----	----	----	82.6	----
Hill	96	99.3	100.0	----	----	----	----
Lotta	--	99.0	97.5	73.0	94.1	----	----
Paul	--	97.3	98.8	96.0	96.1	93.3	95.1
Pennuda*	71	75.3	90.3	68.4	81.2	70.6	73.4
Terra	54	42.8	----	----	----	----	----
Tibor*	94	85.3	90.6	79.4	86.2	90.9	85.5
MF 9018-11801	--	----	----	97.5	98.1	95.8	97.1
MF 9016-26	--	----	----	----	97.6	95.9	----
MF 9116-31	--	----	----	----	89.5	94.5	----
MF 9016-148	--	----	----	----	91.7	90.6	----
NO 51-1	--	----	----	----	98.4	97.7	----
79Ab3811	80	81.8	88.9	----	----	----	----
86Ab1616*	88	86.5	97.1	92.7	93.4	88.5	91.5
87Ab5928	--	98.0	99.2	95.3	94.4	93.0	94.2
87Ab5932*	96	96.0	99.5	97.0	98.3	96.6	97.3
88Ab3073*	96	95.8	99.5	96.7	97.6	96.4	96.9
89Ab1908	--	----	----	97.1	95.5	92.5	95.0
89Ab2861	--	----	----	96.8	96.3	95.0	96.0
90Ab1416	--	----	----	95.6	95.5	96.0	95.7
90Ab1500	--	----	----	98.5	98.5	95.1	97.4
Average*	90	89.5	96.1	88.5	92.5	89.7	

* Average of six entries (*) grown each of six years, 1990-95.

Agronomic and quality data for a number of hulless oat varieties and selections grown under irrigation at the Aberdeen Research and Extension Center or on dryland at the Tetonia Research and Extension Center are summarized in Tables 2 and 3.

Table 2. Agronomic data for selected spring hulless oat varieties and selections grown under irrigation at Aberdeen, Idaho, 1993-95.

Entry	Yield (bu/A)	Groat Yield* (lbs/A)	Test Weight (lbs/bu)	Height (in)	Percent Hulless (%)	Beta- Glucan (%)	Protein (%)
No. Years	3	3	3	3	3	2	2
88Ab3073	161.5	5120	48.3	41	96.9	5.4	19.1
86Ab1616	193.4	6031	46.0	47	91.5	3.6	17.6
90Ab1416	186.5	5891	47.2	41	95.7	3.9	16.8
89Ab2861	200.9	6352	43.6	41	96.0	4.0	17.7
90Ab1500	179.2	5689	47.4	41	97.4	4.8	17.5
Pennuda	129.8	3822	44.7	36	73.4	4.8	21.0
Ogle	227.4	5378	41.8	42	----	4.9	19.2
MF 9018-11801	161.2	5114	51.5	43	97.1	4.9	20.5
Paul	184.1	5805	47.2	49	95.1	4.4	19.1
87Ab5928	194.8	6125	43.3	44	94.2	4.6	18.5
89Ab1908	174.3	5494	44.4	41	95.0	3.6	17.9
Tibor	156.8	4799	44.7	50	85.5	3.9	20.1
Ajay+	228.3	5289	42.2	36	----	4.5	19.0
Monida+	245.2	5759	43.3	46	----	4.4	17.9
Otana+	215.9	5168	42.9	49	----	4.9	18.9

* Assumes 70.0% groat content for hulled fraction of hulless entries.

+ Hulled Variety.

Table 3. Agronomic data for selected spring oat varieties and selections grown on dryland at Tetonia, Idaho, 1994-95.

Entry	Yield (bu/A)	Test Weight (lbs/bu)	Kernel Weight (g/1000)	Heading Date (Julian)	Beta- Glucan (%)	Protein (%)
No. Years	2	2	2	2	1	1
Monida	69.2	41.1	32.8	203	5.6	19.5
Paul*	41.5	48.0	25.4	203	4.4	21.7
86Ab1616*	53.8	47.3	24.4	204	5.5	19.5
88Ab3073*	42.4	48.2	19.6	203	5.5	22.9

* Hulless oat.

Barley landrace cultivars: Source of drought tolerance and disease resistance in semi-arid regions. A.H. YAHYAOUI, Y. UMEHARA, and S. REZGUI, IRESA, Ministry of Ag., Tunisia.

Evaluation of the genetic diversity within a crop is important for its effective utilization by a breeding program. Local varieties (landrace cultivars) can be an important gene pool for breeding more highly adapted varieties, particularly in harsh environments. In Tunisia, semi-arid regions (SAR) cover large parts of the northwest, the center and the south. Total area located in the SAR currently under cereal production represents 71% of the country's total production area. In these regions barley is the most adapted crop and covers about 40% of the cereal growing area in the SAR. The cultivated barleys grown in the center and south are almost exclusively local cultivars which have become, with time, adapted to local growing conditions. The productivity of these landrace cultivars is currently very low, hence, selection for improved agronomic types with consequently higher and more stable yields is needed in order to improve barley production in these areas. Another serious limitation observed when increasing yield under the harsh environment of the SAR is the narrow germplasm base of the high yielding varieties. For the past forty years in Tunisia, only six known barley varieties have been grown commercially - Martin, Ceres, Rihane, Tej, Feiz, Roho. During the past two years only one variety (Rihane) has been commercialized, the remaining are no longer cultivated. About 40% of the barley growing areas in central and southern parts of the country is occupied by landrace cultivars. Farmers in these areas are compelled to keep their landrace varieties because of the useful qualities their cultivars possess and for their yield stability. In some environments new varieties cannot compete with the older ones. We cannot enumerate here all the positive and negative qualities that the landrace cultivars possess, but field experiment results encourage us to further collect land varieties and to support their continued use. A base collection consisting of 309 accessions of 6-row cultivated barleys (*Hordeum vulgare*) was sampled from farmers' fields in the SAR, the oasis, and parts of the coastal region. The barley collection was used for two purposes; (1) to study the diversity in the 6-row barleys encountered in the SAR, (2) to provide breeders with material already adapted to dry land conditions that could be used as a source for drought tolerance and disease resistance. The collection was evaluated for agronomic characters that are sought by farmers and for disease resistance. A few accessions were tested for yield in comparison to commercial varieties. The collection sites can be grouped into six climatic zones (Fig 1b): (1) cold, dry, Mediterranean (Tej), (2) hot, dry, Mediterranean (SBZ), (3) cold steppe (Kas), (4) moderate semi-desert (Gaf), (5) intense desert (Sth), and (6) coastal region (Eco). Among the agronomic traits, the heading period of the barley accessions tested was highly variable with heading dates ranging from 136 to 169

days (Fig 1a). The number of days to heading was recorded as the number of days from the date of planting (in Nov.) until heading (in March-April). Data was collected over a four year time period. The commercial cultivars, Tej, Rihane, and Martin recorded 150, 154, and 160 days to heading. The introduced varieties were either early (Beecher, 136 days) or late (Arig8, 170 days). The heading period varied depending on the geographical zone. The average days to heading (Fig 1b) in zone 5 was the shortest with about 70% of the accessions heading in less than 146 days whereas barley collections from zone 1 were the latest with about 68% heading after 156 days. Barley accessions from the cold steppe region (zone 3) have the narrowest range of heading dates (57% of the accessions heading in 156 to 158 days). The barleys from zone 2 have the widest range varying from 144 to 164 days. The variability in heading among the land race accessions shows the multiline character that allows them to respond to natural selection and farmers preferences in very different places. This genetic variability provides an insurance against possible hazards in a plant's environment (Harlan, 1975).

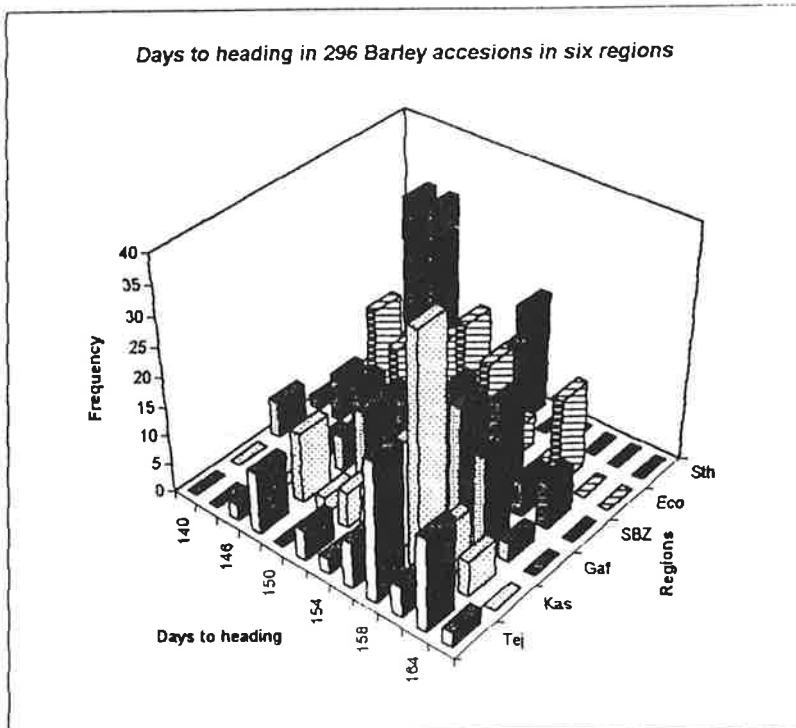
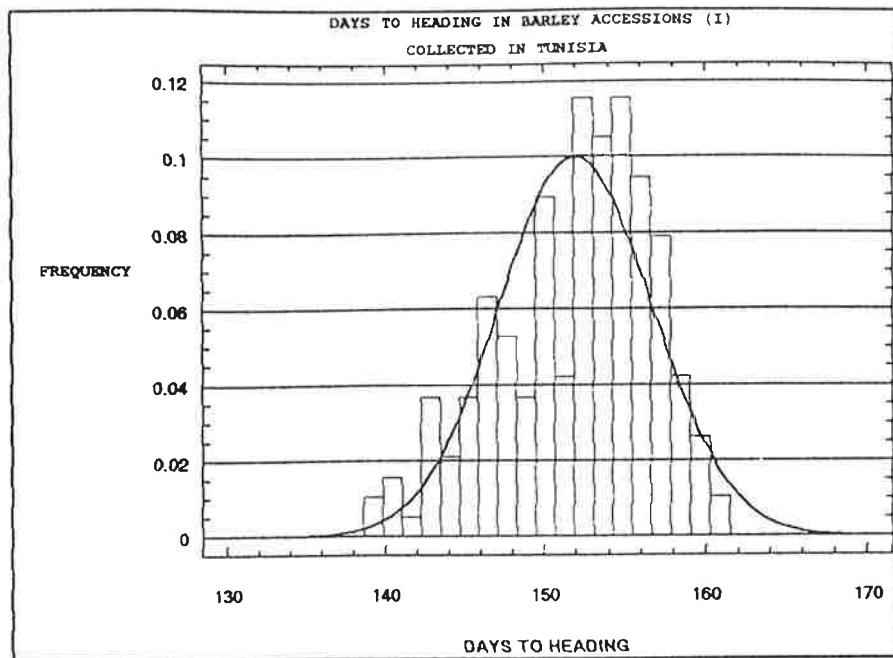
Locally adapted germplasm has a low yield potential and is often susceptible to diseases, particularly when evaluated as populations and grown under favorable climatic conditions. This situation can be changed when individual genotypes, extracted from landraces are evaluated (Ceccarelli et al. 1987; VanLeur et al., 1989). The level of disease resistance varied according to the specific disease. Over the past four years and under field condition, in a disease nursery, only 14 out of the 196 barley accessions screened showed multiple resistance to the major diseases, 78 accessions showed acceptable resistance to scald. Adequate resistance to leaf rust is found in Tunisian barleys. New genes for resistance to Puccinia hordei are common in landraces grown in central and southern Tunisia (Yahyaoui, et al., 1988). The level of resistance of 190 barley accessions to powdery mildew were evaluated in an artificially inoculated disease nursery with a mixture of races. Thirty accessions showed moderate to high level of resistance.

The yield performance of barley lines extracted from landrace populations were slightly higher than commercially grown cultivars when tested in the SAR. The productivity of landrace cultivars can be improved by crossing them to high yielding lines. Eleven accessions from "landrace x high yielding variety" crosses outyielded the variety Rihane.

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Figure 1: Days to heading barley land races



VARIETIES OF CULTIVATED BARLEY AND THEIR DISTRIBUTION IN SICHUAN OF CHINA

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ABSTRACT

197 cultivated barley varieties from Sichuan (not including the naked barley in Western-plateau of Sichuan) belong to the species *Hordeum vulgare* (L.) Hsü with two subspecies: ssp. *H. distichon* (L.) Hsü and ssp. *H. vulgare* (L.) Hsü, and are classified into 65 botanical varieties, among which 11 are new varieties. The studies show that the proportion of poly-rowed barley varieties is 99. 4%, the hulled barley varieties form the greater part than the naked, the majority of varieties are those with lax-spike, extreme-compact spike, long awn and toothed awn, yellow-coloured ear and seed. The widely distributive varieties of poly-rowed barley are *pallidum*, *thysoides*, *parallelum*, *sulphurianum*, *nigribruneum*, *fajanicum* etc. According to the ecological conditions and the distributive characteristics, six barley variety distribution districts in Sichuan have been delimited. The distribution ratios of the yellow-type and poly-rowed barley varieties are the highest in the hilly district in the middle part of Sichuan.

Introduction. Above 300 cultivars of barley have been gradually collected from winter barley region in Sichuan Basin, Qing-Ba mountainous district and Southwest Plateau (Panxi Part) since 1984, among which 197 cultivars have been identified and classified to 65 varieties by being planted and studied for three years in Chengdu plain land. These variety names have been affirmed by adopting the classification system received by Hsü Tingwen (1982), according to international code of Nomenclature for cultivated barley.

Variety Types and Characteristics. These 65 varieties consist of 1 two-rowed barley variety and 64 poly-rowed barley varieties, including 45 hulled barley varieties and 20 naked ones, of which 11 are new varieties. The majority of 65 varieties are those with lax or extreme-compact spike, 29 varieties with lax spike account for 44% of total, 22 varieties with extreme-compact spike account for 34% of total. 6 varieties with broad glume account for 9.2% of total. 35 varieties with long awn account for 63%, 6 varieties with toothed awn account for 9.2%, the others are those with short awn, micro-awn, awnless, medium-lateral florets awn etc; the remarkable characteristics is that those with hook awn are richer, 7 varieties with hook awn account for 12.3%; the majority of hook awn varieties in Sichuan are those with yellow-coloured husk and seed, the minority are those with black-coloured husk and seed. Colour variation of awn, ear and seed involve the type of yellow, brown, purple, grey, green, black, black-brown. 43 yellow-coloured (pale-yellow and brown-yellow) varieties account for 66%, 22 dark-coloured varieties account for 34% of total. Characters of dark (black, grey, furful, etc.)-coloured bran and shell, toothed-awn, lax spike are controlled by dominant genes, besides that dominant genes of early maturing and spring-nature characters exist enormously in these varieties. 3 extreme-early maturing varieties and 101 early-maturing cultivars account for 52.3% of 197 identified cultivars.

Key Words: Barley, Varieties, Distribution, two-rowed barley, Six-rowed barley.

The diversity of variation types and high frequency of dominant genes emerge in distinctive ecological conditions in winter barley region in Sichuan, spring-nature genes and the frequency come to being changes in different regions.

New Varieties. 11 new varieties of the 65 identified varieties comprised 8 poly-rowed hulled varieties and 3 poly-rowed naked varieties, in which, yellow-coloured varieties account for 27.5% of the 11 new ones. The other characters were similar to the old varieties in Sichuan region. The characteristics of 11 new varieties also showed diversity of variation, high frequency of dominant genes and majority of poly-rowed barley. New varieties characteristics described in the following Key to New Variety of ssp. *H. vulgare* (L.) Hsü.

Variety Distribution. The specific geographical and climatological conditions bring Sichuan Basin to special winter barley region. Ganzi and Aba prefecture belongs to Qinghai-Tibet plateau ecological regions. The category and distribution of 197 cultivars and the other 224 cultivars (catalogued in "Variety Record of Barley in China" during 1983-1987) have been synthesized. The studies showed that the distribution of barley variety was intimate relative to the ecological conditions. Six barley variety distributive districts have been delimited in accordance with the amount of barley variety and their characters in the same ecological district. The distributive widely representative varieties are *pallidum*, *glabrigriseum*, *thyrsoidum* in hilly district of the mid-basin of Sichuan; *pallidum*, *glabrigriseum*, *subpiksum* in western basin plain district; *subhorsfordianum*, *trifarcatum* in southeastern hilly district; *himalayense* in northeastern and northern hilly district; *pallidum*, *latiglumalum* in southwestern mountainous district; *violisizangense*, *revelatum*, *asiaficum*, *dupliviolaceum*, *anhuerense*, *trifarcatum*, *pisanjense*, *himalagene*, *coeleste*, *wokonum* in western plateau naked barley district.

* Key to New Variety of spp. *H. vulgare* (L.) Hsü

Spike density (nodes of 4 cm)	Naked or hulled	Glume	Shape of awn	Colour		Var. Nov.
				Ear and awn	Seed	
7 - 14	hulled	narrow ≤ 1 mm	long, tooth	blackish	blackish	<i>nigribrunneum</i> Hsü
7 - 14	hulled	narrow ≤ 1 mm	long, tooth	grey	grey	<i>griseipallidum</i> Hsü
7 - 14	naked	broad ≥ 1 mm	long, tooth	yellow	blue	<i>lafithmalayense</i> Hsü
15 - 19	hulled	broad ≥ 1 mm	long medium awn and awnless lateral florets, tooth	yellow	yellow	<i>latijaponicum</i> Hsü
15 - 19	hulled	narrow ≤ 1 mm	short, smooth	yellow	yellow	<i>glabrihypatherum</i> Hsü
15 - 19	hulled	narrow ≤ 1 mm	long, smooth	grey	grey	<i>glabrigriseum</i> Hsü
15 - 19	naked	narrow ≤ 1 mm	micro	yellow	brown	<i>sichuanicum</i> Hsü
> 19	hulled	narrow ≤ 1 mm	micro medium awn and awnless lateral florets	yellow	yellow	<i>subpilosum</i> Hsü
> 19	hulled	narrow ≤ 1 mm	short, tooth	lilac	lilac	<i>violigriseum</i> Hsü
> 19	hulled	broad ≥ 1 mm	micro medium and awnless lateral florets	yellow	yellow	<i>Latiundarbeyi</i> Hsü et Jang
> 19	naked	narrow ≤ 1 mm	long, tooth	yellow	yellow	<i>brunicongesum</i> Hsü

Section VII: Biotechnology (a) – Markers and Applications

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QTL analysis of agronomic important characters for dryland conditions in barley by using molecular markers. M. Baum, H. Sayed, J.L. Araus, S. Grando, S. Ceccarelli, ICARDA, PO.Box 5466 Aleppo, Syria.

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INTRODUCTION. Genetic improvement of barley in stressful environments within West Asia North Africa (WANA) region is rather slow due to the frequency, timing, duration and severity of a number of climatic stresses (Ceccarelli et al. 1991). Additionally, powdery mildew (caused by Erysiphe graminis f.sp. hordei) and scald (caused by Rynchosporium secalis) are important foliar diseases in this region. The use of DNA-markers combined with multi-location testing over several years might help to identify genetic linkages to qualitative but more important to complex inherited quantitative traits important for dryland agriculture. The collaborative research project between ICARDA and the Technische Universität München-Weihenstephan aims to tag genes of agronomic importance with molecular markers and to allow marker assisted selection for traits that are difficult to select for. A recombinant inbred line population showing variation for traits used for selection in stressful dryland conditions of Syria is being mapped with molecular markers to allow the analysis of qualitative and quantitative trait loci.

MATERIAL AND METHODS. Single seed descent derived recombinant inbred lines (RILs) originating from the cross between Tadmor (a selection from the landrace Arabi Aswad) and an improved breeding line containing 250 lines has been used for the analysis. The cross was originally intended to combine the scald resistance of Tadmor with the powdery mildew resistance of the other parent. Tadmor has been frequently used as a parent and it appears often in the pedigree of lines performing well under drought stress. Accordingly, the recombinant inbred lines are segregating for agronomic characters related to adaptation to stressful dryland conditions such as growth habit, ground cover, days to heading, plant height, grain yield. Field data were collected from unreplicated field plots during 1994 and 1995 (seed increase). In 1996, a replicated field trial at two locations is being evaluated for the disease resistance reaction, physiological and agronomical characters.

RFLP analysis of the population is being carried out in Munich with standard RFLPs, and at ICARDA, with a non-radioactive RFLPs protocol (digoxigenin). RAPD markers are being analyzed at ICARDA for their use in genetic mapping in combination with RFLP markers. T-test analysis and the MAPMAKER QTL software was used to establish linkage between these datasets.

RESULTS AND DISCUSSION. Field performance was evaluated at Tel Hadya (Syria) in 1994 and 1995. The recombinant inbred lines of the cross were being used to cover the barley genome of this cross with well distributed markers. So far, segregation analysis has been performed for 58 molecular markers (29 RFLPs, 29 RAPD-markers). The positions in the linkage map for the RFLP-marker are obtained from the cross Igri x Franka which was mapped in Munich in 1991 (Graner et al. 1991). Additional RAPD

Table 1. Associations between traits and molecular markers as revealed by t-test probabilities in the recombinant inbred line population.(Difference = difference for the trait between parent A and parent B, significance = P=95%, chromosome = chromosome location of the marker)

Trait	Difference	Signific.	Probe	Chromosome
powdery mildew95	+17.24%	0.003	MWG975	3H
powdery mildew	+9.29%	0.032	MWG975	3H
Scald* 95/2	-0.70sp	0.016	MWG569	5H
scald* 95/1	-0.53sp	0.023	MWG569	5H
scald* 95/1	-0.60 sp	0.014	MWG975	3H
scald* 95/1	+0.64 sp	0.021	OPJ19	?
scald* 95	-0.49sp	0.032	MWG975	3H
scald field 95	-0.091sp	0.011	MWG975	3H
scald field 95	-0.72sp	0.040	MWG602	5H
growth habit 95	-0.29 sp	0.041	MWG514	6H
growth habit 95	+0.03 sp	0.045	MWG533	5H
growth habit 94	-0.16 sp	0.036	MWG514	6H
plant height 94	-4.43 cm	0.002	MWG522	5H
plant height 95	+2.99 cm	0.016	MWG571	3H
awns	+0.65	0.036	MWG533	5H
lodging 95/2	+0.44sp	0.034	MWG571	3H
lodging 95/2	-0.48sp	0.049	MWG850	5H
lodging 95/1	+0.59sp	0.008	MWG880	4H
lodging 95	+0.36sp	0.019	MWG440	4H
lodging 95	+0.37sp	0.046	OPJ19	?
leaf roll 95	-0.18sp	0.039	MWG602	5H
leaf roll 95	-0.18sp	0.035	MWG880	4H
ground cover 95	+0.31sp	0.020	MWG949	2H
ground cover 95	+0.31sp	0.018	MWG950	2H
ground cover 94	+0.29sp	0.005	MWG949	2H
ground cover 94	+0.28sp	0.007	MWG950	2H
vigor	+0.37sp	0.008	MWG949	2H
vigor	+0.41sp	0.003	MWG950	2H
days to heading	+1.70d	0.049	MWG975	3H
SPAD	-1.10	0.039	MWG533	5H
SPAD	-1.01	0.028	MWG882	2H
SLDW	+1.74g/m ²	0.015	MWG832	7H
SLDW	+1.65 g/m ²	0.021	MWG836	7H
SPAD/SLDW	-0.038 g/m ²	0.042	MWG836	7H

scald*: artificial field inoculation

markers have been added to the linkage groups by multipoint analysis. We assume the same positions for the previously mapped markers in the cross, if no translocation, inversion or duplication have occurred in the genomes. The already established correlations (t-test) between molecular markers and agronomic characters are shown in table 1. The RILs will be further analyzed in a replicated field trial in 1996 and 1997 at two locations (Tel Hadya and Breda).

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Characterization of QTL affecting heading date in Harrington/TR306 barley.
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Introduction. As reviewed by Laurie *et al.* (1995), some genes affecting heading date may interact with environmental cues such as vernalization or photoperiod, while others may be described as "earliness-per-se" genes. In Harrington/TR306, a two-row spring barley cross, heading date was affected by at least five quantitative trait loci (QTL) (Tinker *et al.*, in press). These QTL showed relatively consistent effects across 29 field environments. These environments ranged in latitude from 42° to 61°, suggesting that the regulation of heading date by these QTL was not affected by differences in photoperiod. It is not known whether this absence of photoperiod response would extend to further extremes, nor is it known which stages of development are affected by these QTL. Here, our objective was to conduct a more detailed investigation of QTL for earliness in Harrington/TR306, by growing the mapping population in controlled environments under different photoperiods, and conducting QTL analyses on the duration of vegetative and early reproductive developmental periods.

Materials and Methods. *Growth Cabinet Studies:* Harrington, TR306, and 139 doubled haploid (DH) lines from the Harrington/TR306 mapping population were grown in four separate growth cabinet trials. Each trial was set at a different photoperiod: 12, 14, 16, and 18 h. The temperature regime used for all trials was 21°C for 12 h (during light) and 16°C for 12 h. One plant per line was grown in Promix soil-less medium in plastic pots 8 cm in diameter, 20 cm in height. Plants were fertilized with 20-20-20 NPK fertilizer once a week and watered as required. The plants were examined two to three times per week, and the numbers of days required to reach various growth stages, including Zadoks Growth Stage (ZGS) 31 (beginning of elongation) and ZGS 54 (mid-heading) were recorded. Duration of vegetative growth was defined as the number of days from seeding to ZGS 31. Duration of early reproductive development was defined as the number of days from ZGS 31 to ZGS 54. Tillers of each plant were counted as they emerged.

QTL Analysis: Analysis of QTL was performed using a 127-marker map (see Tinker *et al.*, in press). The software MQTL (Tinker and Mather, 1995) was used to conduct simple interval mapping for DH line means across photoperiods and within photoperiods. A combined QTL×environment (QTL×E) analysis was also performed, treating photoperiods as environments. Significance was evaluated based on permutation tests to maintain the type-I error rate at 5% per full-genome scan. The positions of QTL were estimated based on the analysis of DH line means across photoperiods. Effects of QTL were estimated within photoperiods for each trait using multi-locus models that included all QTL inferred for that trait.

Results. A total of 11 QTL were detected: three affecting the duration of vegetative development, five affecting the duration of early reproductive development, and three affecting the number of tillers (Table 1). Most QTL were detected in the analysis of DH line means across photoperiods and/or in the combined QTL×E analysis, but two were QTL were detected in only one environment each. One QTL (near markers BCD882 and MWG781 on chromosome 7) affected both the duration of vegetative development and the number of tillers.

Table 1. Estimated effects of QTL on the duration of vegetative development, the duration of early reproductive development, and the number of tillers in Harrington/TR306 barley.

Chromosome	cM position	Nearest marker	Photoperiod			
			12 h	14 h	16 h	18 h
Duration of vegetative development (days)						
3	11	CDO395	-3.7 ^a	-1.5 ^b	-1.3	-0.5
3	120	ABC174	-0.2	1.6	0.5	0.6
7	161	MWG781	-3.7	-2.3	-3.1	-2.6
Duration of early reproductive development (days)						
1	7	BCD129	-	1.2	1.8	2.9 ^b
1	87	MWG003	-	2.6	0.6	0.3
2	83	Pox	-	2.8	2.6	1.0
2	162	BCD882	-	-5.1	-2.9	-4.1
7	143	RZ404	-	0.2	-2.5	-0.8
Number of tillers						
4	6	WG662	-1.1	-1.2	-2.0	-2.2
7	7	MWG502	0.8	-0.3	-0.3	1.1
7	161	MWG781	-0.3	-1.7	-1.7	-2.1

^aEffects are presented as the estimated difference between the Harrington and TR306 homozygotes.

^bThis QTL was detected based only on this environment.

Discussion. We did not expect that the QTL detected for the duration of pre-heading developmental periods in growth cabinets would be identical to the QTL detected for heading date in the field. Growth cabinet environments differ from field environments in light intensity, humidity, temperature, and uniformity. These differences could lead to differential expression of QTL for heading time. Furthermore, with only one plant per pot, genetic potential for tillering will be more fully expressed than when plants are grown at closer spacings in the field, and this could affect developmental rate.

Of the QTL presented in Table 1, those on chromosomes 1 and 7 strongly resemble QTL affecting days to heading or maturity in field studies of Harrington/TR306. Those on chromosomes 2 and 4 do not correspond to QTL detected in the field.

Chromosome 1: The two QTL on chromosome 1 affecting duration of early reproductive development are at the same positions as two QTL affecting days to heading in the field (Tinker *et al.* in press). Since these QTL did not affect duration of vegetative development, we concluded that their primary influence is exerted after ZGS 31.

Chromosome 2: The two QTL on chromosome 2 that affected duration of early reproductive development were not detected in field studies. It is possible that their influence is unique to the growth cabinet environment. These QTL did not affect duration of vegetative development.

Chromosome 4: The QTL on chromosome 4 showed a strong effect on the number of tillers. There was no effect on time to heading or maturity at this position in either the growth cabinet or field environments.

Chromosome 7: The QTL near MWG781, at which Harrington alleles cause a shorter duration of vegetative development, clearly corresponds to a QTL affecting heading date detected by Tinker *et al.* (in press). This effect may be related to a QTL affecting number of tillers at the same position. The QTL near RZ404 affecting duration of early reproductive development may or may not be the same locus. Its effect on duration of early reproductive development was detectable only in the 16-h photoperiod. The QTL near WG622, where the Harrington allele caused fewer tillers, is at the same position as a QTL where the Harrington allele gave earlier maturity in the field (Tinker *et al.* in press).

Expression of some QTL showed a greater dependency on photoperiod than others. For example, for duration of vegetative development, the effect of the QTL near CDO395 was strongest in short photoperiods, whereas the effect of the QTL near MWG781 was relatively constant across all photoperiods. Duration of early reproductive growth was extremely variable in 12-h photoperiods. There was evidence that some of this variability was affected by a different QTL on chromosome 3 (not shown). However, the extremely long vegetative stage in the 12-h photoperiod caused a large amount of environmental variance which could confound this analysis.

Due to differences in mapped markers, it is not possible to say with certainty whether these QTL correspond to loci detected in other genetic studies. Either of the QTL detected on chromosome 7 may be in the vicinity of the *Sh₂* gene. Others might correspond to the *eps* genes detected by Laurie *et al.* (1995). Although some interaction with photoperiod is apparent from these studies, most QTL detected here and/or by Tinker *et al.* (in press) had relatively small but consistent effects on either duration of vegetative development or duration of early reproductive development, or time to heading across environments.

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THE USE OF HORDEINS AS GENETIC MARKERS IN BREEDING SPRING BARLEY IN THE WEST OF UKRAINE. Z.M. COPCHYK, O.T. VRONSKA, A.Y. MARUCHYNAK and G.O. KOSYLOVYCH, Institute of Agriculture and Animal Husbandry of Western Ukraine, Lviv, Obroshyn, 292084, Ukraine.

Introduction. The main task of spring barley breeding at the Institute is creating varieties with good grain productivity and malting qualities with resistance to lodging and powdery mildew caused by Erysiphe graminis DC f.sp. hordei.

The use of molecular and genetic markers is one of the most promising trends in genetics and plant breeding. In the former Soviet Union this problem was first initiated by Academician Sozinov in Ukraine. The Institute of Plant Breeding and Genetics (Odessa) was one of the leaders in the introduction and use of molecular and genetic markers in breeding wheat and barley. The study of storage protein (hordeins) of the grain demonstrated that loci which code for blocks of the hordein components may be effective as genetic markers of some commercially important traits (Sozinov 1985). The electrophoretic hordein spectra are determined by genotype only and do not depend on the environment. At the Institute of Agriculture and Animal Husbandry of Western Ukraine (Lviv) genetic markers have been used in all stages of breeding spring barley since 1986. The applicability of starch-gel electrophoresis of hordeins as a genetic model system makes rapid identification of the hundreds of genotypes possible.

Materials and Methods. The parent material for breeding spring barley was created by interspecific crossing with different complexity levels and subsequent individual selection. Single plant selection was conducted by the pedigree method. The breeding work was carried out in hybrid nurseries (F_1 , F_2 , F_3), selection (F_4 , F_5) and control (F_6) nurseries, preliminary test (F_7). In generations F_8 - F_{10} the concluding tests were conducted and the best entries were passed to state testing. It should be emphasized that selections in F_3 (F_2 progenies) were based on electrophoretic analysis of barley hordein. Some of the seeds of each F_2 progeny of every entry were sown in field conditions and taken for genetic analysis.

Hordein electrophoretic spectra were obtained by a procedure according to O. Sozinov and F. Poperelya (1978) in starch gel columns with a Al-lactate buffer and 3 moles of urea, current 1.5 mA and voltage 300 v on column. Proteins were fixed by 5% solution of TCVA and were coloured by 2% solution of nigrozin. The allelic hordein blocks of hordeins A, B, F and G were singled out of the electrophoretic hordein spectra, according to the published catalogue of hordein blocks (Pomortsev et al. 1985). To determine the hordein spectra of entries of the spring barley collection no less than fifty grains were analysed and in hybrid nurseries no less than five grains were used. The above mentioned procedure makes it possible to evaluate the breeding material with high reliability and to use a new advanced approach for selection of parental forms.

Results and Discussion. The study of hordein polymorphism in entries of the collection is carried out for determining the level of their genetic affinity, registration of varieties, and serves for evaluation of the given genotypes and selection of the best parental pairs for hybridization. 540 entries of the spring barley collection were analysed, 315 of them were homogeneous for hordeins and are represented by single-line varieties; 151 varieties are heterogeneous, they consist of two

hordein allelic blocks. 74 varieties have three or more hordein allelic blocks.

Among homogenous and heterogeneous varieties in the spring barley collection 27% make up the high yielding and available varieties for growing in the west region of Ukraine. They have genetic formulas Hrd 2.19.1 and Hrd 2.8.2. On account of genetic linkage, hordein loci serve as markers of the genes for resistance to powdery mildew. 23 genes are associated with loci Mla and Mlk, which are located near its hordein coding (Islam et al. 1991). Genetic resistance to different races of powdery mildew was found in 43% of investigated genotypes of both homogeneous and heterogeneous varieties. Three varieties of the collection have the highly effective gene Mla1 (Mikro-2, Kushk, Nutans-78), genes Mla12, Mla7, Mlk, Mla13, Mla9, Mla16 are present in 81, 55, 31, 31, 17 and 16 varieties, respectively. 33 varieties have two genes for resistance, Mla7 + Mlk, which are very effective.

There is a relation between Hordein allelic variants that also serve as markers for some malting quality traits of grain, namely by content of extractive substances (Sozinov 1985). High malting qualities are associated with hordein components A2 B19 (98 entries), A1 B21 and A2 B21 (28 entries).

Electrophoretic study of the hordeins increases the efficiency of the breeding work to a great extent, and makes it possible to deliberately select highly productive homozygous lines. Analyses and selections of the constant genotypes were carried out in F_3 (F_2 progenies) for intensifying the breeding process. 9050 F_3 entries, obtained by interspecific crossing in 166 crosses, were studied by electrophoretic analysis in 1991-1995. The homogeneous, according to hordeins, lines with genes for resistance to powdery mildew were discriminated. The genes Mla12, Mla6, Mla13, Mlk, Mla7 + Mlk and Mla9 were in 400, 265, 181, 85, 22 and 3 lines, respectively. The high yielding lines adapted to growing conditions in the western Ukraine were markedly hordein variants 2.1.9 (742 lines) and 2.8.2 (1098 lines) were selected on the basis of electrophoretic analyses. Also, 1054 lines with good malting qualities were detected and those which were not pure for hordein spectra were rejected.

As the result of conventional breeding procedures and the more advanced techniques the new spring malting barley variety Nadiya was developed. The cultivar has high yield potential, and combines high malting qualities with resistance to lodging. It is immune to powdery mildew and leaf rust caused by Puccinia hordei Oth. In preliminary tests at the Institute during 1992-1993 Nadiya outyielded the check cv. Roland by 12.0 - 12.3%.

Thus, the use of wide electrophoretic analyses data for selection and assessment of spring barley genotypes makes it possible to increase information and effectiveness of the breeding work and to develop prospective lines and varieties.

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Mapped Clone Sequences Detecting Differences Between 28 North American Barley Cultivars. L.S. DAHLEEN, USDA-Agricultural Research Service, NCSL, P.O. Box 5677, SU Station, Fargo, ND, 58105.

Introduction. North American barley (*Hordeum vulgare* L.) breeders generally work with a narrow germplasm base to develop new cultivars. Continued improvements in agronomic and quality traits have been possible with this germplasm, indicating that genetic variability is present (Wych and Rasmusson, 1983; Horsley et al., 1995). Barley RFLP marker maps usually have been developed from crosses between diverse parents to maximize polymorphism (Kasha et al., 1995; Kleinhofs et al., 1993; Graner et al., 1991; Heun et al., 1991; Shin et al., 1990). One of the purposes for developing these maps is for molecular marker assisted selection (MMAS) of desirable alleles controlling quantitative and qualitative traits. Clone sequences must detect RFLPs between closely related genotypes for breeders to use these molecular markers in barley improvement programs. The objective of this project was to determine whether the amount of RFLP variation detected between 28 North American (NA) barley cultivars was sufficient for MMAS in NA barley improvement programs.

Materials and Methods. Twenty-eight spring cultivars adapted to the Midwestern and Western regions of the United States and Canada were chosen for study of RFLP markers. The cultivars tested included 15 Midwestern six-rowed cultivars, two Western six-rowed cultivars, four Midwestern two-rowed genotypes, and seven Western two-rowed cultivars.

Leaf tissue was collected in bulk from approximately 20 seedlings of each cultivar at the 3-4 leaf stage, frozen, and lyophilized. DNA was extracted using the methods of Kleinhofs et al. (1993) and 20-30 ug were restriction digested with *Bam*H I according to the manufacturer's protocol. DNA fragments were separated in 0.9% agarose gels and Southern transferred to nylon membranes. Genomic and cDNA clone sequences from the NABGMP were hybridized to the membranes to detect RFLPs between the genotypes (Kleinhofs et al., 1993; Kasha et al., 1995).

The banding patterns detected by each clone sequence were analyzed as genotypes, or single loci, when one variable band or two cosegregating variable bands were present. Patterns were analyzed as phenotypes or multiple loci when two or more independently variable bands were identified. A diversity index (DI) was calculated for the patterns detected by each clone sequence. The DI for those analyzed as genotypes was calculated using Nei's (1987) genic diversity index:

$$DI_i = 1 - \sum p_{ij}^2$$

where p_{ij} is the frequency of the j th RFLP pattern for clone sequence i and the summation extends over all patterns. If the clone sequence identified a pattern monomorphic (identical) for all 28 cultivars, the $DI=0$. If 28 different patterns were identified, the $DI=1$. The DI for the banding patterns detected by clone sequences analyzed as phenotypes was calculated using Shannon's information statistic (Bowman et al. 1971):

$$DI_i = - \sum p_{ij} \ln p_{ij}$$

The resulting values of this calculation range from zero to the natural logarithm of the number of patterns detected. Because the maximum

value of Shannon's statistic varied with the clone sequence used, all results were converted to percentage of the maximum value for comparison of DIs. Readable banding patterns for a minimum of 24 cultivars were obtained before a DI was calculated for a clone sequence.

Pairwise comparisons of cultivars were made for patterns detected by each clone. The percentage of clones detecting polymorphism was calculated for each pair, taking into account unreadable banding patterns (2.25%) for some cultivar-clone sequence combinations.

Results and Discussion. A total of 64 genomic and 36 cDNA clone sequences were used to detect RFLPs between the 28 cultivars. Forty-seven of the 57 useful clone sequences in this study hybridized to single loci and were evaluated as genotypes. The remaining ten hybridized to two or more independent loci, and were evaluated as phenotypes.

Approximately one half (29/57) of the clone sequences detecting RFLPs, and 29% of all clone sequences had overall DIs greater than 0.500. These sequences were located over all seven barley chromosomes. RFLPs were detected equally by genomic (58%) and cDNA (56%) sequences, but the DI values differed. Twenty-two percent of the genomic DNA sequences and 39% of the cDNA sequences detected DIs greater than 0.500. Three sequences that were given a single map location (no cross-overs in 150 doubled haploid lines) on chromosome 3 (Kasha et al., 1995), ABG010, ABG460, and ABG057, detected different levels of diversity.

DIs were calculated for each clone sequence within each geographic/spike morphology group. Three clone sequences that detected RFLPs among the whole group of 28 cultivars detected no variation within any of the geographic/spike morphology groups. Cultivars released over time from the same breeding program showed fewer differences than those released from different programs. For example, the five cultivars released from Minnesota from 1974-1993, Manker, Morex, Robust, Excel, and Stander, are very closely related, as can be seen by their pedigrees and high coefficients of parentage (R.D. Horsley, Pers. comm. 1996). Pairwise comparisons of RFLP banding patterns among these cultivars reveal that at least 4% (Manker vs Robust) and up to 14% (Manker vs Morex) of the clones evaluated separated these cultivars. Clone sequences detecting RFLPs between these closely related cultivars mapped to all chromosomes except chromosome 5, and were associated with traits such as yield, heading date, height, malt extract, lodging, grain protein, alpha-amylase, and diastatic power (Hayes et al., 1993; Tinker et al., 1996). Comparison of cultivars recently released in ND and MN (Foster vs Excel, Stander) indicate that approximately one out of seven or eight clone sequences will be informative using a single restriction enzyme.

The results of this study indicated that NABGMP clones detect reasonable levels of polymorphism among North American spring barley cultivars. More than one fourth of the clones tested have DI values greater than 0.500. Pairwise comparison of North American cultivars indicated that sufficient RFLP variation exists between cultivars or potential breeding parents for North American barley breeders to use these clones for marker-assisted selection of desirable alleles.

associated with yield and malt quality. It is reasonable to assume that more polymorphism will be detected between less closely related parents in barley breeding programs. These crosses often are used for incorporation of disease resistance genes, and RFLPs can be used in MMAS both for the desired gene and against undesirable genes.

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MOLECULAR MAPPING OF GENES FOR DISEASE RESISTANCE IN BARLEY.

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INTRODUCTION. Apart from yield components and quality parameters disease resistance genes have been identified as a target for the application of marker assisted breeding concepts. However, their successful application critically depends on the number of tagged genes. In this context, major emphasis has been put on molecular mapping of genes conditioning resistance to the barley yellow mosaic virus complex, which comprises two unrelated viruses, barley mild mosaic virus (BaMMV) and barley yellow mosaic virus (BaYMV). Of the latter, two related strains (BaYMV-1, BaYMV-2), have been identified that differ in their virulence pattern. Due to the soil-borne nature of the pathogens, plants can not be protected by chemical means and cultivation of barley in infested areas is based on the availability of disease resistant cultivars. In a recent germplasm survey a series of resistant accessions were identified which, apart from their insufficient winter hardiness, are highly susceptible to a variety of diseases including scald (*Rhynchosporium secalis*), net blotch (*Pyrenophora teres*), Typhula blight (*Typhula incarnata*) and powdery mildew (*Erysiphe graminis*). Thus, the successful introgression of novel virus resistance genes requires the combination with resistance genes against these fungal diseases. The present paper summarizes ongoing attempts to (i) investigate the genetic basis of resistance to major pathogens, (ii) to locate the corresponding genes and (iii) to develop selectable, PCR-based markers.

MATERIAL AND METHODS. The winter barley accessions used for RFLP analysis are compiled in Table 1. Igri, Franka and Triton are winter barley cultivars released in Germany. The breeding line W122/37.1 has been derived from the Japanese cultivar "Resistant Ym. No. 1" (Muramatsu, 1976). Tests for resistance to BaMMV, *E. graminis*, *R. secalis* and *P. teres* were performed in controlled environments using defined pathotypes for artificial inoculation of seedlings. Field trials were performed to test resistance against BaYMV-1 and BaYMV-2. Resistance to *T. incarnata* was tested in a field experiment after artificial infection. DNA analyses and genetic mapping were performed using standard procedures (Graner et al., 1991).

Table 1: Accessions used for RFLP mapping.

donor	resistance	gene
H.Hor 3365	BaMMV	<i>ym7</i>
H.Hor 3073	BaMMV	<i>ym7</i>
W122/37.1	BaMMV, BaYMV-1,2	<i>ym5</i>
10247	BaMMV	<i>ym8</i>
Bulgarian 347	BaMMV	<i>ym9</i>
Russia 57	BaMMV	<i>ym11</i>
Muju covered 2	BaMMV	<i>ym12</i>
Iwate Omugi 1	BaMMV	<i>ym4</i>
Franka	BaMMV, BaYMV-1 <i>T. incarnata</i> , <i>R. secalis</i>	<i>ym4</i> <i>Ti</i> <i>Rh</i>
Triton	<i>R. secalis</i>	<i>Rh</i>
Igri	<i>P. teres</i>	<i>Pt.,a</i>
<i>H. bulbosum</i>	<i>E. graminis</i>	<i>MlHb</i>

RESULTS AND DISCUSSION. In order to investigate the genetic basis of resistance to BaMMV, a series of non-allelic, recessive genes were analyzed. According to their phenotype, genes conditioning complete immunity (*ym4*, *ym5*, *ym9*, *ym11*) could be differentiated from those conferring partial resistance (*ym7*, *ym8*, *ym12*). The latter results in a prolonged latent period after mechanical inoculation with BaMMV. Under field conditions, no BaMMV particles could be detected in the donor parent of the *ym7* gene. Since in the field seedlings become infected via their roots and not via their leaves, as it is the case with mechanical inoculation, partial resistance genes seem to be differentially expressed in the corresponding tissues. At present, resistance in adapted barley cultivars is mainly based on the *ym4* gene, which is only effective against BaMMV and BaYMV-1 but not against BaYMV-2. However, the breeding line W122/37.1 shows immunity to all three virus strains. This is due to the presence of the *ym5* gene, which is located in close proximity to the *ym4* gene on chromosome 3L. The availability of closely linked RFLP, RAPD and STS markers will facilitate the rapid introgression of the *ym5* gene. Regarding the distribution of individual virus resistance genes across the genome, major complexes consisting of closely linked genes are present on chromosomes 3L and 4L. However, the genetic fine structure of these remains yet to be determined. In addition to genes conferring resistance to the BaYMV complex, various fungal resistance genes have been mapped (Fig. 1).

The *Rh* gene conferring resistance to *R. secalis* is located in the centromeric portion of chromosome 3L in close proximity to the *Pt,a* gene, which conditions seedling resistance against *P. teres*. Both genes are tightly linked since no recombinant could be identified in a segregating population comprising 112 DH-progeny lines. The availability of a codominant STS marker linked at a distance of less than 1 cM facilitates a PCR based selection for both genes.

A dominant gene from *H. bulbosum* conferring resistance to powdery mildew (*MHb*) has been mapped in a translocation stock of *H. vulgare* to the distal portion of chromosome 2S, where it is tagged by a cosegregating marker (*Chs1b*). Because of the possibility of suppressed recombination around the translocated chromosome segment, the physical distance between the gene and the marker might be much larger than it is suggested by the genetic data.

Due to its environmental variability, resistance to *T. incarnata* is difficult to assess in field tests. Upon artificial inoculation both frequency and rate of infection were scored in a progeny consisting of 69 doubled haploid lines. Both traits were highly correlated and showed a bimodal distribution in the progeny indicating the presence of a single gene (*Ti*) in the cultivar Franka, which is located in the distal portion of chromosome 5S.

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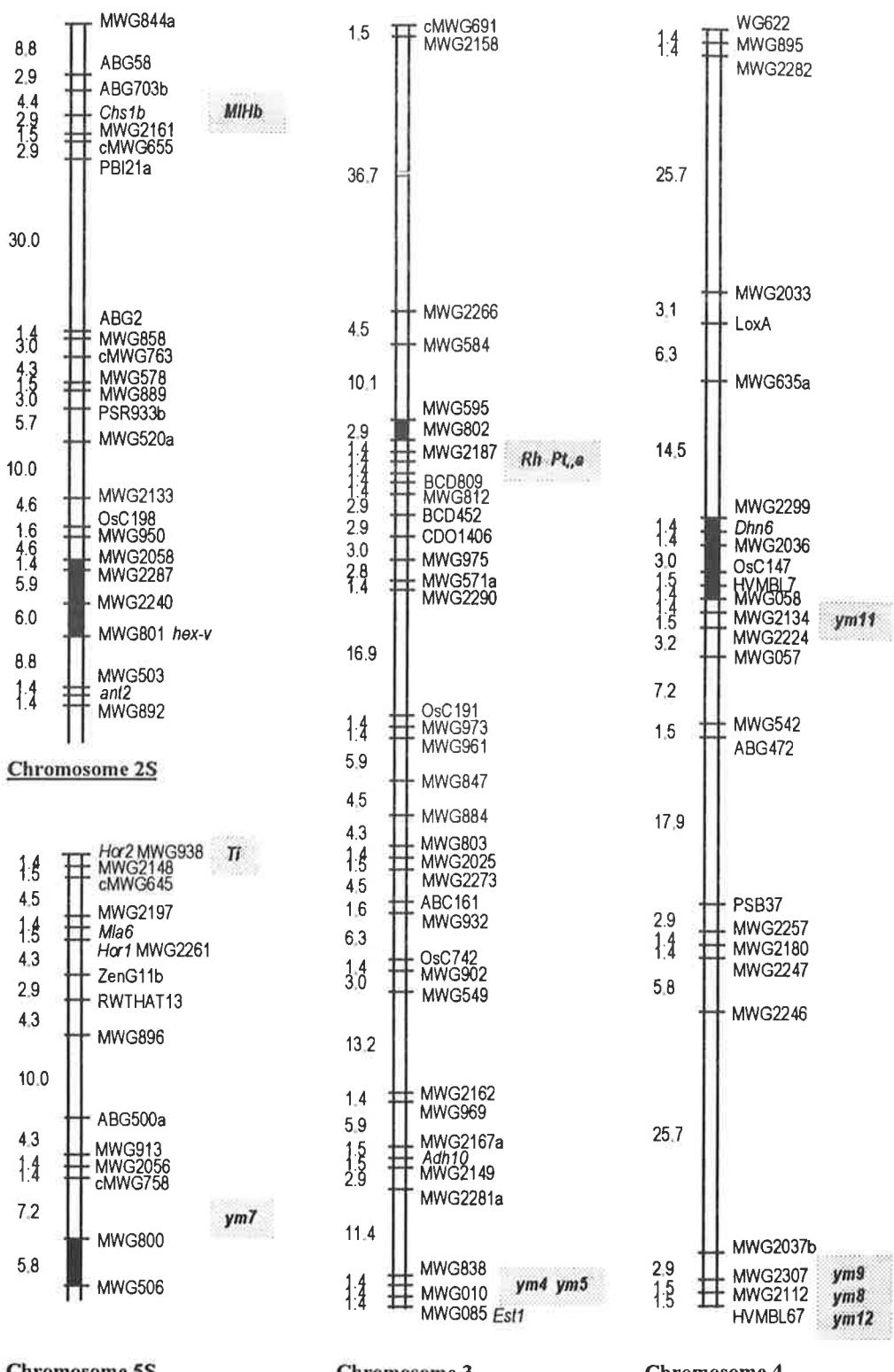


Figure 1: Genetic localization of disease resistance genes. The representation shows selected chromosomes of the Igri x Franka map (Graner et al., 1991). Shaded areas delimit the position of the centromeres. Those resistance genes which were not analyzed in the I/F progeny, were integrated into the map via common markers. Distances are given in % recombination.

**Varietal Identification of Barley and Malt using PCR-STS Markers. D.K.
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Introduction. Currently, variety identification is based on morphological characteristics. The genetic diversity within the 2-row and 6-row germplasm is narrow and limits the usefulness of morphological characteristics. In 1993, we published results suggesting that the use of the sequence-tagged-site (STS) primer sets developed by our laboratory would be sufficient to unambiguously distinguish DNA of most malting and feed barley varieties (Chee *et al.*, 1993). Since 1993 we have developed 400 STS primer sets. We determined the chromosomal locations of about 200 (Blake *et al.*, *in press*). Of the 200 fairly well-characterized primer sets we found 66 to be good indicators of variety in normal barley varieties. In this report we show varietal identification results using a subset of the 66 informative primer sets against a panel of DNAs isolated from either dry embryos or cleaned barley malt.

These results demonstrate that it is practical to verify the varietal makeup of barley seedlots or cleaned malt. While this might not be practically significant when few varieties are grown, we have entered an environment which may spur development and production of many varieties of varied functionality. In this sort of environment, knowing what variety you use may prove critical to meeting market needs.

Methods and Materials. Polymerase chain reactin (PCR) is a process by which a minute portion of the DNA from an organism is selectively synthesized. If that synthesized portion of DNA differs in structure between two barley varieties, that structural difference can provide the basis for varietal identification (Chee *et al.*, 1993). The sequence-tagged-site approach, which we used to identify DNA fragments that differ in structure among genotypes, requires sequences of cloned, mapped DNA fragments. Our laboratory sequenced about 400 of the mapped RFLP clones used in the NABGMP mapping project (Kleinhofs *et al.*, 1993), and synthesized primers (short sequences). These primers, direct the synthesis of the DNA fragment that lies between them (sequence amplification).

Primer sets that permitted the detection of polymorphisms among barley genotypes were used with DNA from 12 barley cultivars and 10 malted barley cultivars, selected for their regional and industrial importance.

We used a modified version of a rapid method to extract plant genomic DNA (Edwards *et al.*, 1991) from dry embryos and malt. Twenty-five embryos were removed from breeders' seed of the harvested barley cultivars. These embryos were crushed within weight paper using a hammer and pliers. A single cleaned malt kernel from each malt variety was also similarly crushed. The crushed samples were collected in sterile Eppendorf tubes. 400 μ l of extraction buffer (200 mM Tris HCl pH 7.5, 250 mM NaCl, 25 mM EDTA, 0.5% SDS) (600 μ l for crushed embryos) were added and each sample and vortexed for 5 seconds. The extracts were centrifuged at 13,000 rpm for 1 minute and 300 μ l of the supernatant transferred to a fresh Eppendorf tube. This supernatant was mixed with 300 μ l of chilled Isopropanol and left at room temperature for 2 minutes. The DNA was hooked with a glass rod and collected with 100 μ l of 70% chilled ETOH. The samples were centrifuged a second time for 5 minutes. The ETOH was poured off and

samples were left at room temperature until all traces of liquid had evaporated. Samples were dissolved in 100 μ l 1xTE.

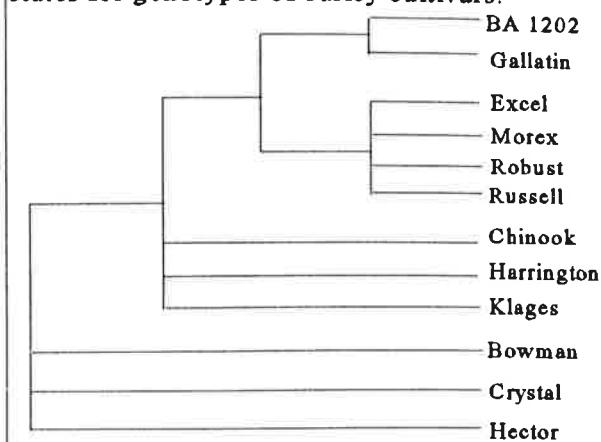
PCR was done as previously published (Blake, *in press* and Chee et al., 1993). All samples were evaluated in 6% acrylamide gels using 1xTBE as gel and tank buffer. Restrictions were performed using commercially available enzymes directly in PCR reaction mix. Data was collected and relatedness estimated using a maximum likelihood algorithm (Fig. 1).

Results and Discussion. See poster for a list of primer sets used in this experiment.

The most informative of the primer sets are described below. Also, see poster for tables reporting allele states for genotypes for barley and malt cultivars and photographs that demonstrate the clarity of amplified restriction site polymorphism analysis.

- ▶ **ABC253**, located on chromosome 1, provides excellent information both cut with *DdeI* and uncut. AB1202 and Harrington contain unusual alleles, the 2-rows and 6-rows are clearly delineated, and Morex appears to be heterogeneous.
- ▶ **ABG54**, located on chromosome 4, shows small size differences among alleles. These 20 basepair differences, seen with this primer, in size are large, relative to many markers of this type. We prefer restriction site polymorphisms to these VNTR polymorphisms.
- ▶ **ABG55** is located on barley chromosome 5. When cut with *AluI*, this marker easily distinguishes Harrington and Oxbow from the other 2-rows, and Argyll from the other 6-rows. A small size polymorphism may distinguish Bowman from the other 2-rows, but this difference is subtle enough to likely be unreliable.
- ▶ **ABG377** resides on barley chromosome 3 and happens to be very close to one of the genes important for yield, especially in 6 row backgrounds (Larson et al., *in press*). The allele found in Morex is particularly unfortunate, resulting in a 10% yield reduction when compared to lines carrying the alternative allele. This marker also shows excellent informativeness. Bowman, Crystal and Harrington carry the Morex allele, while AB1202, Chinook, Harrington and Klages carry the “a” allele. Gallatin is heterogeneous for this marker. All of the 6 row malt varieties evaluated carry the “b” allele, although the “a” allele is found in many feed barleys (data not shown). We are currently evaluating Morex derivatives which carry the “a” allele and show improved adaptation to Montana dryland production environments.
- ▶ **ABG500** is located on barley chromosome 5. The primers produce a single 280bp product which, when cut with *HaeIII* distinguishes 6-rowed from 2-rowed cultivars, and within 6-rows separates Argyll from the US cultivars.
- ▶ **ABG618**, a chromosome 4 marker, utilizes *HinF1* to reveal variation among 2-rows. Crystal is heterogeneous, while Harrington, Oxbow Gallatin carry the “b” allele.

Fig. 1. A strict consensus tree built from allele states for genotypes of barley cultivars.



- ▶ **ABG701** resides on barley chromosome 1. A single 580bp product is produced which when cut with *Dpn II* distinguishes 6 row from 2 row cultivars.

The phylogenetic analysis shown in Figure 1 represents the smallest possible tree of relatedness using the allele states for genotypes for barley. The branch points and primer sets provide indicators of which primer sets may be used to identify specific barley genotypes.

Significance. This project was done as a method to train a new graduate student (D.H.). We didn't expect to see the differences in phenotype for malt and barley that we observed. The use of a single kernel of malt to estimate the genotype of a sample of malt is at best naive, and at worst silly. We should have evaluated each malt sample using a random sample of malt kernels. We will certainly do so prior to submission of a manuscript. The sample of Crystal malt we have may not actually be Klages it may merely contain a few Klages kernels.

Is varietal identity important to the maltster? We in the barley breeding community believe it is. We also believe that much of the grain sold as Harrington may be Lewis, Clark, Crystal, or a mixture. Is varietal identity important to the brewer? Is flavor important to the brewer? Is extract important to the brewer? Are enzyme/total protein and fermentable sugars/nonfermentable carbohydrates important to the brewer? The barley breeding programs in North America have expended significant resources developing tools which permit us to mark and track genes which determine characteristics like these. Our program has specialized in making these tools easy to use. We would enjoy collaborating with maltsters and brewers to deploy these tools to industry. If you would like to receive primer sets or would like to submit blinded samples to our lab, please contact one of the authors. E-mail addresses are as follows:

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Genotype-phenotype associations at major malting quality QTL and the implications for molecular marker assisted selection in barley. F. HAN¹, S.E. ULLRICH¹, I. ROMOGOSA², B.L. JONES³, and NORTH AMERICAN BARLEY GENOME MAPPING PROJECT, ¹Dept. of Crop and Soil Sciences, Washington State Univ., Pullman, WA 99164-6420, USA, ²Univ. de Lleida - IRTA, 25196 Lleida, Spain, ³USDA-ARS, CCRU, 501 Walnut St., Madison WI 53705.

Introduction. Identification of chromosomal locations of individual quantitative trait loci (QTL) provides a potentially powerful selection approach for quantitative traits through linked molecular markers in a breeding program. Generally, three to ten loci are often identified for each quantitative trait with one or two loci having relatively large effects. It is often not feasible or possible to tag all identified QTL due to very small gene effects, coupling or repulsion of favorable alleles, and the expense in genotyping. Therefore, tagging major QTL via linked molecular markers should be desirable in applied breeding programs. The North American Barley Genome Mapping Project has constructed a molecular marker linkage map by using a population of 150 doubled haploid lines (DHL) derived from 'Steptoe' x 'Morex' (S/M) F1s (Kleinjohann et al. 1993). This map was used to identify a number of QTL for malt extract (ME), α -amylase (AA), diastatic power (DP), and malt β -glucan content (MBG) (Hayes et al. 1993; Han et al. 1995). Two QTL regions flanked by *Brz* and *Amy2*, and WG622 and BCD402B on chromosome 1 and 4, respectively, were found to have major effects on these four traits. The objective of this study was to determine the association of marker alleles with phenotypic values at these two major malting quality QTL regions and the implications for molecular marker assisted selection (MMAS) in barley.

Materials and Methods. A second set of 92 DHLs (not used for mapping) derived from S/M F1s (Courtesy of P.M. Hayes) was used as a selection population in this study. The 92 DHLs together with Steptoe and Morex were grown in the summer of 1994 at Pullman, WA, and in the winter of 1994-95 at Lincoln, New Zealand. Each line was grown in a 3 m row at both locations. The DHLs were genotyped with the two pairs of flanking markers, *Brz* and *Amy2*, and WG622 and BCD402B. The DNA extraction, Southern blot, and hybridization procedures were described by Kleinjohann et al. (1993). Due to poor kernel quality of the Pullman grain, malting quality data for each line were obtained by micro-malting seed only from New Zealand by the standard procedures of the USDA-ARS Cereal Crops Research Unit at Madison, WI. Gene interactions for each trait were analyzed by SAS/STAT (SAS user's guide, SAS Institute, Cary, NC, USA, 1991) in both the original mapping population and the selection population.

Results and Discussion. Gene interactions among the major loci. The two major QTL regions on chromosomes 1 and 4 show additive effects on all four traits without apparent epistasis (Table 1). Morex contributes all favorable alleles. In the original mapping population, there were definite significant phenotypic differences with allele substitution for all four traits, ME, AA, DP, and MBG. The DHLs which have all Morex alleles at the two major QTL regions possess better malting quality characteristics. In the selection

population, the substitution of alleles at one or the other QTL did not always show a significant difference. However, the genotypes with all Morex alleles at the two major locus regions showed a significant difference compared to the genotypes with all Steptoe alleles, except for MBG, which didn't show any significant difference among the four classes of genotypes. Fewer significant differences among the phenotypic values for the different allelic constitutions at the two major locus regions in the selection population is likely due to limited data for the selection population (fewer lines and only one malting quality data set). Nevertheless, the trends were the same for both populations.

Table 1. Gene interactions among the major loci for four malting quality traits

Genotype	Malt Extract (%)		α -amylase ($^{\circ}$ DU)		Distatic Power ($^{\circ}$ ASBC)		Malt β -glucan (%)	
	A†	B	A	B	A	B	A	B
SSSS‡	74.0a§	71.7a	26.5a	30.4a	78a	92a	1.3a	1.2a
SSMM	75.0b	72.4ab	29.2b	34.5ab	91b	106ab	1.1b	1.2a
MMSS	74.9b	72.6ab	29.8b	36.4b	89b	119bc	1.0b	1.1a
MMMM	75.8c	73.3b	32.3c	35.7b	102c	125c	0.8c	1.0a

† A--Mapping population, B--Selection population.

‡ S - Steptoe allele, M - Morex allele at the four homozygous QTL flanking marker loci in the order *Brz*, *Amy2*, WG622, BCD402B.

§ Means within each column with different letters are significantly different at $p \leq 0.05$.

Associations of marker alleles and phenotypic values at the major loci. The flanking marker alleles at the two major QTL regions for ME, AA, DP, MBG, and overall malting quality rank of the top 10% of the DHLs within each trait in the selection population are listed in Table 2. For ME and DP, 7 of the 9 lines have the Morex alleles at the locus region on chromosome 1, suggesting that the QTL on chromosome 1 has more impact on ME and DP than the one on chromosome 4. The top 10% DHLs for AA and MBG have Morex alleles at either locus or both, indicating that both QTL have an equal effect on these two traits. The top 10% DHLs with the highest overall malting quality rank also have Morex alleles at either or both loci except one line with all Steptoe alleles at the two loci.

Five different lines with all Morex alleles at the two major loci are included in the top 10% quality lines. There are 12 DHLs total which have all Morex alleles at the two major loci in the selection population. The average malting quality of these 12 lines is significantly higher than those with Steptoe alleles at the two major loci (Table 1). However, by looking at the whole population, we found that some lines, which have all Morex alleles at the two major loci, do not have high malting quality, and some lines, which have all Steptoe alleles at the two major loci, have the relatively high malting quality. The phenomenon could possibly be explained by: 1) the phenotypic value is not accurate due to one year's data, and 2) the function of the major QTL is regulated by or interacted with the allelic state of other minor QTL. In the second case, high malting quality comes from, not only the favorable alleles at the major QTL, but the favorable alleles at the minor QTL.

Table 2. Flanking marker alleles at the major malting quality loci of the top 10% of the S/M DHLs for each malting quality trait and overall quality rank

Geno-type rank†	Malt extract (%)	α -amylase (°DU)	Diastatic power (°ASBC)	Malt β -glucan (%)	Overall quality rank‡
1	MSSMS\$	MMSS	MMSM	MSSM	MMMM
2	MMMM	MMMM	SSMM	MMMS	SSMM
3	MMSS	MMMM	MMSM	MSSS	MMMM
4	MMMS	SSMM	MMMM	MMSS	SSSS
5	MMSS	MMSS	MSSS	MMSM	MSSM
6	MMSS	SMMM	MMMM	SSMM	SSMM
7	SSMM	SSMM	MMMS	SSMM	MMMS
8	MMMM	MMSS	MMMS	SMMM	MMSS
9	MMMS	SSMS	MMSS	MMMM	MMMS

† Genotype rank within each trait of the top 10% of the S/M DHLs.

‡ Based on a calculated index of all quality measurements.

\$ S - Steptoe allele, M - Morex allele at the four QTL flanking marker loci in the order *Brz*, *Amy2*, WG622, BCD402B.

The fact that lines with the favorable alleles at the major loci may or may not possess high malting quality raises a question how to use molecular markers in selection. Since the top malting quality lines indeed have the favorable alleles at the major loci, a selection strategy could be: firstly, to select the lines based on molecular marker data, and then to combine phenotypic data for selected lines to make final decisions. This approach will reduce substantially the number of lines for field planting and malting quality analyses, since genotyping can be done with young vegetatively growing plants at as early as F2 population. The validation of this selection approach is being tested in the selection population.

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Application of AFLP Markers to Diversity Analysis in Barley: P.M. Hayes and J. Cerono, Oregon State University, Corvallis, OR 97331, USA; H. Witsenboer, M. Kuiper, and M. Zabeau, Keygene, Wageningen 6700, The Netherlands.

Introduction. Genetic diversity in barley has been explored by a number of techniques, ranging from morphological markers (Tolbert et al., 1979) to RFLPs (Melchinger et al, 1994; Saghai Maroof et al., 1995). This preliminary report is the result of a collaboration of the North American Barley Genome Mapping Project (NABGMP) and Keygene. Keygene developed and marketed AFLP technology. This technique is based on the selective amplification of subsets of genomic fragments and promises to rapidly generate abundant polymorphism. The NABGMP uses marker-based strategies to the benefit of barley improvement. The objectives of this investigation were to use AFLPs to assess diversity in an international collection of barley genome mapping population parents. We were interested in determining the utility of AFLPs in detecting germplasm relationships and more specifically in determining relationships among genetic stocks that are parents of genome mapping and QTL detection populations.

Materials and Methods. Forty two mapping population parent genotypes of diverse geographic origin, growth habit, end-use, and spike morphology were obtained from various barley mapping projects. For AFLP analysis, templates were made using EcoRI and MseI, essentially as described in the GIBCO/BRL AFLP kit. A total of 322 AFLP loci were scored. Genotypes were scored for the presence or absence of a band at each AFLP. These bivariate data were used as the raw matrix. Subsequently, a square matrix of similarities was computed using Gower's coefficient (Gower, 1971); 0 - 0 matches were not taken as a measure of similarity. The similarity matrix was then used for cluster analysis using the Unweighted Pair Group Method of Averages (UPGMA). Associations among genotypes were also obtained by principal coordinate analysis (PCoA) (Gower, 1972). Eigenvectors were obtained from the similarity matrix used for cluster analysis. The coefficients of the first two eigenvectors were then used as coordinates for the biplot.

Results and Discussion. In the dendrogram shown in Figure 1 genotypes are clustered in a fashion that is in general agreement with their spike morphology, geographic origin, and growth habit. The first cluster consists of spring-habit 2-rows. Within this cluster, there are two principal subgroups. The first consists of European 2-row spring malting types and North American 2-row malting types of the Coors Brewing Company. The Coors Brewing Company has quality requirements distinct from those of other U.S. brewers, who use American Malting Barley Association (AMBA) approved varieties. Based on the pedigrees of Galena and Moravian-14 (Mor-14), achieving this distinct quality profile has meant greater use of European germplasm. The North American 2-row spring types form a second group. Such barleys are often bred with the objective of malting quality, but a selection with exceptional agronomic performance and unacceptable quality may be released as a feed type. Therefore, in this group we see a mix of feed and malting varieties. Interestingly, Baronesse, a German 2-row spring barley that has shown exceptional adaptation throughout the Western U.S., is distinct from both the North American and European 2-rows but appears in the same general cluster. Also loosely allied with this group are Ko A, an older Japanese 2-row malting barley with one European

parent, and Q21861, a spring 2-row accession of unknown pedigree obtained from the ICARDA/CIMMYT breeding program.

Six-row genotypes from the Midwestern U.S., a notoriously inbred group, form the second cluster. Loosely grouped with the contemporary Midwestern 6-rows is Chevron, one of the original land races introduced into the region. Recently, Chevron has been a target of renewed interest in the Midwestern U.S., due to its resistance to head scab. Colter, a Western U.S. 6-row has Midwestern genotypes in its pedigree.

The third cluster consists of a group of winter barleys of diverse geographic origin and spike morphology. The inclusion of Gobernadora, a spring 2-row, in this group may be attributable to the winter barleys in its pedigree. Steptoe, the most loved and hated barley in the Western U.S., is a facultative 6-row. Here, as always, it is in a class by itself.

A number of ICARDA/CIMMYT selections form a fourth, weaker, cluster, together with CI10587, a germplasm of unknown pedigree resistant to stripe rust and Russian Wheat Aphid. The fifth cluster consists of Nepalese, Korean, and Chinese land races and Tambar 500, a winter 6-row from Texas, USA. The presence of "Omugi" in the pedigree of Tambar 500 may account for its presence in this cluster.

When examined in terms of PCoA, there was a striking separation of the Midwestern U.S. 6-rows in the first quadrant. Primarily facultative and winter 6 -rows were found in the second quadrant and spring 2-rows in the fourth. A number of diverse genotypes were found in the third quadrant. The first and second principal coordinates accounted for 13% and 9% of the total variation in genetic similarity estimates.

Both interpretations of genetic similarity support a biologically meaningful basis for AFLP variation. The narrow genetic base of germplasm groups in Europe and North America has been commented upon and lamented. However, germplasm bases continue to erode because breeders are still making progress and exotic germplasm introgression threatens to disrupt carefully constructed genetic backgrounds. In contrast, the ICARDA/CIMMYT program has promoted the idea of mixing gene pools. Selections from this effort are found throughout the cluster diagram and dispersed throughout the third quadrant.

AFLPs are a powerful tool for rapidly generating biologically meaningful similarity matrices. The results of these preliminary analyses indicate that the choice of mapping parents seems to have adequately sampled principal North American and European germplasm pools. A subset of the AFLP loci used for this diversity analysis was mapped in the Harrington x Morex map population. When the allelic relationships of AFLPs in different map populations are established and QTL data are integrated, this marker technology may perhaps be useful in designing matings for accumulating favorable alleles. In this way, exotic germplasm introgression may not be such a risk-prone endeavor. Once tools are available for the routine characterization and manipulation of genetic resources, there will be greater impetus for their conservation.

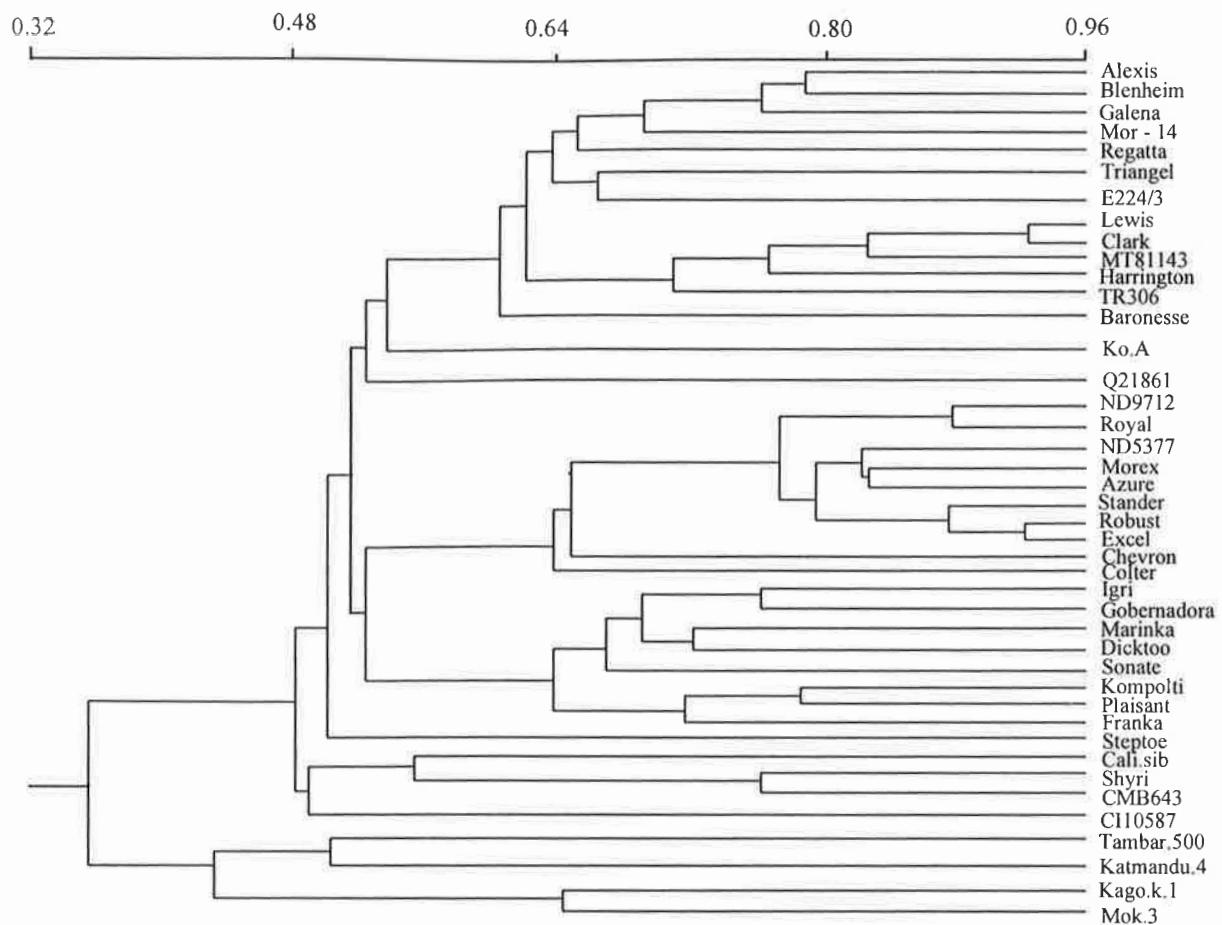


Figure 1. Cluster analysis of genetic similarity based on AFLP analysis of barley genotypes used as mapping population parents.

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Application of AFLP Markers to QTL Detection in Barley. P.M. Hayes and A. Pan, Oregon State University, Corvallis, OR 97331, USA; W. Powell, E. Baird, B. Thomas, R. Waugh, and N. Duncan, Scottish Crop Research Institute, Dundee, DD25DA, Scotland; I. Karsai and Z. Bedo, Martonvasar Research Institute, Martonvasar H2462, Hungary

Introduction. Winterhardiness in cereals is the final expression of a number of interacting component traits (Hayes et al., 1996). Responses to selection for winterhardiness have been limited, leading some to conclude that genetic variation may be exhausted. QTL analysis allows for dissection of complex phenotypes. Determining the genome locations of the components of winterhardiness may allow for selection response. A prerequisite for comprehensive QTL detection is a linkage map providing good marker density and complete genome coverage. RFLP markers have been used to construct excellent barley linkage maps (Graner et al., 1991; Kleinhofs et al., 1993; Kasha et al., 1995). However, RFLP-based map construction is a time-consuming proposition. Amplified Fragment Length Polymorphism (AFLP) technology has the potential to generate significant volumes of marker data in a short time frame. Becker et al. (1995) recently provided a detailed report on the addition of AFLP data to an existing barley linkage map. The objectives of our investigation were to determine the utility of AFLP marker data in developing a robust linkage map and to reanalyze QTL data for a number of critical winter growth habit-related phenotypes with the resulting map.

The Dicktoo x Morex doubled haploid (DH) population has been the subject of extensive collaborative mapping of winterhardiness-related traits (Hayes et al. 1993; Karsai et al. 1995; Oziel et al. 1996; Pan et al. 1994; van Zee et al. 1995). These analyses were based on a 78-point map, developed in the course of several years of collaborative mapping. A number of gaps remained in the map and markers could not be found to increase density in key regions of the genome. In a matter of months, 249 AFLP markers were mapped on a subset of 92 lines.

Materials and Methods. The AFLP methodology was essentially as described by Zabeau and Vos (1993). Template DNA was prepared using the restriction enzyme combination EcoRI/MseI. Two specific double stranded adapters were ligated to digested genomic DNA. Neither of the adapters was biotinylated and the selection step using streptavidin coated magnetic beads was omitted. The digested and ligated DNA was preamplified using an EcoRI directed primer and an MseI directed primer. The primers did not have additional selective nucleotides at the 3' end. Selective amplification was carried out using adapter directed primers. Nineteen different primer combinations were used, each combination consisted of one 'Eco' primer and one 'Mse' primer. All the primers had three selective nucleotides at the 3' end. In each case the 'Eco' primer was radiolabelled using ^{33}P ATP as described by Vos et al. (1995).

Linkage maps were constructed with JoinMap (Stam, 1993) and GMendel (Holloway and Knapp, 1994). JoinMap was used to construct the initial map based on all available data. Using a linkage LOD of 5.0, seven linkage groups were obtained. As these groups each contained markers which had been previously mapped to an individual chromosome (Pan et al. 1994), each linkage group was considered to represent an individual barley chromosome. The robustness of each linkage group was then tested by raising the linkage LOD to 7.0 and any markers which did not remain in the linkage group were excluded. JoinMap was then used to order the loci within each linkage group, using a MAPLOD of 0.05. Some RFLP and AFLP markers were found to

give a significant increase in the total chi-square at the 0.01 level and were removed from the data set. The robustness of the ordering was then tested by comparing the ordering with that produced with a MAPLOD of 0.05. The final maps were based on 289 markers, 216 of which are AFLPs. GMendel was used to construct a skeleton linkage map. All marker data were first assigned to linkage groups. Subsequently, linkage maps were developed separately for each chromosome using the kSAR ordering function. Locus orders were assessed with Monte Carlo and bootstrap simulations. Considerable map expansion was observed when all available data were retained. Data points were then progressively until loci that are in common with other linkage maps are in comparable orders and at similar distances. There are 107 loci in this map, of which 49 are AFLPs. The Simple Interval Mapping (SIM) and Simple Composite Interval Mapping (sCIM) approaches of Tinker and Mather (1995) were used for QTL detection in conjunction with the skeleton linkage map. Each data set was analyzed with 1,000 permutations, a 5 cM walk speed, and a Type I error rate of 5%. Nineteen background markers were specified at an approximately even spacing of 20 cM.

Results and Discussion. The primary map built from 289 data points shows a distribution of AFLPs with clustering typical of high density molecular-marker-based linkage maps. AFLP markers filled critical gaps, joining linkage groups on chromosomes 1, 2, 3, and 5 that had remained unlinked in the 78-point map. Additionally, AFLP markers mapped to regions of key interest and importance, such as the region on chromosome 7 extending from *mR* to *BCD265b*. The skeleton map provides good coverage of all chromosomes (Figure 1) and distance of RFLP loci are in good agreement with published maps. On eight of the fourteen chromosome arms, AFLP markers extend linkage maps beyond reference loci of known position.

The results of SIM and sCIM QTL analysis were similar. The latter analysis provided higher resolution of QTL position than reported by Pan et al. (1994). No new QTL were detected, allele phase remained the same, and both allele value and phenotypic R^2 values were comparable. However, AFLP markers were found in regions of the genome that are of key interest. For example, five AFLPs map in the chromosome 7 interval between *mR* and *BCD265b*.

AFLPs are a useful tool for rapidly fleshing out a linkage map. AFLPs should minimize the effort required to construct genome maps and thus let one focus on the biology that drives these investigations. In that vein, the results of this investigation are of interest and utility from a number of perspectives. They position photoperiod, vernalization, crown fructan content and low temperature tolerance effects in a fashion that allows for direct comparison with other barley mapping populations (i.e Laurie et al., 1995) and provide tools for elaborating on interspecific and intergeneric relationships (i.e. Paterson et al., 1995). They provide a catalog of tools for manipulating components of winter growth habit with the objective of breeding winter habit malting barley. Finally, additional markers may represent leads to unraveling the complexities of the components of winterhardiness.

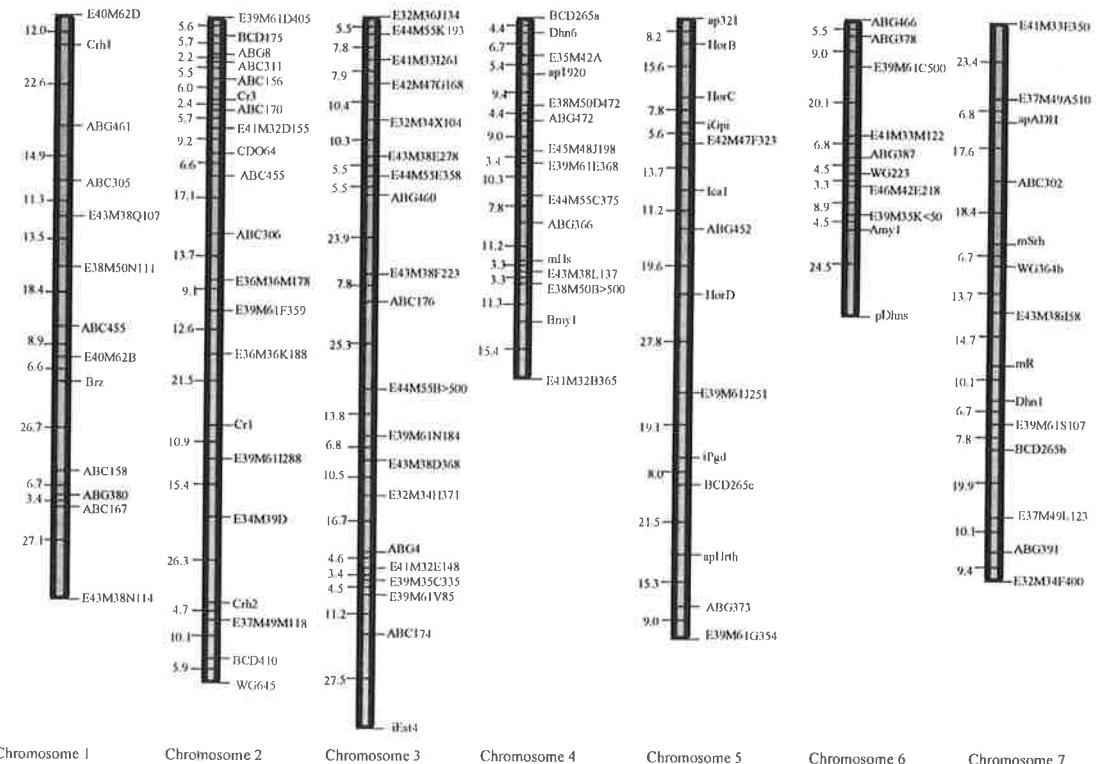


Figure 1. Linkage map based on 91 DH lines from the cross of Dicktoo x Morex.

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Tagging net blotch resistance in barley using RAPDs. L.E. JAMES^{1,2}, K.J. KASHA¹ and S.J. MOLNAR²,¹ Crop Science Dept., University of Guelph, Guelph, Ontario N1G 2W1, Canada; ² Eastern Cereal and Oilseed Research Ctr., Agriculture and Agri-Food Canada, Central Experimental Farm, Ottawa, Ontario K1A 0C6, Canada.

Introduction. Net Blotch is a common disease of barley and is becoming increasingly prevalent in Canada. Complicated genetic interactions determine resistance to the fungal pathogen *Pyrenophora teres* Drechs. f. sp. *maculata* Smedeg and multiple resistance genes have been implicated. Molecular tags for resistance genes would be useful to breeders as they could be used to track resistance during preliminary selections and to pyramid resistance into new populations (Choo et al. 1994). A marker could be used as a diagnostic tool in preference to inoculating the plant with the pathogen and without the need for quarantine facilities. The purpose of this study was to use Randomly Amplified Polymorphic DNA (RAPDs) to obtain markers for resistance to net blotch.

Materials and Methods. Leger is a well adapted Eastern Canadian barley cultivar which is resistant to the spot form of net blotch caused by isolate WRS #857 while CI9831 is an introduction line which is susceptible (Choo et al. 1994). A cross was made between these lines and 125 doubled haploid (DH) lines produced by the Bulbosum method (Kasha and Kao 1970) as described by Choo et al. (1993). Disease testing was done by Dr. A. Tekauz at Cereal Research Ctr., Agriculture and Agri-Food Canada, Winnipeg, as described by Tekauz and Mills (1974) and reported by Choo et al. (1994) and Ho et al. (1996). A reaction rating of 1-3 represents resistant plants, 7-9 susceptible plants and 5 as intermediate phenotype (Tekauz 1985). A set of 93 DH lines (49 susceptible and 44 resistant) gave repeatable scores.

Total genomic DNA was isolated from leaves of seedlings (Molnar et al. 1989). Following the strategy of bulked segregant analysis (BSA)(Michelmore et al. 1991), four DNA bulks were made, two based on susceptibility and two on resistance. Polymorphism was assayed using RAPDs and the polymerase chain reaction (PCR) essentially as described by Williams et al. (1990). Polymorphisms between the two susceptible and the two resistant bulks were tested across all the individuals in the DH population. Linkage was determined using the computer program MapMaker (Lander et al., 1987).

Results. Of the 500 random decamer primers screened against Leger and CI9831, 195 gave useful polymorphic patterns. Seven of these showed an association with resistance or susceptibility on the bulks and were mapped over the entire DH population. The RAPD markers formed three linkage groups of four, two and one marker respectively. The cluster of four also linked to the Vv (row number) and Re2re2 (lemma colour) loci and to an unrelated RAPD marker, indicating that this linkage group is on chromosome two.

BSA was originally designed to recover markers to a single locus by using bulks to mimic near isogenic lines. The molecular alleles present in the individual DHs that constituted each bulk were examined and showed mixed genotypes consistent with a multigenic trait.

Discussion. The DHs, when earlier tested, had a segregation ratio of 1:1, and the F1 was resistant, suggesting a single dominant gene (Choo et al. 1994). However, an F2 population made from the same F1 as the DHs segregated 128R:78S (Ho et al. 1996) which fits a 9:7 ratio, indicative of a two gene model with complementary gene interaction. We propose a genetic model for resistance that is consistent with molecular, DH and F1 data. If resistance is caused by having two out of three dominant genes {ie. any of i) R1R1 R2R2 R3R3, ii) R1R1 R2R2 r3r3, iii) R1R1 r2r2 R3R3 or iv) r1r1 R2R2 R3R3} a 4:4 ratio would be expected in the DH population. This genetic mechanism seems to be the best explanation of the segregation ratios and is consistent with the BSA results. However, the expected ratio for the F2 population would be 54:10 for this genetic mechanism. Clearly additional test crosses will be required to clarify the potentially complex genetics of resistance. In other germplasm, resistance to isolate WRS 857 has been reported to be due to a major gene plus a modifier gene with no resistance of its own in barley CI5791, due to three major genes in barley CI9214 and due to two major genes and a minor gene with some resistance in barley Heartland (Legge and Tekauz, 1993).

In conclusion, this study has shown that it is possible to use RAPDs and bulked segregant analysis to start sorting out complex genetic traits and to obtain markers for separate loci simultaneously. This study has obtained seven markers at three loci, which may represent three separate genes for resistance to net blotch.

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General approaches to the conversion of RFLPs to informative STS-PCR markers for yield, dormancy, and spot blotch QTLs in Barley. S.R. LARSON¹, B.J. STEFFENSON² and T.K. BLAKE¹, ¹ Plant and Soil Science, MSU, Bozeman, MT, 59717. ² Department of Plant Pathology, NDSU, Fargo, ND, 58105

Introduction. Low copy number RFLP markers, detected using Southern analysis, are excellent tools for generating robust linkage maps. With an abundant supply of clones and several restriction enzymes, sufficient polymorphic information content is generally available to construct complete RFLP linkage maps (Botstein et al. 1980) This technique has been effectively used to construct complete linkage maps in relevant crosses of many crop species and to identify genes controlling agronomical traits. However, this technique can be cumbersome when applied to practically-oriented plant breeding programs. The DNA sequences of clones mapped using Southern analysis of RFLPs, can be used to design oligonucleotide primers for PCR. The conversion of these sequence tagged sites into polymerase chain reaction based markers (STS-PCR) has the potential to make these markers more practical tools in crop improvement. However, techniques must be available to develop STS-PCR markers which are informative in the germplasm of interest. These STS-PCR markers must cosegregate with the RFLP locus from which they derive, and should also provide codominant and reliable assays. We describe approaches to convert RFLPs for yield (Hayes et al. 1993, Thomas et al. 1995), dormancy (Ullrich et al., 1992) and spot blotch (Steffenson et al.) QTLs to STS-PCR markers.

Methods and Results. The polymerase chain reaction (PCR) was conducted using standard procedures and 50° C annealing temperatures for all primer sets, and a rapid extraction protocol to prepare DNA samples. The clones used to design STS-PCR primers included six barley genomic clones (ABG) and one barley cDNA clone (ABC). Primers were also designed from the wheat genomic (WG110) and oat cDNA (CDO113) clones and the wheat cDNA clone (PSR128). These clones, described by Kleinhofs et al. (1993), were end sequenced near the vector insertion points so that primer sequences could be selected using OLIGO (National Biosciences, Plymouth MN). Oligonucleotide primers were synthesized using the Model 391 PCR-MATE DNA synthesizer (Applied Biosystems, Foster City, CA). When necessary, the PCR products were cloned using the Invitrogen TA Cloning Kit (San Diego, CA), and sequenced using the Sequenase Kit (USB, Cleveland, OH). GENEPOR software (Riverside Scientific, Bainbridge WA) was used for sequence and restriction site polymorphism analysis. In the case of ABG377, denaturing gradient gel electrophoresis (DGGE) was used to test for polymorphism. Otherwise, sequence polymorphisms were tested using restriction endonuclease digestions or ribonuclease cleavage/protection of heteroduplex PCR molecules. The Mismatch Detect kit (Ambion, Austin TX) was used to test for sequence polymorphism, using a ribonuclease protection assay (RPA), in the WG110 and ABC161 STS-PCR products. All STS-PCR products and RFLPs are analyzed using 6% polyacrylamide gel electrophoresis.

Markers for the chromosome 3P yield QTL (Hayes et al. 1993)

ABG070. PCR-STS primers for the ABG070 clone amplify approximately 430 bp products in Steptoe and Morex. Nine out ten recognition sites identified in a survey of

26 endonucleases, showed polymorphisms between Steptoe and Morex.

ABG057. An estimated 1250 bp amplification product was obtained using ABG057.11 primers. These Steptoe and Morex amplification products were cloned and partially sequenced. A second set of primers ABG057.22 were designed that reliably amplify a 215 bp STS-PCR products containing and an *HphI* restriction site polymorphism, which cleaves the Steptoe product as predicted from sequence analysis.

CDO113. The wheat-barley chromosome addition lines (Islam et al. 1981) were used to identify a CDO113.11 STS-PCR amplification products unique to barley chromosome 3. These primers were designed from the original oat CDO113 clone. A low-amplification 186 bp PCR product unique to chromosome 3 was identified. Homologous, amplification products from Steptoe and Morex were cloned and sequenced. Primers CDO113.23, redesigned from the barley chromosome 3 PCR products, amplify a single 186 bp product. A single base pair polymorphism was observed among these sequences. This polymorphism is recognized by the *BsaMI* endonuclease in the Steptoe genotype and maps near the largest yield QTL reported by Hayes et al. (1993). This STS-PCR marker is not allelic to the intended CDO113 RFLP marker from which it was derived (on chromosome 3M).

ABG396. The STS-PCR primer from the ABG396 clone amplify 388 bp products in Steptoe and Morex. The Steptoe and Morex ABG396 PCR products were cloned and sequenced. A sequence polymorphism in a *HinfI* recognition site was observed in the amplification product from the Morex PCR clone (used in sequencing) but not in the product obtained from amplification of Morex genomic DNA. A second Steptoe/Morex sequence polymorphism for the *MnII* endonuclease has been confirmed.

ABG377. The ABG377 PCR-STS amplifies products of about 520 bp in Steptoe and Morex. This was were surveyed with 17 arbitrarily chosen restriction endonucleases which failed to reveal polymorphism. To determine whether sequence polymorphism actually existed, Steptoe, Morex and Steptoe/Morex duplexes were analyzed using DGGE. The heteroduplex showed reduce electrophoretic mobility, suggesting that sequence polymorphism existed. Steptoe and Morex STS-PCR products were partially sequenced, until restriction site polymorphism (*SspI*) was observed.

Markers for 3M yield QTL (Hayes et al., 1993 and Thomas et al., 1995)

WG110. The WG110.11 primers derived from wheat sequence amplified several products from barley. A 888 bp product that amplified DNA from the barley-wheat 3H addition line (Islam et al. 1981) was cloned and sequenced. A second set of primers (WG110.22) was designed from this sequence. Sequence analysis of the Steptoe and Morex WG110.22 amplification products revealed a single bp mutation. However, this difference was not recognized by the endonucleases searched using GENEPY. A third set of primers, WG110.33, were designed to test this single bp polymorphism using a ribonuclease protection assay. As predicted from the sequence analysis, a heteroduplex of Steptoe and Morex RNA transcripts is cleaved. No polymorphisms were detected in crosses of Blenheim/MT81143 or Baroness/Lewis. Our objective was to test the yield QTL hypothesis, reported by Thomas et al. 1995, in these breeding populations.

ABC161. We also attempted to identify polymorphism in the Blenheim/MT81143 and Baroness/Lewis populations using STS-PCR primers designed from the ABC161 clone. Nested phage primer sets amplify 1.4 kb (estimated) products in Steptoe,

Blenheim, MT81143, Baroness, and Lewis. These same primers amplify a 1.1 kb product in Morex. The size of these products are much larger than the same STS-PCR product amplified from the ABC161 cDNA clone (100 bp), suggesting that one or more introns exist for this locus. The Blenheim/MT81143 and Baroness/Lewis lines parents were screened with 42 restriction endonucleases which failed to detect any sequence polymorphisms. We have also attempted to RPA RNA transcripts from these crosses, however this has been technically difficult with these relatively large RNAs (the kit is designed to test transcripts typically 0.5-1.0 kb).

STS-PCR marker for Morex spot blotch resistance locus on chromosome 5

ABG452. The ABG452 STS-PCR primer set showed efficient amplification of single 600 bp product from Steptoe, Morex, and Harrington DNAs. The PCR products from these three genotypes were screened for restriction site polymorphism using 38 endonucleases. Three restriction site polymorphisms were identified: *MnlI*) one additional site in Morex, *HphI*) one site in Steptoe only, and *DdeI*) one less site in Steptoe.

STS-PCR for two Steptoe/Morex seed dormancy QTLs on chromosome 7

PSR128. The PSR128 RFLP maps to a chromosome 7 locus with major effects on seed dormancy. PSR128 primers were designed from a 446 bp wheat cDNA with a predicted amplification of 385 bp. A small size difference in the single 510 bp amplification products of Steptoe and Morex can be observed. In addition , we identified a *TaqI* RFLP, after screening Steptoe and Morex PCR products with 8 endonucleases.

ABG391. The ABG391 RFLP maps near the second largest dormancy QTL also on chromosome 7. The ABG391 STS-PCR primers amplify a single 600 bp product in Steptoe and Morex. We identified a *RsaI* RFLP, after screening 8 endonucleases.

Conclusion. This paper describes ten well characterized STS-PCR products. All but one of these, CDO113, are allelic to the intended RFLP markers from which they derive. These are reliable, codominant assays that can be used to efficiently screen progeny . Although the approaches described are general, the success varied by locus. The STS-PCR primers which amplify single products are the most reproducible and most likely to show allelism with the intended RFLP markers from which they derive. These products should be the same approximate size as products amplified from genomic DNA clones, or larger in the case of cDNA clones. The informativeness of each primer set varies (compare WG110 and ABC161 vs ABG070). In general we were able to find useable sequence polymorphisms for most STS-PCR products larger than 350 bp but we frequently had trouble detecting polymorphism among smaller STS-PCR products (data not shown). In several cases, we found it useful to employ techniques such as sequencing, DGGE, and RPA in order to detect polymorphism.

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Marker Conversion of RFLP Loci affecting Malting Quality in Harrington/TR306 Cross. SUNG-JONG LEE and GREG A. PENNER, Cereal Research Centre, Agriculture & Agri-Food Canada, Winnipeg, Manitoba, Canada, R3T 2M9

Introduction.

Quantitative trait loci analysis coupled with molecular maps of cereal genomes represents a powerful tool for increased understanding of polygenically inherited traits. To date, cereal maps have been primarily based on RFLP markers (Kleinhofs et al., 1993; O'Donoughue et al., 1995). Within the North American Barley Genome mapping project several RFLP markers have been identified as linked to genes governing grain quality traits. It has proven difficult to apply RFLP analysis in as timely a manner as is required for ongoing plant breeding selection. The objective of this study was to convert the following RFLP markers to allele specific amplicons. Analysis of allele specific amplicons requires very small amounts of relatively impure genomic DNA, resulting in start to finish analysis times of a few days. Analysis of allele specific amplicons also lends itself to automation.

RFLP Marker	QTL	Source of Primer Sequence	Remarks
Nar1	Fine coarse difference	Genomic clone in Barley	1 Intron (1.6 kb)
Amy1	Diastic power	RFLP Probes	3 sets of primers
Nar7	Diastic power	Genomic clone in Barley	2 Introns(117/175 bp)
ABG610	Low Beta-Glucan	RFLP Probe	+/- southern signal
ABC622	Lots of malting quality traits	RFLP Probe	-

Materials and Methods.

The Nar1(nitrate reductase) and Nar7 [NAD(P)H-bispecific nitrate reductase] primers were designed from barley genomic DNA clones (Schnorr et al., 1991 and Miyazaki et al., 1991, respectively). The Nar1 primers amplify over a large intron (1.6 kb), while the Nar7 primers amplify across two small introns(117 and 175 bp). Amy1(alpha-amylase) primers were designed from three different clones, clone-E, clone-46, and clone 6-4 (Rogers and Milliman, 1983; Khursheed and Rogers, 1988). Each amplicon was designed so as to avoid overlap among the clones. The amplification of the ABC622 locus was performed with primer sequences from Tom Blake (Montana State), and the ABC622 amplicons of both Harrington and TR306 were sequenced. The allele-specific amplicon of Harrington was developed on the basis of sequence differences between Harrington and TR306 at this locus. The ABG610 clone detected the presence or absence of a hybridizing genomic fragment during southern analysis of Harrington and TR306. This probe was directly sequenced and locus specific fragments were designed

based on this sequence information. OLIGO(Primer Analysis Software, NBI) was used to design the primers which ranged from 18 to 22 nucleotides in length and 58-61°C annealing temperature.

PCR amplification conditions were 1 min at 94 C, 1 min at 60 C and 1 min at 72 C for 35 cycles in a 25 ul volume at final concentration of 10 pmol of each primer, 30 ng genomic DNA, 200 uM of each dNTP(Pharmacia), 1.5 mM MgCl₂, 1 X Taq activity buffer(Perkin- Elmer), and 1 unit of Taq polymerase. Depending on each primer set, annealing temperature, MgCl₂ concentration, and the number of cycles were changed in the range of 58-64°C, 1.0-1.6 mM MgCl₂, and 35-40 cycles, respectively. PCR products was visualized on 1.6 % agarose gels stained with 4 ug Ethidium bromide/ml of agarose and photographed with Polaroid film. PCR fragments were cloned into pUC 18 by blunt-end ligation(T-tailing Method). These clones were cycle-sequenced by the DNA Service Unit at the Plant Biotechnology Institute (NRC, Saskatoon; Saskatchewan, Canada).

Results and Discussion.

Initial success at RFLP marker conversion (Penner et al., 1995) has been frustrated by a lack of sequence variation and a failure to convert sequence variation, where it has been identified, into allele specific amplification. In this study, attempts were made to specifically amplify intron regions rather than coding sequence (Nar1 and Nar7). In the case of the Amy1 locus, an attempt was made to simultaneously convert three tightly clustered loci into allele specific amplicons. It was thought that an RFLP probe that exhibits variability in ability to anneal to a specific restricted genomic fragment, rather than one that exhibits a co-dominant annealling pattern, might be easier to convert to an allele specific amplicon.

This study was not complete at the time of abstract submission. A complete discussion of results along with copies of the sequences of both the primers developed and the sites that they amplify will be presented in the poster.

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Identification of QTLs controlling abscisic acid concentration in barley leaves.
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Introduction. Abscisic acid (ABA) concentration in plant tissues increases sharply as a result of water stress. ABA accumulation modulates the adaptive response of the plant to drought (Quarrie, 1991) and triggers the synthesis of specific proteins (Bray, 1993). Molecular markers provide a powerful tool to identify the QTLs controlling ABA concentration and verify their possible effects on other agronomically important traits. Our objectives were to identify QTLs controlling leaf ABA concentration and other agronomic traits in a set of barley lines.

Materials and methods. Seventy-one double-haploid (DH) lines, derived from anthers of 'Arda (2-rowed type) x Opale (6-rowed type)' (Noli et al., 1994), were evaluated near Bologna (Italy) according to a RCBD with five reps (3-m-long single-rows; 40 cm between rows) in 1994 and four reps (1.2 m x 3.0 m drilled plots) in 1995. In 1994, ABA concentration in the flag leaf (L-ABA) was determined \approx three weeks after heading; in 1995, L-ABA was determined \approx three weeks before and one week after heading. Heading date and plant height were also investigated in both years. Grain yield and kernel weight were measured only in 1995. ABA concentration was determined with a radioimmunoassay technique on duplicate samples of crude aqueous extracts of leaf disks. Molecular analysis was carried out with 60 RAPDs, 25 RFLPs and 7 STSs for which Arda and Opale showed polymorphism. Details on the construction of the linkage map are reported elsewhere in this volume (Noli et al.). QTL analysis was performed using the computer program qGene, kindly provided by C. Nelson. For each trait analyzed, independent markers with significant effects were identified (F test) and included in a multiple regression equation.

Results and discussion. Ten DH lines which were characterized by poor germination in 1994 were excluded from the analysis. Table 1 reports the results of the ANOVA, means, and broad sense heritability of the investigated traits.

Table 1. Means of the parental lines and derived doubled-haploid lines, F values of the ANOVA among lines and broad sense heritability values estimated on a line mean basis.

	Parental lines		Doubled-haploid lines				
	ARDA	OPALE	Mean	Min.	Max.	F	h^2_B
<u>1994</u>							
L-ABA (ng/g DW)	172	271* (2)	221	132	348	** (3)	0.67
Heading date (d) (3)	30.5	36.8**	35.3	28.4	42.8	**	0.86
Plant height (cm)	69.4	56.8**	58.6	46.9	89.1	**	0.94
<u>1995</u>							
L-ABA-1 (ng/g DW)	243	191	206	143	261	**	0.59
L-ABA-2 (ng/g DW)	352	265**	321	223	452	**	0.47
Heading date (d) (3)	18.2	36.0**	28.0	18.0	40.3	**	0.89
Plant height (cm)	92.1	82.2**	86.8	67.6	113.6	**	0.98
Grain yield (Mg/ha)	5.73	3.38**	4.29	1.73	6.72	**	0.83
Kernel weight (mg)	31.2	26.9	31.1	20.5	47.2	**	0.81

(1) Days from April 1.

(2) *, **: comparison between Arda and Opale significant at P 0.05 and 0.01, respectively.

(3) **: differences among DH lines significant at P 0.01.

Highly significant differences among DH lines were present for all traits. A very strong "DH

"line x year" interaction ($P < 0.01$) was found for L-ABA and the correlation between the DH means in 1994 and the means of the two sampling dates in 1995 was very low ($r = 0.13$ and $r = 0.11$, respectively). The parental lines were also strongly affected by environmental conditions. These findings could be related to differences in growth stage and level of water stress which characterized the plants investigated in the two years. In 1994, due to heavy rainfall before and during heading, collection of leaf samples was delayed until the soil was reasonably dry; in 1995 it was possible to collect samples under sufficiently dry conditions at an earlier growth stage. In 1995, a significant correlation was found among the L-ABA values of the DH lines at the two sampling dates ($r = 0.54$; $P < 0.01$). Broad sense heritability, computed on a mean basis, for L-ABA was lower than that of other traits, thus indicating a stronger influence of the environment on the expression of L-ABA. The genetic map obtained with 94 molecular markers covers 528 cM, which represents $\approx 35\text{-}40\%$ of the length estimated for the barley genome (Graner et al., 1991; Sherman et al., 1995). It should be mentioned that in our case the average distance among sets of linked RFLP markers was $\approx 30\%$ less than that reported in the published maps. The chromosomal location of the markers with significant ($P < 0.05$) effects on L-ABA and the other investigated traits is reported in Fig. 1.

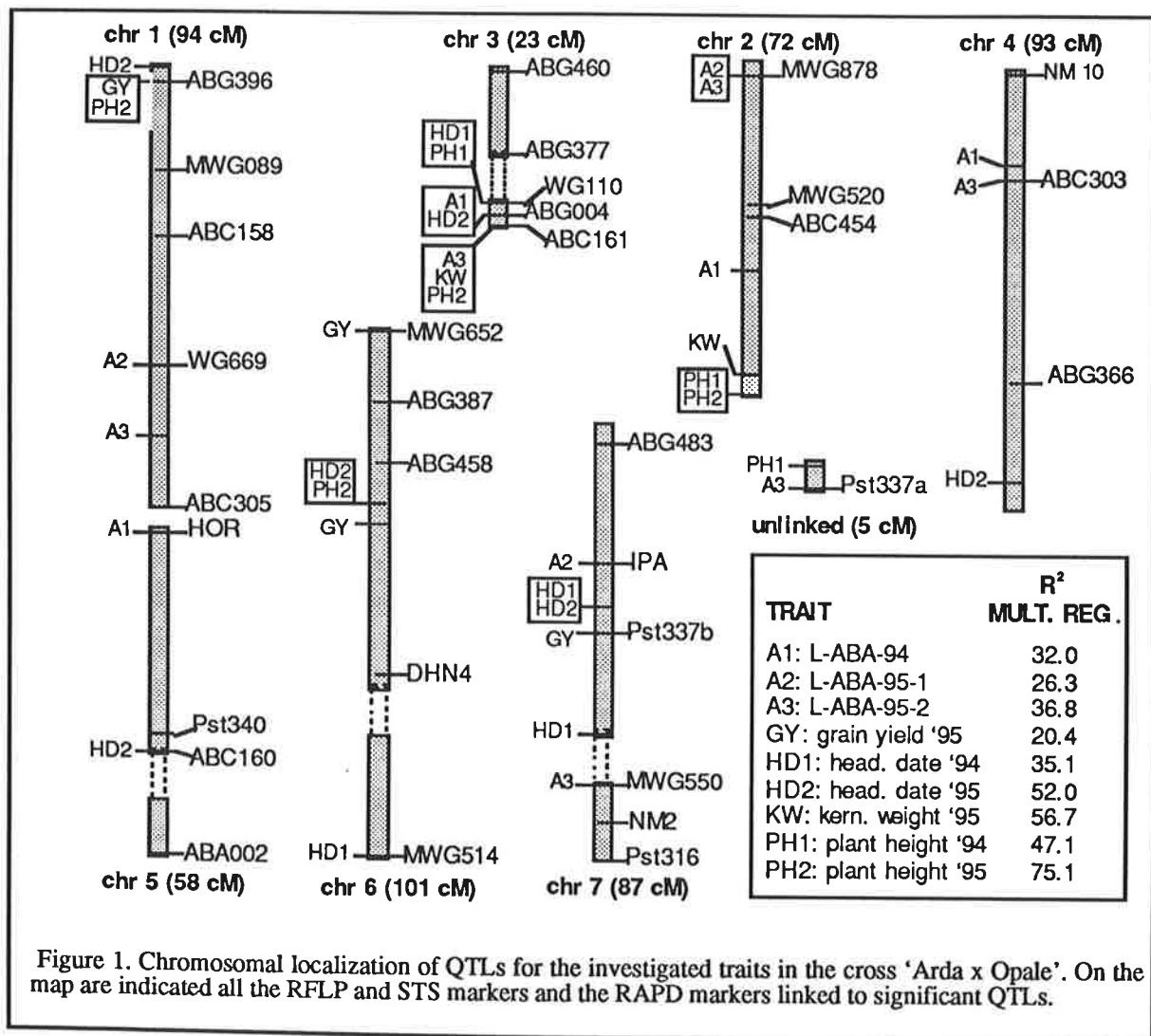


Figure 1. Chromosomal localization of QTLs for the investigated traits in the cross 'Arda x Opale'. On the map are indicated all the RFLP and STS markers and the RAPD markers linked to significant QTLs.

For the sake of clarity, the map only reports the nomenclature of the RFLP and STS markers. If tightly linked markers showed significant effects, we considered only that with the highest F value. The number of QTLs showing significant effects on L-ABA in 1994 and in the two samplings in 1995 were 4, 3, and 6, respectively. None of the QTLs was common to all samplings. One region on chromosome 3 and one region on chromosome 4 showed

significant effects in 1994 and at the second sampling in 1995. One QTL on chr. 2 significantly affected L-ABA at both samplings in 1995. In 1994, Opale contributed 3/4 of the superior alleles at the QTLs influencing L-ABA, while in 1995 Arda contributed a greater portion of such alleles (2/3 and 3/6 at the first and second sampling, respectively). For the region on chr. 3, Opale increased L-ABA in 1994 while in 1995 L-ABA was increased by Arda. These results are in accordance with the L-ABA values which characterized the parental lines. The phenotypic variation among DH lines accounted for by the QTLs included in the multiple regression was equal to 32.0% in 1994 and 26.3% and 36.8% in the 1995 samplings, respectively. In consideration that our map covers only a portion of the barley genome, it is likely that other QTLs will be evidenced once a more complete map will be available. We are currently analyzing other RAPD and RFLP markers in order to extend the length of the map. The different number and location of the QTLs found in the three samplings could be related, not only to effects due to differences in growth stage and environmental conditions, but also to the number of progenies that were tested; recent simulation work has indicated that several hundreds of progenies have to be tested in order to reliably identify the effects and positions of QTLs of low heritable traits (Beavis, 1994). The physiological basis of the QTLs for L-ABA should not be related to the metabolic steps involved in ABA biosynthesis and/or catabolism. In fact, only one biochemical step has been suggested as the main factor limiting the rate of ABA synthesis. If this were the case, then, provided allelic variation was present at the corresponding locus and nearby markers, only one QTL should be evidenced. Previous studies have indicated a polygenic control of the concentration of ABA in cereal leaves (Quarrie, 1991; Sanguineti et al., 1994; Landi et al., 1995). It is more plausible that the QTLs for L-ABA evidenced in this and in previous studies identify allelic variation influencing morpho-physiological traits controlling the water balance of the plant (e.g. root structure, stomatal frequency, etc.) and, consequently, the concentration of ABA in plant tissues. Some of the QTLs may represent loci controlling the level of ABA biosynthesis through the intensity of the transduction signal associated to the loss of turgor, the main determinant of ABA biosynthesis. Some of the markers which showed significant effects on L-ABA, also showed significant effects on other traits (Fig. 1). In 1995, none of the L-ABA QTLs showed partial overlapping with yield QTLs, thus not supporting evidence for variation in grain yield being associated to variation in L-ABA. Indeed, the correlation between L-ABA and yield was not significant.

Conclusions. Our results indicate a strong influence of the environment and/or growth stage on the QTLs controlling the expression of L-ABA in barley. Due to the high "genotype x environment" interaction of L-ABA, it seems that to adequately estimate the effects of the QTLs controlling this trait it will be necessary to analyze a large number of environments.

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Molecular markers for *Pg9* and *Pg13* stem rust resistance genes in cultivated oat.
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Introduction. Stem rust caused by *Puccinia graminis* Pers. f. sp. *avenae* Eriks. & Henn. is an important disease of cultivated oat (*Avena sativa* L.). All the oat cultivars currently recommended for the Canadian eastern prairies carry either the resistance gene combination *Pg2* and *Pg13* or the combination, *Pg2*, *Pg9* and *Pg13*. Genes *Pg9* and *Pg13* have provided effective resistance since the early 1980s so that it is important to retain both in the new cultivars developed for this region. Molecular markers would be particularly useful for *Pg9*, since there are no available stem rust races that will detect the presence of *Pg9* in the presence of *Pg13*. *Pg13* has been shown to be linked to a 56.6-kDa polypeptide locus resolved by SDS-PAGE and *Pg9* to an avenin band resolved using acid-PAGE (3,1). It is known that some of the stem rust resistance genes are clustered within the oat genome and that some are associated with genes conferring resistance to crown rust (*P. coronata* Cda. f. sp. *avenae* Eriks.). Gene *Pg9* is either tightly linked in coupling or pleiotropic to a crown rust gene, designated *PcX*, in Dumont (1).

The objectives of the current study were: 1) to identify RFLP (restriction fragment length polymorphism) and RAPD (random amplified polymorphic DNA) markers for the two stem rust resistance genes, *Pg9* and *Pg13*, and 2) to locate these genes and the associated markers within the hexaploid oat genome by comparative mapping. A detailed account of these and additional studies is in press (8).

Materials and Methods. The cultivar Dumont has both stem rust resistance genes *Pg9* and *Pg13* while line OT328 has no known stem or crown rust resistance genes (3,1). Rodney 0-*Pg9* and Rodney 0-*Pg13* are near-isogenic lines (NILs) developed by backcrossing the resistance genes *Pg9* and *Pg13*, respectively, into the susceptible line Rodney 0 (3). Determinations of linkage relationships between the genes *Pg9* and *Pg13* and putative markers were carried out on segregating F3 families derived from the following three crosses: OT328 x Dumont, Rodney 0 x Rodney 0-*Pg9* and Rodney 0 x Rodney 0-*Pg13*. The F3 families of the OT328 x Dumont cross were taken from the same segregating population used in a previous linkage study of protein markers to these two genes (1).

Segregations of the 92 F3 families of the Rodney 0 x Rodney 0-*Pg9* cross for *Pg9* and *PcX* resistance were tested with the stem rust race NA27 and the crown rust isolate CR192, respectively. Segregation of the 95 F3 families of the Rodney 0 x Rodney 0-*Pg13* cross for *Pg13* resistance was tested with stem rust race NA55. The absence of *Pg9* and *Pg13* resistance in the cultivars Kanota and Ogle was determined by inoculation with race NA27. The methodology and results of rust resistance evaluations of the 88 F3 families from the OT328 x Dumont cross segregating for the genes *Pg9*, *Pg13* and *PcX* using NA27, NA25 and CR192 have been described (1). As there are no stem rust races that will detect the presence of *Pg9* when *Pg13* is also

present, the tight linkage (in coupling) of *PcX* to *Pg9* allowed identification of *Pg9* in F3 families that were homozygous resistant or segregating for *Pg13* resistance.

A set of 174 clones which had been mapped in cultivated oat by O'Donoughue et al. (1995) were used to detect RFLPs. In addition, because of the known association between endosperm proteins and the *Pg9* and *Pg13* genes, pOP6, an oat prolamin (avenin) genomic clone and MOG12, an oat globulin cDNA clone, kindly provided by Dr. Illimar Altosaar of the University of Ottawa, Ottawa, Canada, were also used.

To identify putative markers for *Pg9* and *Pg13*, survey filters carrying single digests (using *Dra*I, *Eco*RI or *Eco*RV) of DNA were prepared. Clones exhibiting RFLPs between the NILs were considered putative markers. Dumont and OT328 were checked simultaneously for the presence of the same polymorphisms. DNA extractions and digestions, Southern blotting, and hybridizations were performed as described by O'Donoughue et al. (9,10). DNA from the NILs was also surveyed with 200 random primers. Any putative markers were then checked for polymorphisms between Dumont and OT328. Bulked segregant analysis (BSA) (7,2) was also performed using 127 random primers and bulks differing for resistance at the *Pg9* and *Pg13* loci. Primers detecting a polymorphism between the differential bulks were considered putative markers. The RAPD protocol used was as described in Wight et al. (12).

Linkage between the stem rust resistance genes and markers was determined using DNA from individual F3 families (pooled from 6 to 12 individuals per family) from the segregating populations. The scores for the 56.6 kDa polypeptide locus and the avenin B2 band, in the OT328 x Dumont population (1), were kindly provided by Dr. James Chong and coauthors. Linkage maps were obtained using the program Mapmaker V2.0 (4). Localization of the stem rust resistance genes and associated markers was achieved by comparative mapping with the *A. byzantina* C. Koch cv. Kanota x *A. sativa* L. cv. Ogle map (9) and a diploid oat map (10).

Results and Discussion. In this study, five new markers were identified for the *Pg9*-*PcX* complex. In total, six markers are now available for *Pg9*, including the avenin B2 marker first reported by Chong et al. (1), three RFLPs and two RAPDs. Two of the three RFLP markers (Xcd01385F and XpOP6(A)) were identified using NILs and the RAPD marker Xacor458A using BSA. The RFLP marker Xumn101A was discovered through comparative mapping with the Kanota x Ogle. Primer UBC195 (Xacor195A; identified as ACOpR-2 in ref.11) was surveyed with DNA from all parental lines because of its known association with *Pg3* (11). Interestingly, the marker band is linked in coupling to *Pg9* in the OT328 x Dumont cross and was previously shown to be linked in repulsion to *Pg3* in a Rodney 0 x Rodney 0-*Pg3* cross, consistent with the report that *Pg3* and *Pg9* are either allelic or tightly linked in repulsion (5). The five new markers exhibit from 0 to 2.7% recombination with the *Pg9* locus. All markers either cosegregated with the *Pg9* locus or mapped to one side. Flanking markers were not identified. The association of *Pg9* with an avenin marker in the OT328 x Dumont cross has been demonstrated by Chong et al. (1). In the present study, an oat avenin genomic clone, pOP6, also detected an RFLP linked to *Pg9*.

Eleven new markers linked to the *Pg13* locus were identified in the present study. Nine of the new markers are RFLPs and two are RAPDs. Seven of the new

markers were mapped in both populations segregating for *Pg13* resistance. In total, twelve markers are now available for this locus, including the 56.6 KDa protein marker. All RFLP and RAPD markers for *Pg13* were identified using NILs except for Xacor254C, which was identified by BSA. The markers exhibited from 0 to 22.7% recombination with the *Pg13* locus. Again, most markers identified in this study cosegregated with, or mapped to one side of the *Pg13* locus. However, the 56.6 KDa protein marker and the RFLP marker identified with the globulin cDNA clone MOG12 mapped to the other side of the locus in the OT328 x Dumont population, thereby providing flanking markers for the *Pg13* locus. Two percent recombination was detected between the 56.6KDa protein and the Xmog12B RFLP loci. It appears that these markers are derived from two different loci, although they may represent separate members of a globulin gene family. This constitutes another example of the association of loci for seed storage proteins and rust resistance. The utilization of mapped probes in our initial surveys of NILs allowed comparative mapping with the existing cultivated oat map. Thus, the *Pg9* locus can be mapped to group 4 and *Pg13* to group 3 of the Kanota x Ogle map.

The availability of a molecular linkage map of cultivated oat has allowed the rapid identification and localization of several markers for two stem rust resistance genes. The information gained in this study and in other ongoing studies about the organization of rust resistance genes within the oat genome will prove useful for the long term goal of combining several resistance genes into well-adapted common cultivars. Furthermore, information on the organization of rust resistance genes may in time provide insight into how resistant genes have been evolving in *Avena*.

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RAPD characterization of barley germplasms resistant to soil-borne mosaic inducing viruses (BaMMV, BaYMV, BaYMV-2). F. Ordon, A. Schiemann and W. Friedt, Institute of Crop Science and Plant Breeding I, Justus-Liebig-University, Ludwigstr. 23, D-35390 Giessen, Germany

Introduction. Soil-borne mosaic inducing viruses, i.e., barley mild mosaic virus (BaMMV), barley yellow mosaic virus (BaYMV) and BaYMV-2 (Huth 1990, Huth & Adams 1990) cause one of the most important diseases of winter barley in Europe. Resistance of all commercial German barley cultivars resistant to BaMMV and BaYMV is presumed to be due to the gene *ym4* which was located on the long arm of chromosome 3 with the help of telotrisomic analysis (Kaiser & Friedt 1989, 1992) as well as by RFLP (Graner & Bauer 1993) isozyme (Le Gouis et al. 1995) and RAPD analysis (Ordon et al. 1995). Besides *ym4* different genes conferring resistance at least to BaMMV are present within the barley gene pool (Götz & Friedt 1993, Ordon & Friedt 1993, Erdogan et al. 1994). Resistance to these viruses is mainly derived from East Asia (cf. Ordon et al. 1993) but has also been found in accessions of *Hordeum spontaneum* Koch derived from Turkey (Erdogan et al. 1994) and Israel (Nevo, pers. comm.). Attempts to characterize these exotic germplasms by isozyme electrophoresis were not very efficient as out of 15 isozyme systems corresponding to 26 loci tested only 3 were polymorphic and 12 were monomorphic (Le Gouis et al. 1995). Because of this low level of isozyme polymorphism within exotic germplasms on the one hand and in comparison to German commercial cultivars on the other hand the RAPD-technique (random amplified polymorphic DNA) was used to characterize these germplasms on the DNA-level.

Material and Methods. Thirty-six genotypes of *Hordeum vulgare* derived from Germany (i.e., Alraune, Brunhild, Corona, Gerbel, Colombo, Jana, Igri, Trixi, Sonate, Magie, Ogra, Labea), East-Asia (Kanto Nijo 19, Misato Golden, Resistant Ym No. 1, Chikurin Ibaraki 1, Ea 52, Muju covered 2, Namji Milyang Native, Rokkaku 1, Ou 1, Zairai Rokkaku, Taihoku A, Mihori Hadaka 3, Mokusekko 3, Iwate Omugi 1), the former USSR (Russia 32, Russia 57), Austria (9043, 9048), the former Yugoslavia (10247, Krasnodar 1920, Maksimirski 452), Turkey (Turkey 235), Bulgaria (Bulgarian 347), USA (Anson Barley) as well as 12 accessions of *Hordeum spontaneum* derived from Turkey (Candarli, Icemeler, Kupalan, Menemen, Pinarbasi) and Israel (25-30, 09-01, 09-35, 09-39, 09-43, 09-09, 09-H27) were assayed for RAPD-polymorphism. DNA-isolation was carried out according to Bernatzky & Tanksley (1986) using equal quantities of leaf tissue from 10 plants of each variety grown in the greenhouse. PCR reaction mixtures (25 μ l) contained 25ng genomic DNA, 0.4mM dNTPs, 6mM MgCl₂, 0.3 μ M of a random 10mer primer (Operon Technologies Inc.) and 0.2U Taq DNA-polymerase (Red Goldstar, Eurogentec Inc.) with the corresponding reaction buffer. The mixture was overlaid with mineral oil and amplification was carried out in a DNA thermocycler 480 (Perkin Elmer) using the following temperature profile: an initial denaturation step (94°C/4min) was followed by 45 cycles of 94°C/1min, 36°C/1min, and 72°C/2min. The polymerization step was extended for 3sec/cycle. Fragments were separated on a 2% agarose gel (NuSieve agarose, FMC), stained in ethidium bromide and visualized on an UV-screen (254nm). RAPD-patterns were scored using the software package RFLPscan 2.0 and genetic similarity was estimated according to Nei

& Li (1979). Based on these data UPGMA-clustering was carried out using the software package NTSYS-pc 1.7.

Results and Discussion. Based on the analyses of 20 RAPD-primers according to 544 distinct bands varying from 2691 to 280 bp genetic similarity estimates according to Nei & Li (1979) were found between 0.685 ('09-09' *versus* 'Corona') and 0.964 ('09-39' *versus* '09-43'). The results of the UPGMA-clustering based on these data are shown in Figure 1.

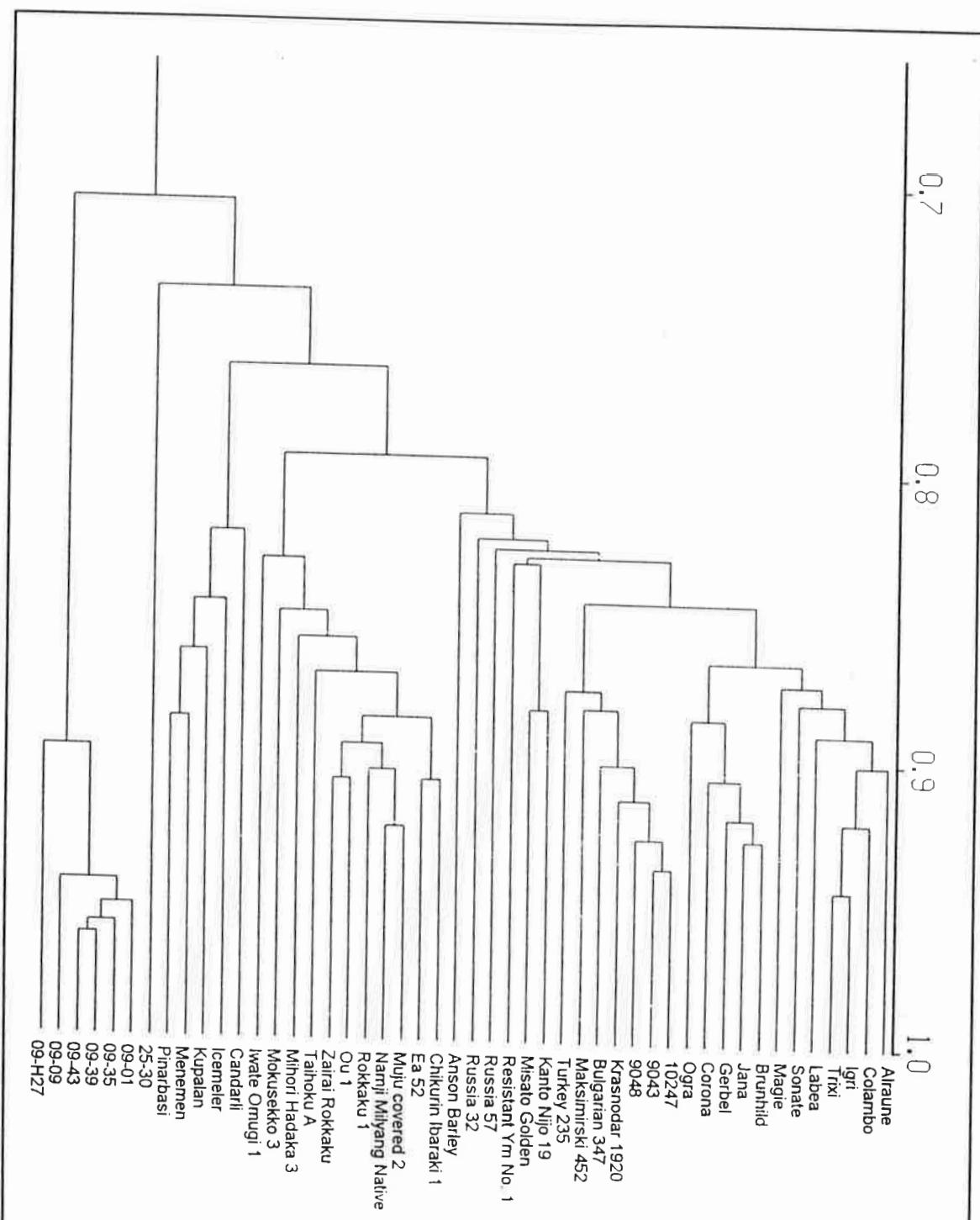


Fig. 1. Dendrogram constructed from RAPD-based genetic similarity (Nei & Li 1979) of 48 barley genotypes, *H. vulgare* and *H. spontaneum*, respectively.

In general seven groups of genotypes can be distinguished. The first one consists of *H. spontaneum* accessions derived from Israel ('09H27'-'09-09'), whereas the variety '25-30' - although deriving from Israel - does not belong directly to this cluster. This may be due to the fact that varieties from '09H27' to '09-09' derive from the same region (Mt. Meron) while '25-30' comes from Atli (Nevo, pers. comm.). This cluster is followed by the Turkish *H. spontaneum* accessions ('Pinarbasi' to 'Candarli') and the resistant *Hordeum vulgare* varieties derived from East Asia ('Iwate Omugi 1' to 'Chikurin Ibaraki 1'). Next to this group are three more or less isolated genotypes, i.e. 'Anson Barley' from the USA and 'Russia 32' and 'Russia 57' from the former USSR. The fourth cluster comprises three Japanese malting barley cultivars ('Resistant Ym No. 1' to 'Kanto Nijo 19'). It is interesting to note that these varieties are more closely grouped to European barley cultivars than the other East-Asian varieties. This may be due to the fact that these varieties obtained their malting quality from European barley cultivars (cf. Muramatsu 1976, Kobayashi et al. 1987). The last three groups consist of European barley varieties. On the one hand these are resistant varieties derived from the Eastern part of Europe ('Turkey 235' to '10247') and on the other hand resistant and susceptible German cultivars which can be subdivided into six-rowed ('Ogra' to 'Brunhild') and two-rowed cultivars ('Magie' to 'Alraune').

The results elucidate, that RAPD-PCR is a very suitable and efficient tool for the genetic characterization of barley, since all the varieties are correctly grouped according to their origin. Despite the results of isozyme electrophoresis (Le Gouis et al. 1995) RAPD-analysis reveals a large genetic diversity within varieties resistant to the soil-borne mosaic inducing viruses, which can be exploited by practical plant breeding. In this case it is interesting to note, that those *H. vulgare* varieties more closely related to German cultivars are in general higher yielding and better adapted to German growing conditions (Ordon & Friedt 1994). These varieties may be better suited for the rapid incorporation of different resistance genes while the others may be used in recurrent selection programmes. RFLP-markers for different resistance genes are available now for an acceleration of the breeding procedure (Graner et al. 1995).

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The use of molecular markers to improve the efficiency of doubled haploid breeding. C. OTI-BOATENG¹, C.E. SCHMERL², R.C.M. LANCE³, A.R. BARR¹, P. LANGRIDGE¹ and P.A. DAVIES²

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Introduction Anther culture (AC) is commonly used for the accelerated production of homozygous doubled haploid (DH) barley breeding lines. The efficiency of DH breeding can be enhanced by identifying and pre-selecting donor plants which have alleles for superior malting quality, agronomic performance and/or cereal cyst nematode (CCN) resistance before anthers are collected for culture. Molecular genetic markers linked to genes of interest can be used to identify plants carrying these desirable genes. This technique is known as "marker assisted selection".

Table 1 illustrates the genetic advantages of using marker assisted selection in sexual seed production, or in combination with pre-selection of anther culture F₂ donor plants carrying the desired alleles either as homozygotes or heterozygotes. The data illustrate two important points. Firstly, marker assisted selection of F₂ seed parents (+ selⁿ (F_{2/3})) produces progeny homozygous at *n* loci as efficiently as DH production from either F₁ or F₂ donor plants in which marker assisted selection is not used. Secondly, when the marker assisted selection is used to select F₂ donor plants for doubled haploid production, the greatest genetic efficiencies are achieved.

Table 1. A comparison of the expected proportion of progeny homozygous for the desired allele at *n* unlinked loci from F₁ or F₂ parent or anther culture donor plants. Data are presented for progeny of F₂ plants either selected (+ selⁿ) or unselected (-selⁿ) as having the desired allele in either the homozygous or heterozygous state.

No. of genes	Sexual seed production			DH seed production		
	F ₁ parent	F ₂ parent		F ₁ donor	F ₂ donor	
	(F ₂)	-sel ⁿ (F _{1/3})	+sel ⁿ (F _{2/3})		-sel ⁿ	+sel ⁿ
<i>n</i>	(1/4) ^{<i>n</i>}	(3/8) ^{<i>n</i>}	(1/2) ^{<i>n</i>}	(1/2) ^{<i>n</i>}	(1/2) ^{<i>n</i>}	(2/3) ^{<i>n</i>}
1	0.25	0.375	0.50	0.50	0.50	0.67
2	6.25 × 10 ⁻²	0.141	0.25	0.25	0.25	0.45
3	1.56 × 10 ⁻²	5.27 × 10 ⁻³	0.125	0.125	0.125	0.30
5	9.77 × 10 ⁻³	7.42 × 10 ⁻⁴	3.13 × 10 ⁻²	3.13 × 10 ⁻²	3.13 × 10 ⁻²	0.14
10	9.54 × 10 ⁻⁷	5.50 × 10 ⁻⁵	9.77 × 10 ⁻⁴	9.77 × 10 ⁻⁴	9.77 × 10 ⁻⁴	1.82 × 10 ⁻²

Implementation of marker assisted selection in combination with anther culture will reduce the number of spikes to be cultured to produce the required number of regenerants with desired characters. A further advantage of AC from F₂ donor plants is that an extra cycle of recombination is achieved to increase the likelihood of breaking undesirable linkages.

This report describes the use of a CCN resistance RFLP marker, AWBMA 21, which maps 3.5cM from the CCN resistance gene and was isolated from barley root cDNA (Langridge, pers. comm.) to identify CCN resistance in barley donor plants prior to AC.

Materials and Methods Plants were grown under controlled conditions in pots in a growth room with a 14h-photoperiod and a 17°C/14°C day and night temperature respectively. The genotypes tested were WI2875 (Waite breeding line), Chebec (CCN resistant cultivar) and 18 anther culture donor plants (F₂) from a cross between WI-2875 and Chebec. At the five leaf stage, leaf tissue was harvested from each plant and DNA extracted using the method of Rogowsky *et al.* (1993). The restriction endonuclease EcoRI was used to digest 6µl of DNA per plant. DNA was loaded onto 1% agarose/TAE gels, run at 15mA overnight and transferred onto a Hybond N+ charged nylon membrane. Labelling and hybridisation procedures were carried out as described by Rogowsky *et al.* (1993).

Results and discussion. Polymorphisms obtained with the donor plants are shown in Figure 1. Four plants (6,7,8,15) were found to be homozygous for the RFLP marker linked to CCN resistance, 12 were heterozygous (2,3,4,5,9,10,11, 12,13,14,16,18) and 2 (1,17) homozygous for the RFLP marker linked to CCN susceptibility.

We are currently pre-screening AC donor F₂ plants for this CCN linked RFLP and a β-amylase isozyme using isoelectric focusing. In an F₂ population (Table 1) we would expect to find plants homozygous for both the desirable β-amylase allele and the CCN resistance gene at a frequency of 6.25×10^{-2} (6.25%). In an unselected F₃ population (F_{1/3}) this frequency increases to 14.1%. The strategy of pre-selecting AC donor plants which are either homozygous or heterozygous for CCN resistance and a particular β-amylase allele will produce a DH population in which 45% of the progeny are homozygous for the desirable alleles at both of these loci. The alternative strategy of selecting donor plants which are homozygous for the desirable allele at both loci would, of course, produce a doubled haploid population in which 100% of these plants were homozygous for these alleles. However, our goal is to maximise the number of plants which are not only homozygous for these alleles but also have the

maximum probability of containing combinations of genes conferring improved yield, agronomic characteristics, disease resistance and grain quality. By selecting donor plants both homozygous and heterozygous for the preferred alleles, the risk of discarding donor plants with favourable combination of genes conferring these and other important characteristics is minimised.

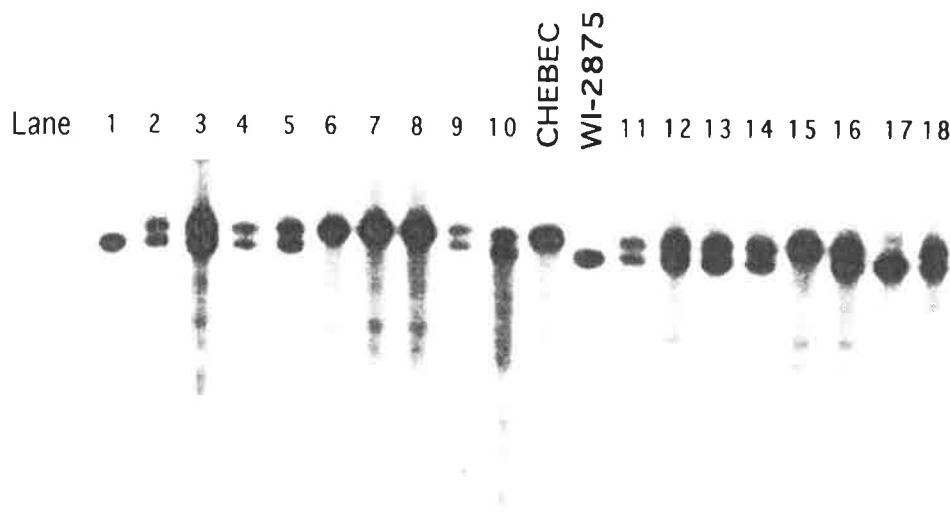


Figure 1. Restriction fragment length polymorphisms of parents and F₂ lines from a cross between WI-2875 and Chebec.

The relative efficiency of DH breeding over sexual seed production increases with the number of gene loci that can be selected. Research into the development of new molecular markers for both agronomic and quality traits in barley is continuing in the Plant Science Department of the University of Adelaide. Quantitative trait loci (QTL) markers linked to α -amylase, β -amylase and β -glucanase activity are also being investigated. We are also interested in developing markers linked to genes conferring high DH regenerability which could be backcrossed into breeding lines to improve the efficiency of DH production.

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Molecular markers: their potential use to barley breeding in north-east Australia.

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Introduction. A barley breeding program has been located at Hermitage Research Station since the early 1970s. Its role has been to breed spring type malting and feed barley cultivars for winter cereal cropping areas of Queensland and northern New South Wales. This is a predominantly subtropical region from 22°S to 33°S with an average annual precipitation ranging from 500 to 950mm.

The program's breeding objectives fall into three categories; agronomic traits, disease resistance and grain quality. High grain yield is the principal selection criteria. Time to flowering and crop maturity are also important; for example, long season cultivars such as Triumph are unsuitable. Good straw strength is highly desirable, and finally, plump grain is an essential trait for malting cultivars.

Barley leaf rust (*Puccinia hordei*) and stem rust (*P. graminis*) are the major foliar diseases. Severe epidemics in the 1980s caused significant yield losses and poor grain quality. Several races of leaf rust occur (Cotterill et al, 1994), suggesting that gene pyramiding or slow rusting resistance strategies are warranted. *Rpg1* is currently effective against stem rust in Australia, however the emergence of the QCC race in north America indicates a need for alternative sources of resistance. Net blotch (*Drechlera teres*) and spot blotch (*Bipolaris sorokiniana*) are becoming increasingly important, in part due to the adoption of minimum tillage practices. Field collections have indicated the existence of several biotypes of net blotch, including the spot form (Platz et al, 1996). Powdery mildew (*Erysiphe graminis*) also occurs, but not at economically damaging levels. The economics of broadacre chemical treatments for these diseases are unreasonable and so genetic resistance is the main control option. Two soilborne diseases, crown rot (*Fusarium graminearum* group 1) and common root rot (*B. sorokiniana*), are significant throughout the region and may cause substantial yield losses. Genetic resistances against them are currently being sought.

Heavy emphasis is placed on selection for malting quality. Guidelines outlined by the Malting and Brewing Industry Technical Committee have been used to establish the current micromalting and grain quality assessment protocols (Inkerman et al, 1995). The region's best malting cultivar, Tallon, is used as the main malting quality benchmark. Early generation testing focuses on hot water extract, α -amylase and diastatic power. In later trials malt β -glucan, Kolbach index, viscosity, free amino nitrogen, malt friability and wort haze and colour are added to the selection criteria. Adequate grain dormancy is essential because of the high risk of rain during crop maturation.

To meet these objectives it is necessary to have practical screening methods, accurate selection techniques and rapid progression of experimental lines towards release. New technologies can therefore play a significant role in focussing the program's methodology. Two significant recent advances have been the use of anther culture to produce double haploids and the development of molecular marker technology. While

double haploid technology can be used to speed up the release of new cultivars, molecular markers are being adopted to enhance selection of specific traits. The Grains Research and Development Corporation and the Cooperative Research Centre for Tropical Plant Pathology are supporting the program's adoption of these technologies. This paper discusses current and projected molecular marker work.

Materials and Methods. For DNA extraction, we used barley leaves and a slight modification of the CTAB method described by Graham *et al* (1994) with storage of DNA in TE buffer containing RNAase. The polymerase chain reaction (PCR) (Saiki *et al*, 1988) with RAPD primers (Williams *et al*, 1990) has been the principal technology used. Full details have been published elsewhere (Poulsen *et al* 1995, Poulsen *et al* 1996).

Discussion.

Using PCR for germplasm identification. In a series of experiments, PCR was used to identify different types of germplasm (Poulsen *et al* 1996). Barley α -amylase primers were used to confirm that five plants were F_1 progeny of the cross Tallon/Kaputar. Confirmation of the F_1 plants identity was required before using them in anther culture. In the other experiments, RAPDs were used to distinguish between two F_2 populations, correctly identify seed lots of a breeding line and confirm the presence of off-type plants in a seed production block. In each case, the use of marker technology meant that a solution was obtained quickly, saving the considerable time and effort needed to grow and observe the material in the field. The results from the experiments were sufficiently accurate to give a high level of confidence in the outcomes.

Using marker assisted selection (MAS)

The most significant use for molecular markers is as a selection tool; particularly for gene pyramiding, recovery of recurrent parent genotypes in backcrossing and breeding for traits with low heritability. This is because MAS can be applied without the restrictions of conventional field and glasshouse based selection techniques. For example, disease resistant plants can be selected in the absence of pathogens or potential grain quality of new lines can be assessed without the need for substantial grain quantities for detailed analysis.

We are initially applying MAS to selecting disease resistance genes. It is not possible to simultaneously select for resistance to all of the diseases of interest to our program because of the lack of suitable markers. However, some progress has been made and a number of markers linked to useful resistance genes, including the stem rust resistance gene, *Rpg1* (Kilian *et al*, 1994), have already been identified. Q21861, an accession originating from CYMMIT, is known to possess resistance to leaf rust, stem rust and powdery mildew. We have identified a RAPD marker linked to the Q21861 leaf rust resistance gene at a distance of approximately 12cM (Poulsen *et al*, 1995) and are attempting to clone it to develop SCAR primers. However, the RAPD marker may be of immediate use for selecting from a Q21861 derived backcross population. Members of the North American Barley Genome Project (NABGMP) have also been working on Q21861 and markers have been developed for a stem rust resistance gene, *rpg4*, which confers resistance to race QCC (Borovkova *et al*, 1995). This is of particular significance to our program as race QCC has not been recorded in Australia. We hope

to use the *rpg4* marker to select for resistance to race QCC in its absence, and therefore have locally adapted, resistant material on hand for when the race is found in Australia. Flanking markers linked to the leaf rust resistance genes *Rph3* and *Rph7* would be useful as both are effective against all of the known races in Australia, but are found in relatively unadapted germplasm. The slow rusting resistance from Vada and its progeny would be another useful target. This material has been used widely in our program, and an efficient means of detecting the resistance would be very welcome. Other leaf and stem rust resistance markers could be used to strategically deploy genes in our material.

Because it is our desire to select for multiple disease resistances at an early stage in the breeding program, PCR based markers for resistances to net blotch and spot blotch would also be highly desirable and we hope that markers generated by NABGMP may be of use. Selection for resistance to crown rot and common root rot is a high priority, and here again MAS would have great value as screening procedures are, at present, too complicated for large scale testing of breeding populations.

Enhancing the selection of malting quality traits is also a goal of the breeding program. This is a longer term goal because of the complex genetics of the traits. We expect that data from NABGMP projects will be useful. However, because of genotype-environment interactions, the combined Australian barley breeding programs intend to map quality traits in locally adapted material under Australian conditions. This Australian Barley Genome Mapping Project is currently collecting data to map a wide range of traits, including malt extract and significant enzyme activities. The first year of field trials has been conducted and quality results will be obtained in 1996.

Conclusions. Adoption of MAS by our barley breeding program is progressing and we expect to commence selection with PCR markers for leaf and stem rust resistances in 1996. Selection for additional traits, particularly disease resistances and grain quality parameters will be added as appropriate markers become available.

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Identification of RAPD markers linked to genetic factors controlling hull content in oat (*Avena sativa* L.). P.S. RONALD, G.A. PENNER, P.D. BROWN
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Introduction

Hull content expressed by measurements such as percent hull, caryopsis percentage and groat/hull ratio, is the most important physical parameter of oat grain quality.

Hull refers to the lemma and palea that enclose the mature oat caryopsis. The fibrous hull has minimal nutritional value but does serve a purpose in maintaining the groat in a clean state free of molds and other fungi. It is therefore desirable to develop improved oat varieties with low hull content while maintaining high grain yield.

Selected recombinant inbred lines (RILs) from the cross Cascade / AC Marie have been used in conjunction with arbitrarily primed PCR in an attempt to identify RAPD markers linked to genomic regions controlling hull content in oat. Such markers would be valuable in any oat breeding program, allowing selection for low hull content to be conducted at the seedling stage based on genotype.

Hull content in oat is thought to be a quantitatively inherited trait with continuous variation. Past research has suggested that multiple quantitative trait loci (QTL) with primarily additive effects could account for the observed variation in hull content. Quantitative traits do not represent a barrier to random amplified polymorphic DNA (RAPD) analysis.

Methods

Phenotypic Assessment

A rapid, accurate assessment of the hull content of an oat sample can be obtained using 50 primary grains. Percent hull was determined by comparing hull weight to total kernel weight. Determination of hull content was also achieved by dehulling 2 g of randomly selected kernels or non-destructive NIR analysis of approximately 20 g of randomly selected kernels.

Genotype Scoring

Based on hand- and NIR-determined percent hull values for the F_5 - F_7 generations of the segregating population, 26 RILs displaying consistently high or low percent hull values were selected. In order to identify individual quantitative trait loci of major effect, seven RILs representing the extremes of the distribution for percent hull were used to construct two DNA pools.

These DNA pools have been amplified with more than 300 arbitrary primers. Amplification products were separated on acrylamide gels for improved resolution using the temperature sweep gel electrophoresis (TSGE) technique. Polymorphic products will be tested for linkage to genomic regions controlling hull content by genotyping members of the recombinant inbred population.

Results and Discussion

Phenotypic Data

Hull content has been determined for more than 2000 samples from F_5 - F_8 RILs of the cross Cascade / AC Marie.

Hull content determination using 50 primary kernels was highly repeatable. Measurement of percent hull was repeated on 24 samples with an average difference between paired measurements of 0.34%.

Histograms have been prepared for all measurements including groat / kernel, hull / kernel and percent hull (**Figure 1 a-c**). Among Cascade / AC Marie progeny, substantial variability was observed for hull and groat / kernel values, both of which contribute to percent hull. Transgressive segregation is apparent at both extremes of the hull / kernel and groat / kernel distributions.

RAPD Data

Of the 300 primers screened across the DNA pools, 12 produced detectable polymorphisms. Furthermore, these 12 primers amplified 25 polymorphic loci, seven of which cluster in three separate groups. Several polymorphic loci were scored on five plant pools for the 14 RILs with hull values in the tails of the distribution pattern. For three loci the dominant amplification product was present in Cascade, the remaining four were present in AC Marie (**Table 1**). The linkage group including the E9a and M18a loci demonstrates that AC Marie, while relatively low in hull, carries alleles for high hull content.

Nine marker loci were tested for possible cosegregation with measured grain characteristics by scoring 80 randomly selected lines from the recombinant inbred population. Using Mapmaker/QTL available data for nine polymorphic loci has been related to groat / kernel, hull / kernel, and percent hull values from F_8 RIL grown at two locations in 1995. No marker indicated the presence of a major gene for hull / kernel, groat / kernel and kernel weight. However six of the nine marker loci explained a significant proportion of the variation in percent hull.

Summary and Future Work

1. The discovery of groups of clustered, as well as several unclustered polymorphisms may represent the identification of genes controlling hull content in oat. Polymorphisms would be expected to cluster as more than one would be linked to each genetic factor for hull content.
2. Future work will focus on the use of conventional genetic analysis to uncover many of the important parameters of the hull content trait including its heritability and the number of loci that contribute to the expressed phenotype.
3. The potential linkages identified between markers and variation for hull content will be clarified by scoring individual plants for the 80 selected lines. This procedure will delineate lines that are heterogenous for any given marker locus.

Figure 1: Histograms of mean groat / kernel, hull / kernel, and percent hull for 223 F8 Cascade / AC Marie RIL and 16 parental checks grown as a RCBD (3 replicates) at Portage, MB. in 1995.

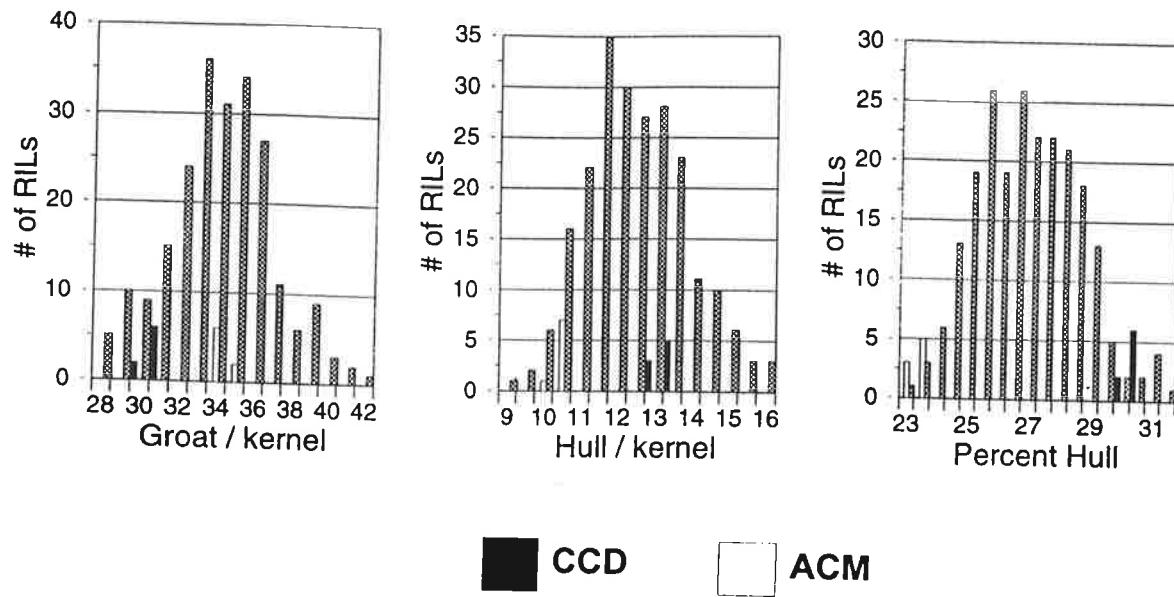


Table 1. Allelic composition at seven RAPD loci of seven individuals used to construct two DNA pools representing high and low percent hull values. The mean and standard deviation of the High and Low bulks are given.

RAPD Locus	Parents		High Bulk		Low Bulk	
			30.09 (0.62)		23.95 (0.88)	
	CCD	ACM	CCD	ACM	CCD	ACM
OPD 20	+	-	5	2	0	7
OPE 9a	-	+	0	7	4	3
OPE 9b	+	-	5	2	1	6
OPG 12e	-	+	3	4	1	6
OPK 1a	-	+	5	2	0	7
OPM 18a	-	+	0	7	4	3
OPN 3a	+	-	3	4	0	7

Direct comparisons of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs and RAPDs. J.R. RUSSELL, J.D. FULLER, M. MACAULAY, B.G. HATZ*, A. JAHOOR*, W. POWELL and R. WAUGH, Department of Cell and Molecular Genetics, Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, UK; * Technische Universität München, Lehrstuhl für Pflanzenbau und -züchtung, 85350, Freising-Weihenstephan, Germany.

Introduction. Presently more than 250,000 *Hordeum* accessions are held in genebanks throughout the world, and the number is increasing. However, what this vast number of accessions represents in terms genetic diversity is largely unknown. In order to effectively manage such a collection requires accurate and appropriate methods of measuring and monitoring genetic variability. DNA-based assays provide the most accurate measures of variability. The number of molecular assays available has increased dramatically, each method differing not only in technical aspects but also in the type and amount of polymorphism detected. Given the wealth of available genetic markers a comparison of techniques to characterise barley genetic resources is timely. The objective of this study was to determine whether the genetic relationship among cultivated barley differed based on analysis with RFLPs, RAPDs, AFLPs and SSRs.

Materials and Methods. DNA from 18 barley accessions, which were representative of the cultivated gene pool, was isolated using the following procedure described by Saghai-Maroof *et al.* (1984). RFLPs analysis was performed according to the protocol described by Graner *et al.* (1991). RAPDs profiles were generated using the conditions described by Barua *et al.* (1993). AFLP markers were generated using the procedure developed by KeyGene (Wageningen The Netherlands; Zabeau & Vos, 1993). Screening and isolation of SSR-containing clones, sequencing, and primer design were described by Powell *et al.* (1996). Database derived SSRs were described in Heun & Becker (1995). SSR assays were performed as described by Morgante *et al.* (1994).

Results and Discussion. Fingerprinting. All of the molecular approaches used in this study were able to uniquely fingerprint each of the 18 cultivated barley accessions. Phenotype diversity indices ($1-S \Pi_2$, where π_i is the phenotypic frequency for each probe/enzyme combination (RFLPs), primer (RAPDs), primer combination (AFLPs) and primer set (SSRs) were calculated. Overall the highest diversity index was observed for AFLPs (0.937), with three of the six primer combinations distinguishing between all 18 accessions. In contrast RFLPs gave the lowest diversity index (0.322), several probes were required to detect differences between all 18 genotypes. RAPDs and SSRs were intermediate with values of 0.521 and 0.566 respectively.

Comparison of genetic similarity. The cultivated barley gene pool can be divided into spring and winter types. The latter group consists mainly of fodder barleys which can be further divided into two-rowed and six-rowed barley. Spring cultivars are mainly malting barleys. Genetic similarity between two accessions, i and j, was estimated according to Nei & Li (1979) as $G_{ij} = 2N_{ij}/(N_i + N_j)$. The mean similarity values varied for each molecular assay, from the highest of 0.891 for AFLPs and 0.886 for RAPDs to the lowest of 0.701 for SSRs and 0.729 for RFLPs. As expected from previous RFLP studies (Melchinger *et al.* 1994) genetic similarity estimates between spring and winter types revealed that spring types were more similar to other springs than to winter types for all of the assays studied.

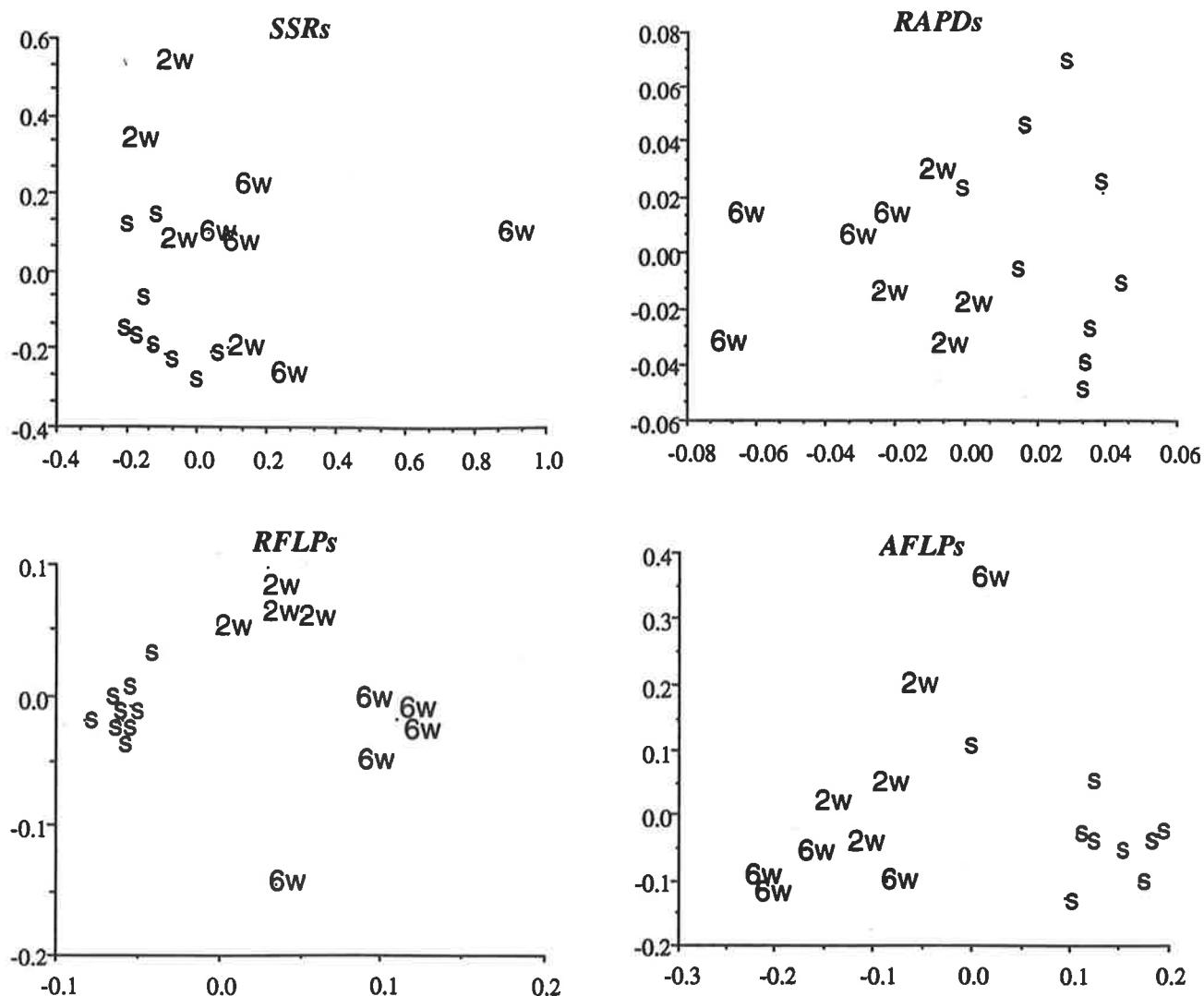


Figure 1. PCoA performed on genetic similarity estimates for 18 cultivated barley accessions for each different molecular assay.

Comparison of genetic relatedness. Associations among the 18 accessions were revealed by Principal Coordinate analysis (Pcoa) for each of the molecular assays in this study (Figure 1). Spring types were clearly separated from the winter types in both the PCoAs generated by RFLP and AFLP data. Furthermore the two-row winter types formed an intermediate group between the springs and six-row winters. Using RAPD data three distinct groups were observed, although the springs are spread further apart than with the previously mentioned PCoas. This was not unexpected as the primers selected for RAPD analysis were previously used to map a spring population. Only a small group of springs clustered together using SSRs data, two-row and six-row winters were widely dispersed throughout the Pcoa.

Comparison between assays. Each of the four assays are very different, not only do they differ in the technical aspects but also in the type and amount of polymorphism detected. For example, with only six primer combinations 297 fragments were detected using AFLPs, in contrast 114 probe/enzyme combinations (43 probes and three enzymes) were required to generate 299 fragments using RFLPs. Thirteen SSR primer sets generated 70 different alleles and 25 single 10-mers amplified 109 RAPD fragments. The levels of polymorphism were also very different for each assay. All of the 13 SSR primers were polymorphic, with an average of 5.7 alleles per primer set. In contrast, nearly 54% (158) of the fragments generated using AFLPs were monomorphic. Can a direct comparison of such different techniques be made? For example, are accessions which have the highest similarity estimates always the same accessions for each assay? Using Spearman Rank Correlation which compares how each assay ranks pairwise similarities (GS). AFLPs and RFLPs ranked over 70% of the pairwise comparisons in the same order. This is not unexpected as both these techniques are based on restriction site changes, the major difference is that PCR is used in AFLPs rather than Southern analysis for detection of restriction fragments. As the inventor of AFLP says 'The resemblance with the RFLP technique was the basis to choose the name AFLP' (Vos *et al.*, 1995). In contrast SSRs have the lowest values when compared to the other three assays. This is also not unexpected, considering the high levels of polymorphism and hence the lack of similarity between pairwise comparisons.

In conclusion, SSRs are the most polymorphic, yet were not particularly useful for assessing genetic relationships among cultivars. RFLPs were particularly valuable for assessing genetic relationships, but required several probe and enzyme combinations to discriminate between accessions. Both of these assays require some initial investment in terms of sequence information, and according to Vos *et al.* (1995) the ideal fingerprinting assay should require no prior sequence knowledge. Only AFLPs and RAPDs meet these requirements.

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QTL analysis for net blotch resistance in barley. K. SATO¹, K. TAKEDA¹ P.M. HAYES², ¹Research Institute for Bioresources, Okayama University, Kurashiki, 710, Japan. ²Crop and Soil Science Department, Oregon State University, Corvallis, Oregon, 97331, USA.

Introduction. Net blotch is a common foliar disease of barley in temperate and humid areas of the world. This disease is caused by the fungi *Pyrenophora teres* f. *teres* (net type symptom) or *P. teres* f. *maculata* (spot type symptom). Both major and partial resistance genes have been reported for net blotch (Jørgensen 1992, Arabi et al. 1990). Moreover, as several pathotypes have been reported for this disease (Steffenson and Webster 1992), expression of resistance may be different among the combinations of resistance genes and isolate pathotypes. We studied resistance to net blotch using a Quantitative Trait Locus (QTL) approach, diverse isolates, and dihaploid (DH) barley populations.

Materials and Methods. Two sets of DH populations of 150 lines each - Steptoe/Morex (SM) and Harrington/TR306 (HT) - and the four parents were inoculated in a series of four experiments with four isolates of *Pyrenophora teres*. Isolates were from Japan and Canada and included net types (K105, WRS102) and spot types (WRS857, WRS1566). Barley populations were provided by the North American Barley Genome Mapping Project and Canadian isolates were kindly provided by Dr. Tekauz, Agriculture Canada. Second-leaf stage seedlings with two replications were inoculated with conidia suspensions, and reactions were scored after 14 days using the disease rating of Tekauz (1985) from 1 (resistant) to 10 (susceptible). The disease phenotypes and the basemap genotypes for each population (Mather 1995) were analyzed using MAPMAKER/QTL1.1 (Lander and Botstein 1989). QTL were considered significant at LOD > 2.0.

Results and Discussion. There were clear differences in the reaction of parents to WRS102. Steptoe and TR306 both received a rating of 4, while Morex and Harrington received a rating of 8. However, the reactions of the parent genotypes were not obviously different against the other isolates. Although monofactorial inheritance was observed in the SM population against WRS102 ($\chi^2=3.27$, 1 resistant : 1 susceptible), continuous distributions were observed in the other combinations of populations and isolates. When the SM population was inoculated with K105 and WRS1566, there were positive and negative transgressive segregants, indicating each parent carried some resistance factors.

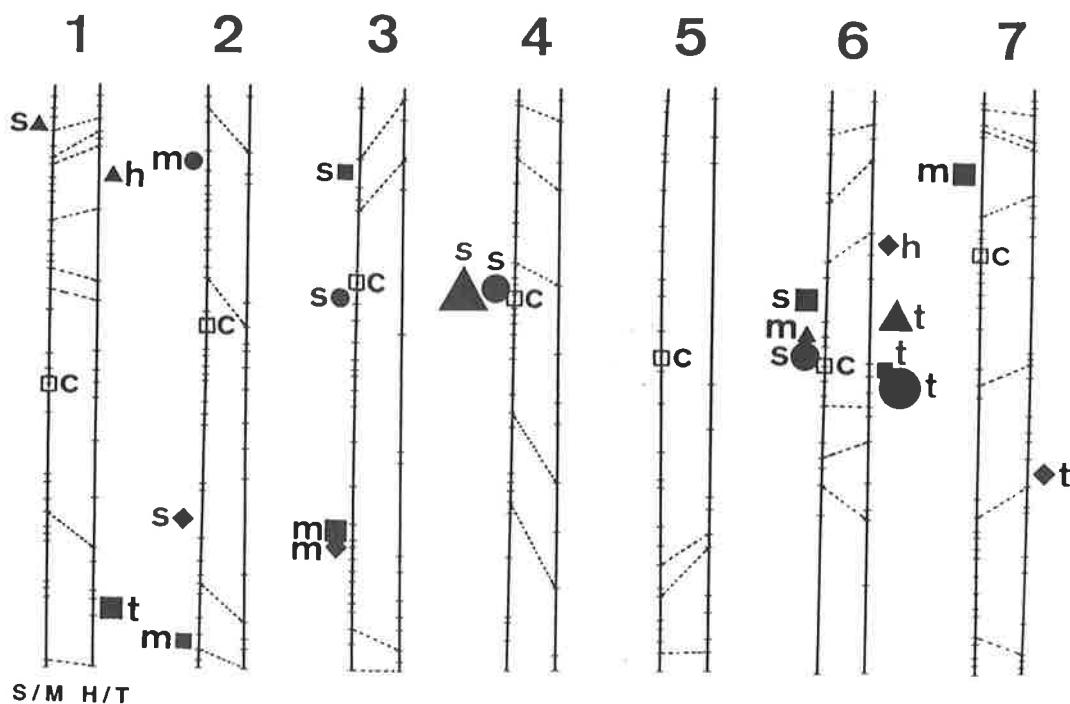


Fig. 1. Chromosomal location of QTL for resistance to four *P. teres* isolates on the maps of Steptoe/Morex (left) and Harrington/TR306 (right). Dotted lines indicate corresponding loci between maps. Symbols indicate QTL for resistance to isolates K105(●), WRS102(▲), WRS857(■) and WRS1566(◆). Size of the symbols shows the magnitude of LOD score: 2.0-4.9 (small), 5.0-14.9 (middle), and >15.0 (large). Letters after symbols indicate the parents that contribute the resistant allele (s:Steptoe, m:Morex, h:Harrington and t:TR306).

Fig. 1 shows the position and significance of QTL for the four isolates. Several highly significant QTL (with LOD scores greater than five) were concentrated within the same chromosome regions. The QTL on chromosome 4 were highly significant for both K105 and WRS102, but existed only in the SM cross for the net type isolates. On the other hand, QTL were detected on chromosome 6 in both cross combinations and were associated with most of the isolates tested. Highly significant QTL controlling resistance to WRS857 were found on chromosomes 1, 3 and 7. Other QTL were less significant and usually determined resistance to only one isolate. Although isolate-specific, the low significance of these QTL may indicate they are not race-specific resistance genes per se.

Four major genes have been reported for resistance to *Pyrenophora teres* f. *teres* (Jørgensen 1992) and three of them have been located to chromosomes by trisomic

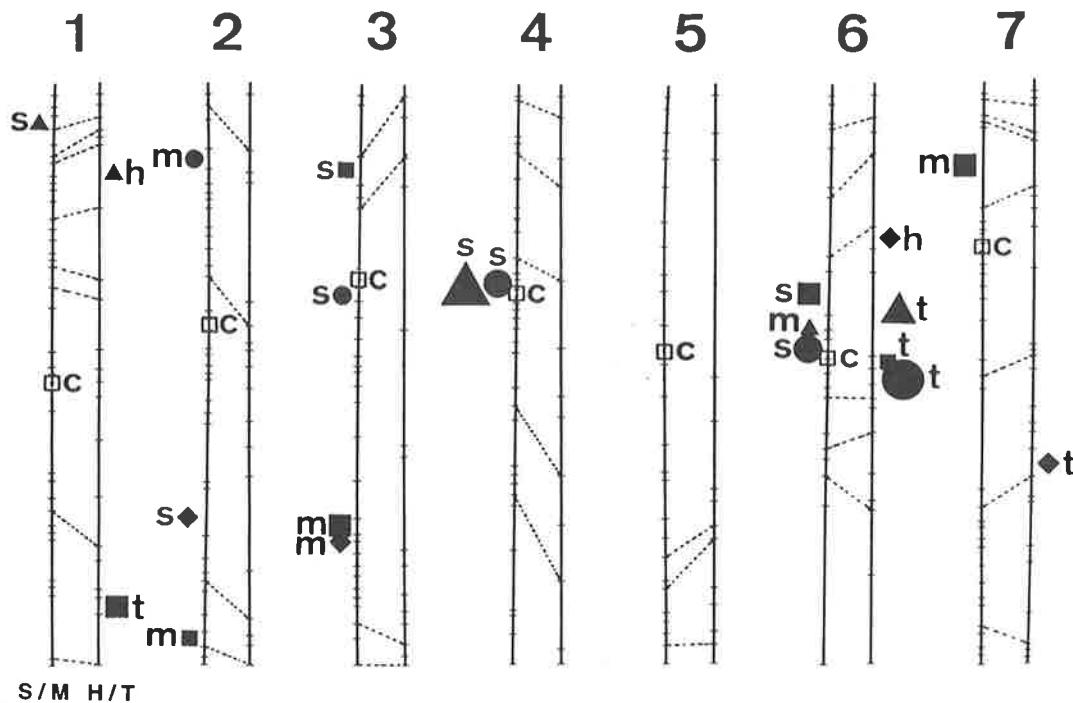


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analysis: Pt on chromosome 3, Pt_2 on chromosome 5 and Pt_3 on chromosome 2 (Bockelman et al. 1977). Using the SM population and an isolate from North Dakota, Steffenson et al. (1996) found QTL for seedling resistance on chromosome 4 and 6, which agrees with our data. As the Japanese and Canadian net type isolates used in this experiments have different virulence spectra (Sato and Takeda 1993), these two resistance QTL may have wide effectiveness for net type isolates. QTL on chromosome 6 may confer resistance to a wide range of isolates, including spot types. Our data support the existence of more net blotch resistance genes than was previously reported. Mapping in other populations may reveal additional resistance genes.

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The use of AFLP-markers to estimate genetic similarities. Johan W. Schut, Department of Plant Breeding, Wageningen Agricultural University, P.O.Box 386, 6700 AJ Wageningen, The Netherlands

Measuring relatedness of different genotypes has always had great interest of plant breeders. For instance, some authors reported genetic similarities of two parent lines of a hybrid to have good predictive ability with regard to the level of heterosis (Smith et al., 1990). Analogously with the breeding of hybrid varieties it is expected that in the breeding of pure line varieties genetic similarities can also be used for cross prediction (Cowen and Frey, 1987a). The analogy implies that low degrees of relatedness between parent genotypes are expected to result in a high level of variation between descending inbred lines. This variation may contribute to an increased transgression in the offspring, i.e. a higher probability of outperforming their best parent and it may therefore be quite relevant to a breeder.

Relatedness measures may be based on different kinds of information. Pedigrees are often used to estimate a coefficient of parentage (Van Hintum & Haalman, 1994). Other measures are usually based on genotypic differences in agronomic and morphological traits, isozymes, storage proteins, etc. (Cowen & Frey, 1987b). More recently differences have also been observed directly at the DNA-level using molecular markers, e.g. RFLPs (Melchinger et al., 1994) and RAPDs. A relatively new PCR-based marker technique, called AFLP (Vos et al., 1995), can be added to this list. This technique may be quite efficient in detecting polymorphism in populations with a relatively narrow genetic base. AFLPs may therefore be useful to improve efficiency and accuracy of genetic similarity estimation in barley. This could support breeders in their prediction of cross performance.

To investigate their usefulness more than 200 AFLP markers are being detected in a group of 21 European two-row spring barley lines. AFLP-based genetic similarities are being estimated and compared with coefficients of parentage and genetic similarities based on morphological traits. Results are being analysed at the moment of this writing.

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Preparation of non-radioactive DNA probes specific to *Rhynchosporium secalis*. T.R. Sharma, J.P. Tewari , P.A. Burnett¹, and K.G. Briggs. Department of Agricultural, Food, and Nutritional Science, University of Alberta, Edmonton, Alberta T6G 2P5, Canada; ¹Research Centre, Agriculture and Agri-Food Canada, P.O. Box 3000, Lethbridge, Alberta T1J 4B1, Canada.

Introduction. Molecular diagnosis and classification of plant pathogens have great significance in the prediction of disease epidemics and in deciding among disease management options. Scald caused by *Rhynchosporium secalis* (Oud.) Davis is an important disease of barley in central Alberta and many other parts of the world and the seedborne nature of this pathogen may play an important role in long distance dispersal (6, 7). Early detection of this pathogen is highly desirable. Although *R. secalis* can be identified on the basis of symptoms and signs on the host, minor infections are hard to detect. It would be nice to have probes to follow the development of disease in infected fields. DNA probes specific to *R. secalis* will be useful in such cases. DNA probes are generally labeled with radioactive substances like ³²P and ³⁵S for obtaining autoradiographs upon hybridization with the DNA of unknown organisms on the nylon membrane. However, with the increasing awareness about environmental hazards and health risks posed by the radioactive substances, efforts are being made to replace them with non-radioactive substances. In DNA fingerprinting, distinct RAPD fragments can be suitably labeled and amplified with PCR (6) and used as probes for *in situ* hybridization or Southern blotting (9, 10). There are some reports on the use of RAPD fragments for making probes in phytopathogenic fungi (1, 2, 3, 5, 10). However, to our knowledge this has so far not been done in *R. secalis*. The objective of this study was to synthesise non-radioactive DNA probe(s) specific to *R. secalis*.

Materials and Methods. Isolates of *R. secalis* used in this study were collected from commercial cultivars of barley from different geographical regions of Canada. Single spore cultures of all the isolates were maintained on Lima Bean Agar (Lima bean infusion 62.5 g/l, Bacto agar 15 g/l) in Petri dishes at 15 °C. Three other fungi, *Alternaria brassicae*, (Ab17), *A. brassicicola* (Abc7) and *Leptosphaeria maculans* (Lm6), used for the restriction analysis, were cultured at 22 °C on V-8 juice agar supplemented with 0.004% rose bengal. Discrete RAPD bands of 0.40, 0.60, 1.1, 1.5, and 1.6 kb were separately isolated from the 1% agarose gel in microcentrifuge tubes and purified by using GENECLEAN II kit (BIO 101 Inc., La Jolla, CA, U.S.A.) following the supplier's instructions. Details of the origin of probes are given in Table 1. Simultaneous amplification and labeling of probes with PCR was performed in a thermocycler (Thermolyne, Temp. Tronic, Barnstead/ Thermolyne Corporation, Dubuque, IA, U.S.A.) at the following temperature profiles: 94 °C for 5 min to denature genomic DNA; 35 °C for 1 min for low stringency annealing of the primer, and 72 °C for 1 min for primer extension. Forty additional cycles were run at 95 °C for 1 min, 35 °C for 1 min, and 72 °C for 2 min. An additional cycle at 72 °C for 5 min was run at the end of these cycles. The probes were labeled with alkali labile Dig-11-dUTP(Boehringer Mannheim) in a 50 µl volume containing 5µl 10X PCR buffer (200 mM Tris-HCl, pH 8.4, 50mM KCl), 1.5 µl of 50 mM MgCl₂,

0.25 μ l (1-1.5unit) Taq DNA polymerase, 1 μ l of each of 10 mM dATP, dGTP, dCTP, 10 μ l Dig-11-dUTP+ dTTP (1:4) (all Gibco BRL), 2.5 μ l of PCR product-specific 10 mM primer and 2.5 μ l (10-15 ng) template DNA. The reaction mixture devoid of Dig-11-dUTP was used as control. Estimation of the yield of Dig-labeled probe was done as per the method given in Genius System User's Guide for Membrane Hybridization, Version 3.0 (Boehringer Mannheim Corp., Indianapolis, IN, U.S.A.). The genomic DNA (2-4 μ g) of *R. secalis*, *A. brassicae*, *A. brassicicola*, and *L. maculans* were digested with endonuclease *Pst*1 (Gibco BRL) at 37 °C for 1 h in a buffer supplied by the manufacturer. Southern hybridization and chemiluminescence detection were performed according to the instructions given in Genius System User's Guide for Membrane Hybridization.

Results and Discussion. Five discrete polymorphic RAPD bands from 106 isolates of *R. secalis* were excised from the agarose gel, purified and labeled with Dig-11-dUTP using PCR. The yield of Dig-labeled probes, RsP1 and RsP2 was found to be 10 ng/ μ l whereas it was 100 ng/ μ l for the probes RsP3, RsP4, and RsP5. The concentration of these probes was standardized between 10-25 ng/ μ l for Southern hybridization and the probe solution was reused in subsequent hybridization experiments. The amplified PCR products were used as hybridization probes in Southern analysis. Details of the five probes along with their sizes are given in Table1. All five probes were used to hybridize with

Table 1. Sources of RAPD bands used for probe synthesis

Probe	Isolate No.	Primer sequences	Probe size (kb)
RsP1	98	OPA 01 (5'- TGCCGAGCTG -3')	0.4
RsP2	100	OPA 02 (5'- TGCCGAGCTG -3')	0.6
RsP3	103	OPA 05 (5'- CAGCACCCAC -3')	1.1
RsP4	98	OPA 13 (5'- AATCGGGCTG -3')	1.5
RsP5	106	OPA 20 (5'- AATCGGGCTG -3')	1.6

the Southern blots of the *Pst*1 restriction digests of four fungal species namely, *R. secalis*, *A. brassicae*, *A. brassicicola*, and *L. maculans*. Out of these, three probes i.e. RsP1, and RsP3 specifically hybridized with the single copy sequences and RsP2 with repetitive copy sequences of the restriction digests of *R. secalis* but not with the restriction digests of other species. However, probes RsP4 and RsP5 non-specifically hybridized with the restriction digests of *R. secalis* as well as with those of *A. brassicae* and *L. maculans*. During this study, three probes specifically hybridized with the *Pst*1 restriction digests of *R. secalis* but not with the restriction digests of other species tested. However, these probes need to be tested further using a greater number of species for restriction

analysis in order to establish a high level of specificity. Being non-radioactive, these probes could be used for the identification of *R. secalis* both in culture, infected tissues and seeds without any additional facilities being required for maintaining radioactive probes. Since RAPD fragments have already been used as hybridization probes in different organisms (1, 2, 3, 5, 10), this method holds great promise in the case of *R. secalis*, where early detection of the pathogen in the seed or at the seedling stage of the host will be useful in planning disease control strategies. Therefore, availability of specific DNA probes would be helpful in the molecular detection of this important pathogen of barley. Species-specific probes made during this study would also be useful in various molecular biology studies of *R. secalis* and for the chemical synthesis of genome-specific primers, after determining their base sequences.

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Proposal for barley fingerprinting by using RFLP, STS and robotized RAPD. V. TERZI and N. PECCHIONI, Experimental Institute for Cereal Research, Section of Fiorenzuola d'Arda, I-29017, Via S.Protaso, 302, Fiorenzuola d'Arda (PC), Italy

Varietal characterization of small grain cereals has been based, since the beginning of the century, upon morpho-physiological traits, isozymes and seed storage proteins. New and powerful tools based on molecular markers have been proposed for a more precise assessment of genetic similarity among varieties.

For barley varietal identification, we proposed RFLP analysis of highly polymorphic loci, as those encoding for seed storage proteins and thionins. By using three probe/enzyme combinations 51 varieties have been identified (Pecchioni et al., 1993). A sequence tagged site (STS) has been developed from a B-hordein cDNA sequence and its efficiency in discriminating barley genotypes has been evaluated in comparison with twenty-two morpho-physiological traits, four RFLP probes and hordein A-PAGE using Procrustes analysis (Faccioli et al., 1995). RAPD analysis, helped by laboratory robot, is proposed as speed up of fingerprinting of barley, triticale and oat varieties. From the comparison of the proposed methods, it can be concluded that varietal identification could be based on a strong integration of the different systems.

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RAPD tagging of barley chromosome 3L with special consideration of the BaMMV/BaYMV resistance gene *ym4*. J. Weyen¹, E. Bauer², A. Graner², W. Friedt¹ and F. Ordon^{1, 3, 4}, ¹ Institute of Crop Science and Plant Breeding, Justus-Liebig-University, D-35390 Giessen, Germany, and ² Federal Centre for Breeding Research (BAZ), Institute of Resistance Genetics, D-85461 Grünbach, Germany. ³ Corresponding author

Introduction. The Barley Yellow Mosaic Virus Complex, i.e. barley yellow mosaic virus (BaYMV), barley mild mosaic virus (BaMMV) and BaYMV-2 causes high yield losses in winter barley. Different strains of this complex were found up to date in Asia and nearly all central European barley growing countries. In contrast to Barley Mild Mosaic Virus (BaMMV) and Barley Yellow Mosaic Virus, BaYMV-2 is able to infect cultivars carrying the resistance gene *ym4* (Friedt et al. 1990, Huth 1990), which is presumed to be present in all BaMMV/BaYMV resistant cultivars released in Germany up to now. Due to the fact that BaMMV is efficiently transmissible mechanically - in contrast to BaYMV and BaYMV-2 - it can be handled in the greenhouse under controlled conditions required for genetic studies (Friedt 1983). Genetic analyses revealed that different recessive genes conferring resistance to BaMMV are present within the barley gene pool (Götz & Friedt 1993, Ordon & Friedt 1993). Although germplasms resistant to BaYMV-2 were detected soon after the first appearance of this strain (Huth 1991, Ordon et al. 1993) little is known about the mode of inheritance of resistance to this virus up to now. The BaMMV/BaYMV resistance gene *ym4* has earlier been located on chromosome 3 (Kaiser & Friedt 1992). Furthermore, a closely linked RFLP-marker has been developed by Graner & Bauer (1993) and linkage to the *Est1-Est2-Est4* isozyme cluster was detected by Le Gouis et al. (1995). RAPD-analysis was carried out in order to achieve a further marker saturation of the chromosomal segment around the *ym4*-locus and to establish a marker-assisted selection procedure fitting the high throughput requirements of breeding programmes.

Material and Methods. The mapping population consisted of a progeny comprising 283 F₁ anther derived DH-lines of a cross between cv. 'Franka' (*ym4*) and cv. 'Igri'. Reaction to BaMMV was estimated in two replications, each comprising five plants per line by mechanical inoculation according to Friedt (1983) followed by DAS-ELISA. DNA-extractions were carried out according to Graner et al. (1991). Screening for RAPD-polymorphism was performed by bulked segregant analysis (Michelmore et al. 1991) with bulks containing DNA of 15 DH-lines, each. PCR reaction mixtures contained 1.5 U of AmpliTaq® DNA polymerase, Stoffel fragment (Perkin-Elmer), with 3 µl of the Stoffel reaction buffer. Amplification was performed in a volume of 25 µl containing 25 ng template DNA, 6 mM MgCl₂, 0.3 µM of a random 10mer primer (Operon-Technologies Inc.) and 0.4 mM dNTPs. The reaction mix was overlaid with mineral oil. Reaction products were resolved by electrophoresis in 2.0% agarose gels and treated with ethidium bromide and visualised by UV illumination (286nm). RAPD-PCRs were conducted using a thermal cycler (TC480, Perkin-Elmer), according to the reaction and amplification conditions described by Sobral & Honeycutt (1993). Following an initial denaturation step at 94°C/4 min the cycler was programmed for 1 min at 94°C followed by 45 cycles of 94°C/1 min, 36°C/1 min and 72°C/2 min, respectively. The heating rate was fixed at 5°C/min. The polymerisation step was extended for 3 sec/cycle at 72°C. The extension step in the last cycle was 4 min. To determine the linkage relationships of markers that were detected by this approach, the programme MapMaker (Lander et al. 1987) was used. Crossover units were converted into map distances (centi-Morgans, cM) by the application of the Kosambi function (Kosambi 1944).

Results and Discussion. Out of 310 RAPD-primers tested four showed linkage to the *ym4* gene (Fig.1), i.e., OPZ04H660 (5'AGGCTGTGCT3'), OPK15H1230 (5'CTCCTGCCAA3'), OPL14H910 (5'GTGACAGGCT3'), and OPN11H800

(5'TCGCCGCAAA3'). While OPZ04H660 and OPL14H910 are obviously closely linked to *ym4*, the markers OPK15H1230 and OPN11H800 are located about 24 cM proximal of *ym4* Fig. 2).

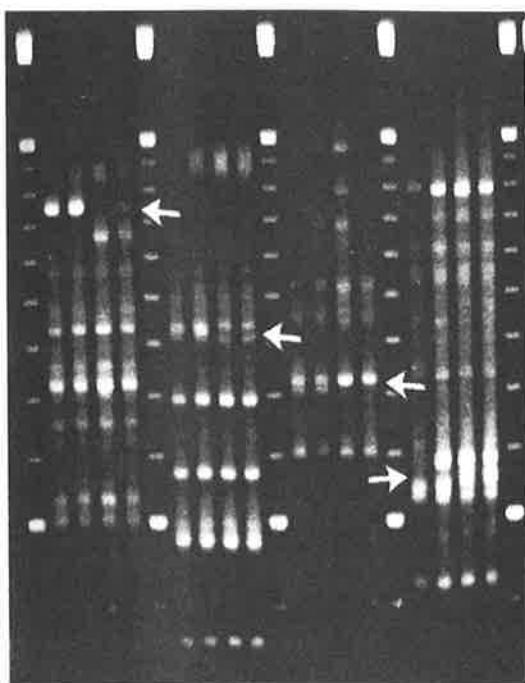


Fig. 1: Polymorphic RAPD-patterns of cv. 'Franka' (lanes 2, 7, 12, 17), resistant bulks (lanes 3, 8, 13, 18), cv. 'Igrí' (lanes 4, 9, 14, 19) and susceptible bulks (lanes 5, 10, 15, 20) with OPK15H1230 (lanes 2-5), OPL14H910 (lanes 7-10), OPN11H800 (lanes 12-15) and OPZ04H660 (lanes 17-20). Lanes 1, 6, 11, 16 and 21 represent 100 bp DNA ladder.

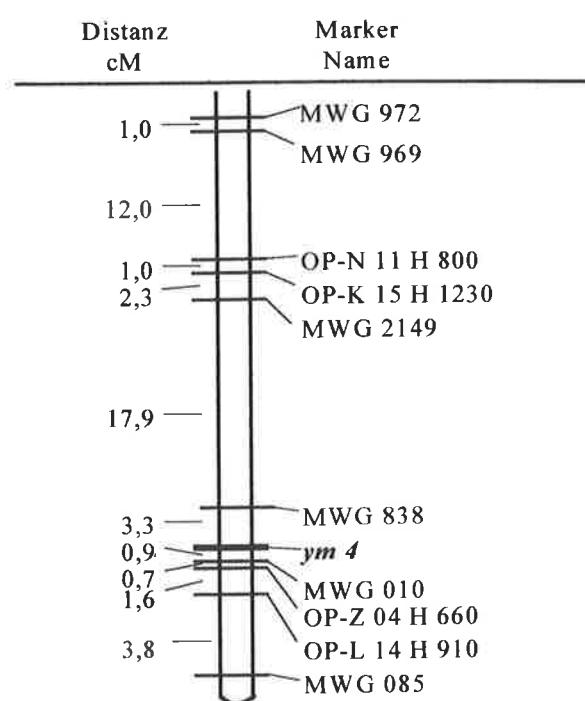


Fig. 2: Genetic map of barley chromosome 3L including *ym4*.

Especially the closely linked markers OPZ04H660 and OPL14H910 are well suited to apply marker-assisted selection for BaMMV-resistance encoded by *ym4*. Due to the PCR-procedure which requires only minimal amounts of DNA, these markers facilitate an efficient selection in early generations. Using DH-lines DNA may be extracted directly from plants developed *in vitro* giving reliable results on the presence or absence of *ym4* in an early breeding stage. The conversion of OPZ04H660 to a codominant PCR-marker for the detection of the resistance gene in its heterozygous state is in progress. Furthermore, in a germplasm survey, OPZ04H660 was shown to efficiently discriminate between susceptible and resistant German (*ym4*) barley cultivars (Ordon et al. 1995). Previous studies have revealed a large genetic variability for resistance to the BaMMV/BaYMV complex (GÖTZ & FRIEDT 1993, Ordon & Friedt 1993). Future work will concentrate on the detection of RAPD-markers for resistance genes different from *ym4*. Finally, specific markers will offer the opportunity of the marker-assisted combination of different resistance genes in one breeding line or variety. Combining different genes in one cultivar may prevent the selection of new virus strains as already reported from Japan, where seven strains of BaYMV and two of BaMMV have been described (Kashiwazaki & Hibino 1995).

Besides the establishment of marker-assisted selection procedures, the saturation of the genetic map around the *ym4* locus is a prerequisite for the development of a high resolution map as well as for attempts of positional cloning of the *ym4* resistance gene (Bauer & Graner 1995, Graner et al. 1995).

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IDENTIFICATION OF RAPD MARKERS LINKED TO *Pc-91*.

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Introduction

The crown rust resistance (CRR) conferred by the Amagalon gene (*Pc-91*) is the most effective gene in oat breeding germplasm of the northern plains. As such, considerable research effort has focused on incorporation of *Pc-91* into elite germplasm, and identification of linked molecular markers.

Combining effective resistance genes may increase the durability of single-gene resistance and confer some protection if resistance is overcome. DNA marker-assisted selection will facilitate the combination of resistance genes in the next generation of cultivars. The efficacy of marker-assisted selection depends on the reliability of the marker to identify the resistance gene and the ease of evaluation.

The objectives of this study were to (1) identify RAPD markers linked to oat crown rust resistance gene *Pc-91* and (2) evaluate the reliability of RAPD marker-based selection for crown rust resistance in oat breeding populations.

Materials and Methods

A leaf disc extraction method (Edwards et al., 1991) was used to isolate genomic DNA for PCR amplification. PCR was performed in 25 μ l reactions (1X Tfl buffer, 0.188U Tfl DNA polymerase (Epicentre Tech.), 200 μ M dNTP's, 1.5 mM MgCl₂, 200 μ M primer, and 25-40 ng genomic DNA) on an automated thermocycler (Perkin Elmer Cetus) programmed for 45 cycles of 94 °C 1 min, 35 °C 1 min, and 72 °C 2 min.

Amplification products produced by 517 random primers (Operon Tech.) were evaluated to detect polymorphic amplifications. Linkage of polymorphic amplification products was evaluated in $F_{5:6}$ lines and a BC population segregating for *Pc-91*.

Crown rust resistance conferred by *Pc-91* was introgressed into the cultivated oat from the tetraploid oat *A. magna* (Rothman, 1984). In this study, RAPD markers linked to crown rust resistance gene *Pc-91* were evaluated in the tetraploid *A. magna*, early generations of the synthetic hexaploid, and Amagalon-derived germplasm lines obtained from P. Rothman and H. Rines at the University of Minnesota, St. Paul, MN.

Results

Identification of RAPD Markers Linked to *Pc-91*

Seedling classification after inoculation with a CR composite avirulent on *Pc-91* identified two infection types (IT): an immune IT with no visible signs of infection and susceptible (IT 3 or 4). Single gene segregation (3:1) was observed in 1 of 4 segregating families.

Resistant and susceptible segregates were evaluated with 517 random primers to detect amplification differences. Two primers, AG12 and W17, produced polymorphic amplification products. AG12 produced six amplification products ranging from 300-2100 bp in size. The largest band (2100bp, referred to as R-2100) was differentially amplified only in the resistant segregates. W17 produced a range of products (200-700 bp). An intense 850 bp (referred to as R-850) amplification product exhibited putative linkage to *Pc-91*.

Both differential amplifications were present in resistant individuals and absent in susceptible individuals, indicating coupling linkage to *Pc-91*. Linkage of the R-2100 marker appeared to be complete or very tight. Linkage estimates of the R-850 marker placed it approximately 4.5 cM from *Pc-91*. Linkage relationships were confirmed in a BC population segregating for *Pc-91*.

Evaluation of R-2100 and R-850 in Amagalon Germplasm

Both markers were evaluated for the presence/absence of each marker in a selection of germplasm lines that possess *Pc-91*. R-2100 and R-850 were identified in all the Amagalon germplasm lines, early generation Amagalon germplasm, and the wild tetraploid *A. magna* accession (Table 1).

Table 1. Evaluation of germplasm lines developed by Rothman (1984, 1986) for the presence of R-2100 and R-850.

<u>Pedigree</u>	<u>Selection number</u>	<u>Present (+)</u>	
		R-2100	R-850
<i>A. magna</i>	CI 8330	+	+
Amagalon (early)	RL3191-6	+	+
Amagalon/2/A. sterilis/Kyto	RL3414-6	+	+
Amagalon	R801441	+	+
Amagalon/*4Ogle	R16-35	+	+
Amagalon/Starter/Ogle F ₆	RBT1114	+	+
Amagalon/Marvellous	R795026-4	+	+
Amagalon/Black Mesdag/Aojss	R805065-5	+	+

Amagalon-derived germplasm has been utilized in the oat breeding program at North Dakota State University. The reliability of both markers was evaluated in independently derived lines in the breeding program. The linkage relationship between both markers and *Pc-91* was confirmed in populations derived from RBT1114 and R801114, but deviated in two other populations (Table 2).

Table 2. Evaluation of linkage relationship in breeding lines with *Pc-91* resistance.

<u>Population</u>	Amagalon germplasm number	<u>Marker present/total</u>	
		R-2100	R-600
1	RBT1114	11/11	11/11
2	R801114	11/11	11/11
3	R805065-5	3/7	3/7
4	R805065-5	0/3	0/3

In populations 3 and 4 (derived from R805065-5), resistant plants were identified in the field that lacked R-2100 and R-850.

Aojss Resistance

Further evaluation of population 3 for additional resistance genes was performed using crown rust evaluation and RAPD marker analysis. A single line in population 3 that was homozygous for CRR in the field evaluation was selected for analysis. Eight resistant individuals were evaluated for the presence of both markers. One individual was identified that lacked both markers indicating recombination between *Pc-91* and R-2100 or the presence of an additional CRR gene.

Single panicles from this line were selected, and segregation for CRR was evaluated in the greenhouse. Inoculation with a CR composite avirulent on *Pc-91* identified a single $F_{4:5}$ line segregating 15R:1S. A 15:1 segregation indicated that a CRR gene in addition to *Pc-91* was present and segregated independently. The presence of an additional CRR was verified in other $F_{4:5}$ lines. This gene was likely contributed by the Aojss germplasm developed by Rothman (1986). The two gene combination has been identified in advanced lines within the breeding program.

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Composite interval mapping of QTL in barley: effects of map density.

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Introduction. In barley (*Hordeum vulgare* L.) and other plant species, highly saturated marker maps have been developed. Researchers using such maps to detect quantitative trait loci (QTL) sometimes use only a subset of the available marker data, often aiming for a "skeletal map" of fairly evenly spaced markers. Clearly, retaining data for more than one co-segregating marker is unnecessary. Furthermore, any questionable (ie. possibly mis-scored) marker data should be removed or corrected. Beyond this, is it useful or harmful to eliminate markers from a map? Is it better to use a dense map or a sparse map to detect QTL and to estimate their positions and effects?

Darvasi et al. (1993) conducted simulation experiments with individual unlinked QTL at various positions on marker maps of varying density, and used simple interval mapping to analyze the data. They found little advantage to increasing the marker density to more than one marker per 10 cM. Hyne et al. (1995) conducted simulation experiments with 40 markers spaced at 5 cM, and with 10 markers spaced at 20 cM, and found similar results, using 'marker regression' analysis to detect QTL. Neither of these previous studies included linkage blocks with more than one QTL, and neither used the recently proposed methods of composite interval mapping (Zeng 1993, 1994).

To further investigate the effects of marker density on QTL mapping, we simulated dense and a sparse genome maps, placed individual QTL and QTL linkage blocks at different positions in the genome, and used composite interval mapping to detect the simulated QTL. In addition, we used dense and sparse versions of the Steptoe/Morex marker map to analyze real quantitative data from the Steptoe/Morex cross.

Materials and Methods. Like barley, the simulated genome had seven pairs of chromosomes. The simulated marker map was either dense, with 4-cM intervals between markers, or sparse, with 20-cM intervals between markers. QTL were assigned to six of the seven chromosomes. Four chromosomes contained one QTL each. One chromosome contained a linkage block with QTL alleles in coupling phase and one chromosome contained a linkage block with QTL alleles in repulsion phase. All QTL were positioned so that they were midway between two markers on the dense map. Different positions relative to the markers on the sparse map were tried and compared. QTL with different effects were also compared. Mapping populations of 150 DH lines were simulated, with 500 repetitions for each set of parameters that was simulated.

From North American Barley Genome Mapping Project marker data available in January 1995 (A. Kleinhofs, personal communication), we derived a 223-marker 'base map' of Steptoe/Morex (<http://gnome.agrenv.mcgill.ca/info/basemaps.htm>), with markers chosen to provide 2- to 5-cM intervals between markers (where possible) and to provide a stable marker order in Monte Carlo evaluations with a simulated annealing mapping algorithm (Holloway and Knapp, 1994). The average interval between markers was 5.5 cM. From this dense marker map, we derived an sparse marker map with an average of 19.2 cM between markers. Distances on the dense map were assumed to be correct and additive, so distances on the sparse maps were obtained by summing intervals from the

dense map, not by remapping with a subset of marker data.

Simulated and real data were analyzed by composite interval mapping. Permutation was used to establish significance thresholds with a 5% genomewide Type I error rate. In the simulation experiments, the power of detection was determined for each simulated QTL. Realized type I error rates were assessed in two ways: by recording the frequency with which QTL were detected on the chromosome that contained no QTL, and by recording the frequency with which the number of QTL present was overestimated on each of the six chromosomes that contained QTL.

Results and Discussion. In the simulations, the two maps were about equally accurate in estimating the positions and effects of individual unlinked QTL. QTL with the small effects were detected slightly more frequently with the dense marker map than with the sparse map. For linked QTL, the dense marker map was more efficient than the sparse map at detecting the presence of both QTL and at estimating their positions. This was true for both coupling- and repulsion-phase configurations. The linkage phase affected the power of QTL detection for all three marker maps. Linkage between QTL interfered strongly with estimation of QTL effects.

In the analysis of real quantitative-trait data from Steptoe/Morex, we detected more QTL with the dense map than with the sparse map. Most of the additional QTL detected with the dense map were estimated to have small effects.

Based on the simulation experiments and the Steptoe/Morex data analysis, we concluded that, with composite interval mapping, a sparse skeletal map permits the detection of QTL with large effects, but a dense map allows for a more thorough genomic search for QTL, including linked QTL and QTL with small effects.

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Section VIII: Biotechnology (b) – Maps and Chromosomes

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HIGH RESOLUTION MAPPING AND RICE SYNTENY AROUND THE YM4 VIRUS RESISTANCE LOCUS ON CHROMOSOME 3L.

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INTRODUCTION

Resistance of European winter-barley to barley mild mosaic virus (BaMMV) and barley yellow mosaic virus strain 1 (BaYMV-1) is dependent upon the single recessive gene *ym4*, which confers complete immunity. Virus particles are delivered into root cells of susceptible plants via the soil-borne fungus *Polymyxa graminis* under low temperature conditions in fall and winter (Toyama and Kusaba, 1970). The *ym4* gene was mapped to the distal portion of chromosome 3L, where it is tightly flanked by two RFLP markers (Graner and Bauer, 1993). Since there are no indications about the molecular mechanism leading to immunity, a map based cloning approach has been chosen to facilitate isolation and functional analysis of the resistance gene. As a first step, a high resolution map around the *ym4* locus is being constructed and additional, closely linked markers are being identified by using the AFLP technique.

Compared to barley, the rice genome has a considerably lower complexity and contains less repetitive DNA. Also, large insert libraries have been established in this species. As a precondition for using the rice genome as a platform for the physical isolation of the *ym4* gene synteny between barley chromosome 3 and rice chromosome 1 has been investigated.

MATERIALS AND METHODS

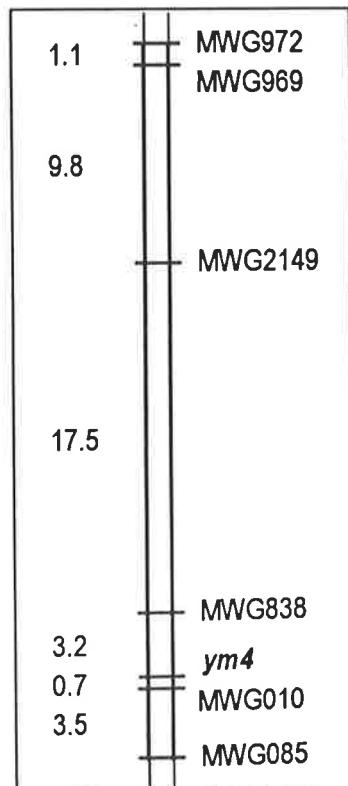
Linkage analysis around the *ym4* locus was carried out in a F₁ anther-derived doubled haploid (DH) population from a cross between the German cultivars Igri (susceptible) and Franka (resistant to BaMMV/BaYMV-1). Further analysis was made in a F₂ progeny of the same cross. DNA extraction, digestion, hybridization and PCR analysis of marker MWG838 were performed as described earlier (Graner et al., 1991; Bauer and Graner, 1995). Mechanical transmission of virus-particles (BaMMV) to 6-10 plants of each DH-line followed the procedure described by Friedt (1983).

RESULTS AND DISCUSSION

Map construction

In order to confirm the exact map position of the RFLP-markers flanking the *ym4* gene, genetic analysis was carried out in a DH-population consisting of 287 lines. As a prerequisite for map based cloning a genetic resolution exceeding 0,1% recombination is required. We therefore developed a strategy for the generation of a corresponding

Figure 1. Partial map of the distal portion of chromosome 3L based on the analysis of 287 DH-lines. Recombination values are given on the left side.



population comprising 1040 F₂ plants. In a first step, RFLP analysis was carried out using the two flanking markers MWG010 and MWG838 (Fig. 1). The distance between these markers in the F₂ population was calculated to be 2.5 ± 0.3 cM, which is slightly less than in the DH-population (3.9 ± 1.1 cM), but still within the limits of the standard deviation.

Plants carrying recombinant chromosomes in the target region were selfed to generate F₃ families. Identification of homozygous, recombinant plants allowed a mapping population to be established that consisted of 50 informative lines carrying homozygous crossovers in the target interval (MWG838-MWG010). These lines can be stably propagated and subsequently used for repeated resistance tests in F₄ and later generations. The genetic resolution within the target region will be less than 0.05% recombination \pm sd.

The identification of additional molecular markers within the interval MWG010-MWG838 is required for later identification of clones from large insert libraries carrying the *ym4* gene. To this end, Amplified Fragment Length Polymorphism (AFLP) analysis of phenotypic pools, each consisting of 10 resistant and 10 susceptible lines from the DH-population was performed. Altogether, some 35,000 loci were surveyed by running 350 primer-enzyme combinations, yielding 30 reliable markers that differentiated the pools.

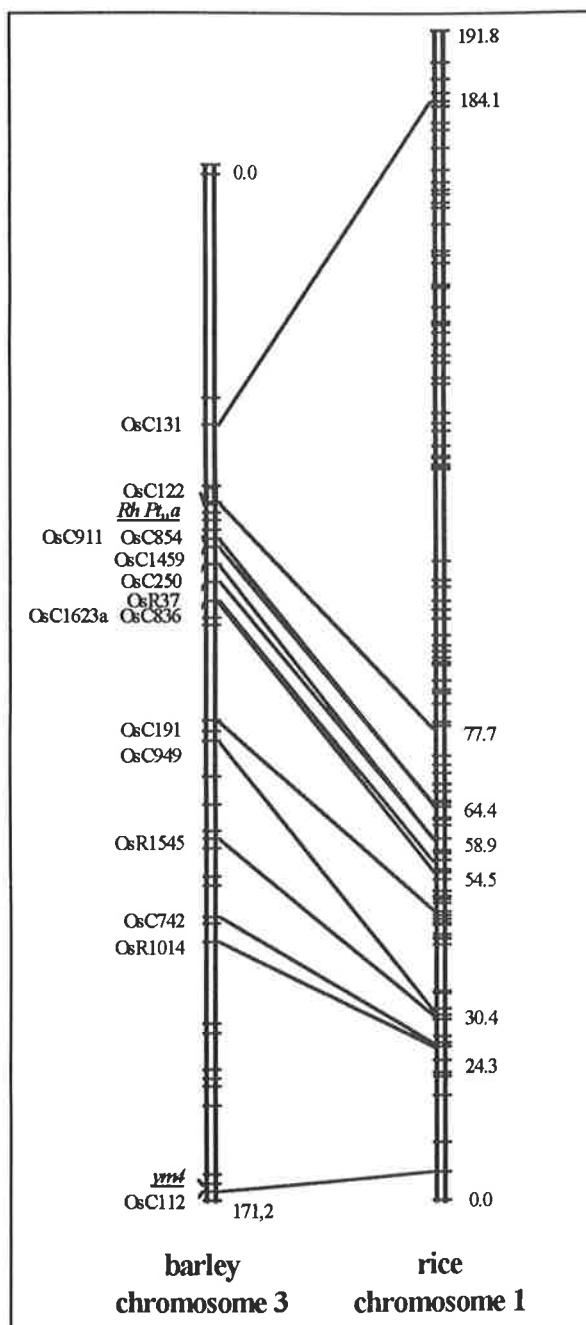


Figure 2. Comparison of the RFLP maps of rice (Kurata et al. 1994a) and barley. Cumulative distances are given in cM. The barley chromosome has been oriented with the short arm on top. The crosshatched region indicates the approximate position of the centromere of the barley chromosome. The position of the centromere in rice is not known. Common rice markers have been connected by lines.

With a size of 21 cM, the genetic window of the pools comprises about 1.4 % of the total genome length (1500 cM). Given an AFL polymorphism of 10% between the two parents, the actual number of detected markers slightly below expectation (49). AFLP analysis will be continued on the level of individual progeny lines to determine the degree of linkage between individual AFLP markers and the *ym4* gene. Also, the conversion of usually dominant AFLP markers into PCR-based, codominant STS (sequence tagged site) markers will be attempted to generate additional, user friendly markers suitable for breeding programs.

Synteny barley-rice

Since the homoeologous group 3 of the *Triticeae* largely corresponds to rice chromosome 1 (Kurata et al. 1994b) attempts are being made to integrate rice markers into the Igri x Franka chromosome 3 map. Out of 60 cDNAs tested, 15 were polymorphic and could be mapped in the standard population consisting of 71 DH-lines, revealing that chromosome 3L shows nearly perfect synteny to rice chromosome 1 (Fig. 2). Both chromosomes have a similar length (171 vs. 192 cM), however, individual distances vary considerably. For example, the interval OsC131-OsC122 represents less than 10% of the genetic length of the barley chromosome, whereas it comprises more than 50% of the rice chromosome. One rice marker (OsC112) could be identified, which cosegregates with MWG010 and, therefore, might represent a starting point for a chromosomal walk in the rice genome. However, the corresponding chromosomal region is not well saturated with markers. Hence, additional efforts will be required to establish a physical map of this region of rice chromosome 1.

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OCCURRENCE AND CAUSES OF UNREDUCED MALE GAMETE FORMATION IN HEXAPLOID OATS. M.B. DILKOVA and R.A. FORSBERG, Department of Agronomy, University of Wisconsin, 1575 Linden Dr., Madison, WI 53706, USA.

INTRODUCTION. Cytological investigations of species such as *Dactylis glomerata* subsp. *lusitanica* (2) provided evidence for meiotic abnormalities resulting in formation of pollen grains with somatic chromosome number. Disturbances in spindle formation at the second meiotic division and lack of cytokinesis at the end were identified. The nuclear content of the dyads and triads formed was genetically equivalent to second division restitution (4). Ellison (1) studied polyploid gamete formation in progeny of interspecific diploid *Avena* hybrids. He observed PMCs containing more than one nucleus due to failure of cytokinesis in the mitotic division prior to meiosis. Katsiotis and Forsberg (5) explained that the presence of 2n gametes in tetraploid *A. vaviloviana* was caused by premeiotic doubling. Regular meiosis then followed giving rise to pollen grains with 2n nuclear content. If mechanisms for sexual polyploidization produce viable gametes, they can be used for crop improvement purposes (4).

The major objective of the present study was to determine the formative causes of n+n and 2n gametes and the frequency of this phenomenon in hexaploid oat breeding materials.

MATERIALS AND METHODS. Plant material included F_1 hybrids from crosses between four crown rust (*Puccinia coronata* Corda var. *avenae*) susceptible breeding stocks, X5968-3, X5974-4, P7869D1-5-17-3 and PA8494-4099, and resistant selection MAM17-4 from the Wisconsin research and oat breeding programs and their F_2 , F_3 , and F_4 progenies. The genes for resistance carried by MAM17-4 have wild *A. strigosa* origin. Their initial incorporation into cultivated *A. sativa* was achieved with the derivation of translocation lines N770-165-2-1 and DCS1789 (3). Line MAM17-4 has both selections in its pedigree. Majority of the rust readings were done on an individual plant basis. PMCs came from an average of two plants per phenotype and represented the entire spectrum of rust reactions: highly resistant, resistant, moderately resistant, and susceptible. Panicles were fixed in Carnoy solution and stored in 70.0% ethanol. Pollen mother cells (PMCs) were examined from temporary squash preparations stained in 1.0% acetocarmine. Irregularities were recorded as a percentage of the total number of PMCs examined. Data were collected from at least 100 PMCs with ongoing meiotic division, a minimum of 100 microspore tetrads, or near 1000 pollen grains.

RESULTS AND DISCUSSION. Abnormalities that resulted in the formation of n+n and 2n microspores and pollen grains were observed prior to meiosis and through the duration of the microsporogenesis. Cytomixis was another manifestation of disturbed meiosis creating irregular male gametes.

Premeiotic doubling Disturbances in the preceding mitosis such as lack of cytokinesis, predetermined the formation of polyploid male gametes by introducing PMCs with doubled nuclear contents (Fig. 1a). The true identity of these 42 bivalent cells was revealed at diakinesis. At metaphase I, chromosomes were arranged in two overlapping plates. Although the division progressed regularly, it took much longer for these cells to complete meiosis compared to regular ones.

First meiotic division The failure of spindle formation or function prevented homologous chromosomes from separating in a regular fashion. Cytokinesis, however, did take place and resulted in the formation of dyads with one cell containing no nucleus and another one carrying the entire amount of chromatin. Division progressed with no further disturbances. Nonnucleated microspores continued to exist at the tetrad stage along with two 2n microspores (Fig. 1b) and were seen even later as pollen grains with only cytoplasmic content.

Second meiotic division There were dyads with an extremely unsynchronized second division due entirely to spindle failure. In some cases chromosomes remained in late prophase unable to approach the cell equator. In other cells, the entire mass of chromosomes was concentrated at the cell center with no further arrangement at the plate. Finally, the absence of spindle fiber activity prevented perfectly well arranged metaphase II chromosomes from moving toward the poles (Fig. 1c). The meiotic outcome was always a triad with two haploid microspores and one with somatic chromosome number. The absence of cytokinesis was another cause of second division restitution. It led to the formation of n+n type of microspores (Fig. 1d).

The investigation of parental materials and their F₁, F₂, F₃ and F₄ progenies strongly suggested that there was a connection between the incorporated *A. strigosa* chromosome segments carrying crown rust resistance genes and the constantly exhibited meiotic irregularities: multivalents, univalents, lagging chromosomes, bridges, micronuclei and unreduced gametes. Only 0.4% of the examined pollen grains from line MAM17-4 and none of the microspores from the susceptible breeding stocks contained multiple sets of chromosomes. The formation of n+n and 2n gametes was promoted in 1.9%, or less, of the tetrads from the investigated F₁ hybrids. This phenomenon was observed more often in F₂ populations where the maximum values, 3.8% and 5.4%, were recorded for the PMCs from moderately resistant materials. The percentage declined toward F₃ generation reaching nearly equal and low values for all phenotypes and all crosses. It did not exceed the 2.5% margin. At the F₄ progeny level, the average occurrence of n+n and 2n gametes was reduced to 1.0%.

The low frequency of the unreduced gametes limits the practical significance of this phenomenon. Pedigree selection for resistance to *P. coronata* in early generations with an emphasis on plant fertility will result in the identification and selection of stable, superior progenies with adequate regularity of microsporogenesis from crosses with MAM17-4 line

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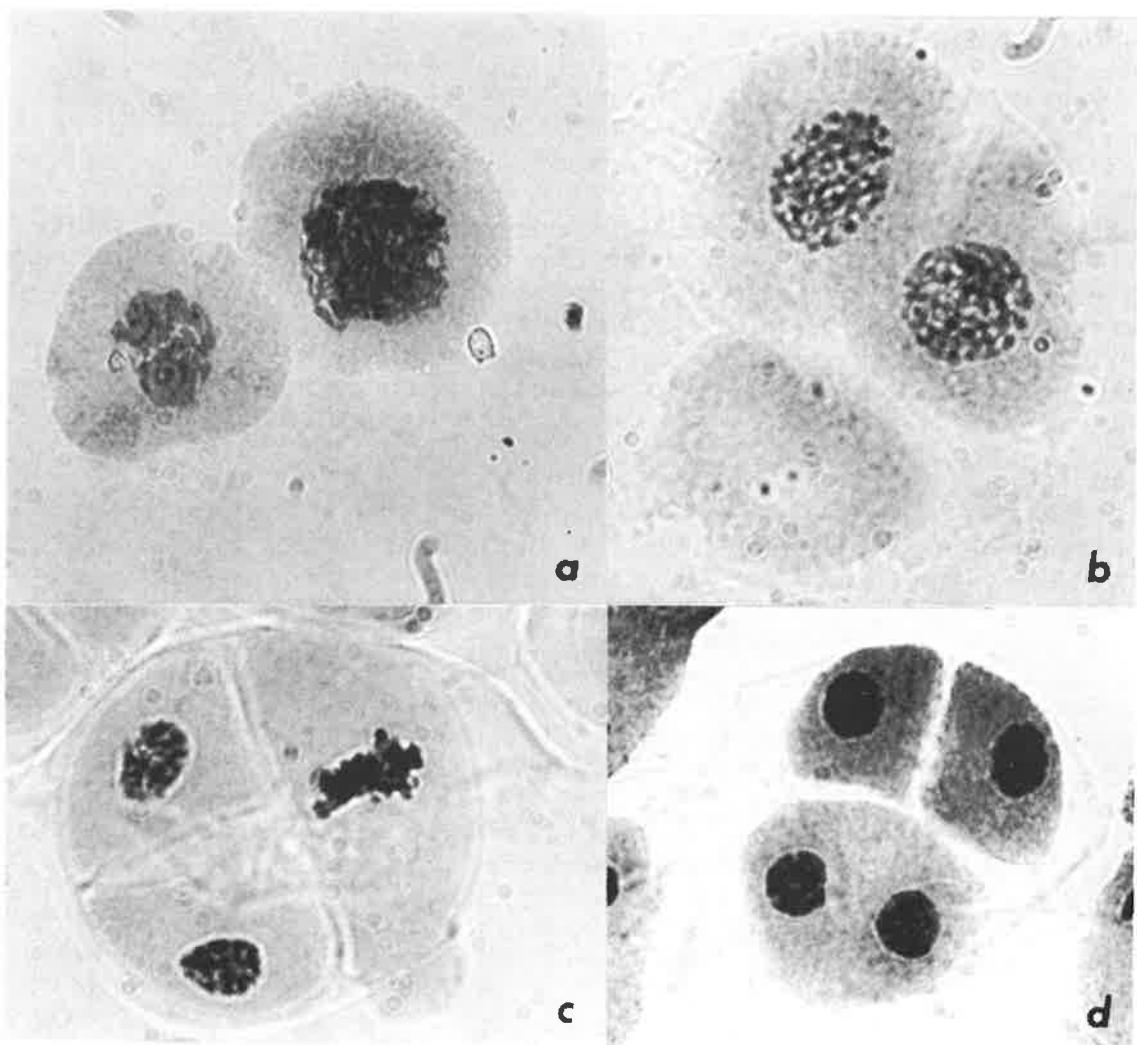


Fig. 1 a. PMCs with regular (left) and doubled (right) amount of chromatin at early prophase; b. One nonnuclear and two 2n spores resulted from a spindle failure at meiosis I; c. Unsyncronized second division due to spindle failure and lack of chromosome movement; d. Binuclear microspore as a result of lack of second cytokinesis.

Analysis of hybrids between barley and species of Elymus. GEORGE FEDAK and RENE PETROSKI, Eastern Cereals and Oilseeds Research Centre, Agriculture and Agri-Food Canada, Ottawa, Ontario K1A 0C6 Canada

Elymus is an extremely large and diverse genus consisting of several hundred species at several ploidy levels. The wide geographical distribution has produced a genus with a wide range of tolerances to biotic and abiotic stresses that could be used to widen the gene pool of crop plants.

Various species of the genus have already been crossed to wheat and this study represents an attempt to hybridize a range of Elymus species to barley. A total of eight hybrid combinations were produced. The barley cultivars used predominantly were Betzes and Elrose and to a lesser extent Bonus and Bomi. The Elymus species that hybridized with the barleys were: Elymus alatavicus, arizonicus, barbicallus, curvatus, donianus, interruptus, laxiflorus and sinicus. E. alatavicus was a hexaploid species and the remainder were tetraploids. Hybrids were produced at frequencies ranging from 0.8 to 3.6 plantlets as a percent of pollinated florets. The hybrids in most cases were vegetatively very vigorous with the Elymus traits predominating in spike characteristics.

Chromosome pairing in most hybrids was very low ranging from .06 to 2.60 bivalent configurations per meiocyte. The one exception was the hybrid between Elrose and E. alatavicus where the average meiotic configuration was $20.95^I + 3.03^{II} + .21^{III} + .09^{IV}$. In the latter hybrid as in all others, fluorescent in situ hybridization using Elymus DNA as the probe confirmed that the majority of chromosome configurations were autosyndetic.

In these hybrids as with virtually all intergeneric hybrids involving barley, the plants were vegetatively virogous but totally sterile and backcrosses to barley were not successful.

Monosomics and RFLP linkage group associations in oat. S.L. FOX¹, E.N. JELLEN², S.F. KIANIAN³, R.L. PHILLIPS³ and H.W. RINES^{3,4}. ¹Agriculture and Agri-Food Canada, Semiarid Prairie Agricultural Research Centre, P.O. 1030, Swift Current, Saskatchewan, S9H 3X2, Canada. ²Dept. of Plant Pathology, 4024 Throckmorton Hall, Kansas State University, Manhattan, Kansas 66506, USA. ³ Dept. of Agronomy and Plant Genetics, University of Minnesota and ⁴USDA-ARS, St. Paul, Minnesota 55108, USA.

Introduction. Based on a mapping population of 71 F_{6:7} recombinant inbred lines from the cross 'Kanota'/'Ogle', an oat molecular map has recently been completed consisting of 561 markers mapped to 38 linkage groups (O'Donoughue et al., 1995). However, 21 linkage groups are expected for allohexaploid *Avena sativa* L. (2n=6x=42).

By using chromosome C-banding (Jellen et al., 1993 and unpublished), an oat monosomic series has been assembled (Table 1) consisting of monosomic lines obtained from the cultivars 'Kanota' (Morikawa, 1985), 'Sun II' (Riley and Kimber, 1961; Hacker and Riley, 1963; Leggett and Markhand, 1995) and from progeny of 'Sun II' oat polyhaploids developed from oat/maize crosses (Rines and Dahleen, 1990). Oat does not tolerate nullisomy well; fertile nullisomic lines have been isolated for only two chromosomes (7C and 18). Analysis of RFLP autoradiograms can be used to make associations between linkage groups and chromosomes by detecting the complete loss of bands in DNA from nullisomic lines or reduced signal strength in bands representing hemizygous loci in DNA from monosomic lines. The detection of reduced signal strength can be impeded by autoradiogram quality.

An F₁ monosomic series has been developed to associate oat molecular linkage groups with specific oat chromosomes. This series involved all of the monosomic lines indicated above and was used to detect the loss of maternal alleles.

Materials and Methods. Members of the various monosomic series were crossed to Ogle. The Sun II-derived materials were crossed to Kanota as well as Ogle. Monosomic F₁ plants were selected using chromosome counts of root tip cells at mitotic metaphase and were grown for seed and tissue for DNA extraction. F₂ progeny from monosomic F₁ plants also were grown for tissue. Tissues from eight F₂ plants were combined on an equal dry weight basis prior to DNA extraction to represent immortal F₁s. Genomic DNA was extracted from lyophilized tissues using a modified method from Kim et al. (1990).

Restriction enzymes *Eco*RI, *Hind*III, and *Dra*I were used to digest genomic oat DNA. Digested DNA was electrophoresed in 1% agarose. For Southern blotting, gels were treated with 0.25 N HCl for 10 minutes, followed by 30 minutes denaturing solution (0.5 M NaOH, 1.5 M NaCl), 30 minutes neutralizing solution (1 M tris pH 8.0, 1.5 M NaCl) and then transferred to Imobilon nylon membranes using 5X SSC.

From the oat molecular map, polymorphic probes were selected that represented different linkage groups and for which the maternal form of the polymorphism was visible. Thirty nanograms probe DNA/240 cm² blot was oligolabeled with ³²dCTP and allowed to hybridize to blots for 12-24 hours. Blots were washed for 30 minutes in a solution of 2X SSC and 1% SDS at room temperature and again at 65°C. These washes were then repeated using a solution of 0.1X SSC and 1% SDS. X-ray film was exposed at -70°C using intensifying screens.

Table 1. RFLP linkage groups (O'Donoughue et al. 1995) associated to specific chromosomes based on the analysis of an F_1 monosomic series.

Mono Aneuploid ^a	Group	Mono Aneuploid ^a	Group
1C K1, K2 (Mor)	21, 31	13 K16	6
2C K3	15	14 K7, K13 (Mor)	16
3C Sn276C-R2-4-13	20	K7	23
4C Sn1a 4-4, Sn10-2 1-5		K13 (Mor)	38 ^b
5C K8	5	15 K10, K20	7, 10
6C Sn5 1-3, Sn1a-R2 8-13		K20	28
7C SVII, SXIV	3	16 K18 (Fom)	20
8 SXVI, SXVII, SXVIII	23	17 K11, K17	3
9 Sn10-2 3-3	17	K17	24
10 Sn1a 3-1		18 K21	33
11 SI, K19	13	19 K12 (Mor), K14 (Mor), SXII	22
12 SV	2	20 Sn3b-R2 7-10	
		21 SVIII, SIX, SX	4, 12

^a K refers to aneuploids derived from the cultivar Kanota. S and Sn refers to aneuploids derived from the cultivar Sun II. Mor refers to Kanota aneuploids obtained from T. Morikawa. Fom refers to Kanota aneuploids obtained from A. Fominaya.

^b Requires remapping of loci for confirmation of linkage group

Results. In a monosomic F_1 , presence of both alleles of a polymorphic locus indicates that both homologues of a chromosome carrying that locus are present. The presence of only the paternal form of the polymorphic locus indicates that the maternal chromosome of the homologous pair was not contributed to the zygote and serves to associate the RFLP locus with the monosomic chromosome. Twenty-three linkage groups were associated with 17 different chromosomes using this technique (Table 1). Lines that were identified with C-banding as having the same chromosome deficiency were confirmed as such with RFLP data from monosomic F_1 s developed from the duplicate lines. Four translocations have been identified involving chromosome pairs 7C/17, 8/14, K7 and K13 forms of chromosome 14, and K11 and K17 forms of chromosome 17. The former two involved linkage groups with clustered loci, indicating reduced recombination that may be due to proximity to the centromere or structural differences (O'Donoughue et al. 1995). A translocation difference between the K7 and K13 forms of chromosome 14 had been previously noted by Jellen et al. (1993).

Discussion. C-banding has facilitated the assembly of a complete oat monosomic series. This procedure is effective in distinguishing between oat chromosomes as well as detecting translocations between A/D and C genome chromosomes. New deficiencies and lines duplicating already available deficiencies can be perceived.

To make associations between molecular linkage groups and specific chromosomes, both cytogenetic and molecular genetic information is needed. Prior to the development of a F_1 monosomic series, detection of lines monosomic for different chromosomes was done using C-banding and limited RFLP analysis of available nullisomic lines. Sun II monosomic lines were crossed with both Kanota and Ogle so that any probe on the oat map could be used in this study. Development of an F_1 monosomic

series allows for analysis of all chromosomes. The eight-plant F₂ bulks adequately mimicked the actual F₁ plant. Occasional contamination of the F₂ bulks was detected indicating that outcrossing needs to be controlled. Detection of contamination is simplified once chromosome - linkage group associations are known. F₂ bulks from the same monosomic F₁ plant can be created many times depending on seed production and allows for larger quantities of high quality DNA to be extracted from young tissues, a limiting factor when using the actual F₁ plants.

Because both Kanota and Sun II monosomics were obtained by screening respective cultivars, the genetic background of these monosomic lines is not uniform. In some cases, the monosomic line has the same allele as the paternal parent causing certain probes to be uninformative. Additionally, translocation polymorphisms have been noted among sets of lines in both genetic backgrounds (Jellen et al., 1993 and unpublished; Leggett and Markhand, 1995). Thus, it is necessary to demonstrate that the parents of each cross contribute different alleles so that type II errors are avoided.

Prior to the advent of molecular genetic methods and C-banding of chromosomes, verification of chromosomal differences between aneuploid lines was not reliable in oat. The use of an F₁ monosomic series has been effective in condensing the oat molecular map, identifying duplicate aneuploid lines, and identifying translocations. Comparison of linkage groups and their chromosome associations suggests that intact homoeologous chromosomes in common oat are rare due to substantial numbers of rearrangements. However, these rearrangements may allow novel recombination of traits.

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Barley *SNF1*-related protein kinase genes

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Introduction

Sucrose non-fermenting 1 (SNF1)-related protein kinases have been identified in a wide range of eukaryotes (1). The first member of the family to be characterised genetically was SNF1 itself from budding yeast (2), the gene for which was isolated in a screen for mutants defective in the regulation of the invertase gene *SUC2*. It was subsequently shown to be required for the derepression of a wide range of genes in response to glucose deprivation, and to regulate the activity of a number of metabolic enzymes, including glycogen synthase and acetyl-CoA carboxylase. No role in the regulation of gene expression has been demonstrated for the mammalian member of the family, AMP-activated protein kinase (AMPK) (3), but it does regulate acetyl-CoA carboxylase and other metabolic enzymes, including HMG-CoA reductase.

The first plant *SNF1*-related sequence to be isolated was a cDNA (*RKIN1*) from rye (4) and a functional relationship between SNF1 and RKIN1 was demonstrated, in collaboration with Dr. J.R. Dickinson (University of Cardiff, UK), by expressing an *RKIN1* cDNA in a yeast *snf1* mutant strain. SNF1 function was restored, allowing the yeast to grow on non-fermentable carbon sources. Plant SNF1-related protein kinases therefore potentially have roles in the regulation of gene expression in response to cellular glucose levels, and in the regulation of metabolic enzymes. This paper summarises our studies of the *SNF1*-related gene family of barley.

Materials and Methods

Southern blot analysis was performed using the methods described by Sabelli and Shewry (5). A genomic library of barley cv. Sunbar was supplied by Clontech and screened using the methods described by Halford (6). BKIN2 and BKIN12 PCR products were amplified from total RNA by reverse transcription followed by a PCR reaction of [2 minutes at 94°C, 1.5 minutes at 50°C and 3 minutes (plus 1 second per cycle) at 72°C] x 30, followed by 7 minutes at 72°C. DNA sequencing was performed by the dideoxy method and sequences were analysed using the Wisconsin programs (7). S1 mapping was performed using the methods described by Huttly *et al.* (8). Northern blot analysis was performed on total RNA, using the methods described by Sabelli and Shewry (5) for the hybridisation. Purification of HMG-CoA reductase kinase from barley endosperm was carried out using a procedure based on that described by Ball *et al.* (9) and MacKintosh *et al.* (10) and immunodetection was performed using the horseradish peroxidase (HRP)-based enhanced chemiluminescence (ECL) system (Amersham).

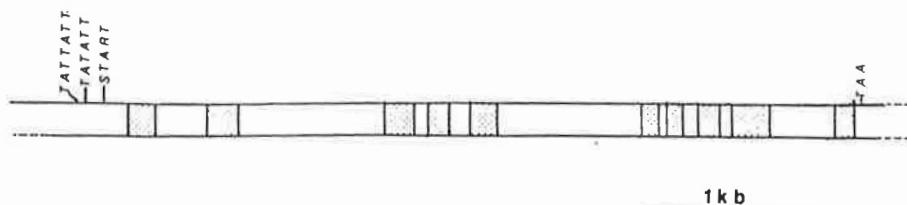
Results and Discussion

Barley SNF1-related gene family

Southern blot analyses of barley genomic DNA probed with a plant *SNF1*-related sequence showed that barley contains a multigene family of 10-20 *SNF1*-

related genes (11). Fifteen *SNF1*-related clones were isolated from a barley genomic library and two sub-types were identified and named *BKIN2* and *BKIN12*. The *BKIN2* clone was disrupted and lacked exon 2, but otherwise the two genes shared the same structure (Figure 1). The transcription start site of *BKIN12* was identified by S1 mapping and full-length PCR products for both types were amplified from reverse-transcribed RNA templates (11, 12). The *BKIN2* and *BKIN12* nucleotide sequences have been deposited in the EMBL database under accession numbers X65604 (*BKIN12* PCR product), X65606 (*BKIN12* gene) and X82548 (*BKIN2* PCR product).

Figure 1. Structure of the *BKIN12* gene. Exons are shaded.



Protein sequences

Comparison of the *BKIN2*, *BKIN12*, AMPK and *SNF1* amino acid sequences confirms that they are closely related protein kinases. Their catalytic regions are aligned in Figure 2. The barley proteins show 78% amino acid sequence identity to each other and 68% identity to *SNF1* and AMPK in this region. Protein kinases with this degree of sequence conservation are expected to have similar substrate specificities and functions.

Figure 2. Catalytic regions of *BKIN2*, *BKIN12*, AMPK and *SNF1*

BKIN2	L G I G S F G K V K	I A E H I I T G H K	V A I K I L N R R K	I K S M E M E E K V	K R E I K 48
BKIN12	L G L G T F G D V K	V A E H K L T G Q R	V A I K I L N R R K	M E T M E M E E K A	N R E I K 67
AMPK	L G V G T F G K V K	I G E H Q L T G H K	V A V K I L N R Q K	I R S L D V V G K I	K R E I Q 66
SNF1	L G E G S F G K V K	L A Y H T T T G Q K	V A L K I I N K K V	L A K S D M Q G R I	E R E I S 105
BKIN2	I L R L . . . F M H	P H I I R L Y E V I	D T P A D I Y V V M	E Y V K S G E L F D	Y I V E K 90
BKIN12	I M R L F I D F I H	P H I I R V Y Q V I	E T P K D I F I V M	E Y C N N G E L D	Y I I E N 112
AMPK	N L K L . . . F R H	P H I I K K L Y Q V I	S T P T D F F M V M	E Y V S G G E L F D	Y I C K H 108
SNF1	Y L R L . . . H R H	P H I I K L Y D V I	K S K D R I I M V I	E Y A . G N E L F D	Y I V Q R 146
BKIN2	G R L Q E E E A R R	F F Q Q I I S G V E	Y C H R N M V V H R	D L K P E N L L L D	S K C N V 135
BKIN12	G R L Q E D E A R R	I F Q Q I I L A G V E	Y C H R I M V V H R	D L K P E N L L L D	S K Y N V 157
AMPK	G R V E E V E A R R	L F Q Q I I L S A V E	Y C H R H M V V H R	D L K P E N V L L D	A Q M N A 153
SNF1	D K M S E Q E A R R	F F O Q I I S A V E	Y C H R H K T V H R	D L K P E N L L L D	E H L N V 191
BKIN2	K I A D F G L S N V	M R D G H F L K T S	C G R P N Y A A P E	V I S G K L Y A G P	E V D V W 180
BKIN12	K L A D F G L S N V	M R D G H F L K T S	C G S L N Y A A P E	E I S S K L Y A G P	E V D V W 202
AMPK	K I A D F G L S N M	M S D G E F L R T S	C G S P N Y A A P E	V I S G R L Y A G P	E V D E W 198
SNF1	K I A D F G L S N I	M T D G N F L K T S	C G S P N Y A A P E	V I S G K L Y A G P	E V D V W 236
BKIN2	S C G V I I L Y A L L	C G T L P F D D E N	I P N L F K K I K G	G I Y T L P S H L S	P L A R D 225
BKIN12	S C G V I I L Y A L L	C G S V P F D D E N	I P S L F R K I K G	G T Y I L P S Y L S	D S A R D 247
AMPK	S C G V I I L Y A L L	C G T L P F D D E H	V P T L F K K I R G	G V E Y I P E Y L N	R S I A T 243
SNF1	S C G V I I L Y V M L	C R R L P F D D E S	I P V L F K N I S N	G V Y T L P K F L S	P G A A G 281
BKIN2	L I P R M L V V D P	M K R 238			
BKIN12	L I P K H L N H D P	M K R 260			
AMPK	L L M H M L Q V D P	L K R 256			
SNF1	L I K R M L I V N P	L N R 294			

Several *SNF1*-related genes, cDNAs and PCR products have been isolated from other plant species, including *Arabidopsis* (*AKIN10*) (13), tobacco (*NPK5*) (14)

and potato (*PKIN1*) (manuscript in preparation). All the plant sequences encode proteins with M_r s of approximately 58,000, making them slightly smaller than the catalytic subunit of AMPK (M_r 63,000) and SNF1 (M_r 72,000).

Northern blot analyses

BKIN2 and *BKIN12* contain divergent sequences in the C-terminal regions (12) and these were used to probe northern blots of RNA isolated from different barley tissues. *BKIN2* transcripts were detectable in all the tissues, while *BKIN12* transcripts were only detectable in the seed. The significance of this differential tissue-specific regulation of gene expression within the gene family is not known.

Immunological evidence that barley HMG-CoA reductase kinase is a member of the SNF1 family

Two protein kinase activities (HMG-CoA reductase kinase (HRK)-A and -B) with similar biochemical properties to AMPK had been partially purified from cauliflower and other plant species prior to the discovery that AMPK is a member of the SNF1 family (9, 10). Both phosphorylate HMG-CoA reductase and a synthetic peptide (the SAMS peptide) derived from the rat acetyl-CoA carboxylase phosphorylation site. In collaboration with Professor Grahame Hardie and co-workers at the University of Dundee, UK, we have shown that an M_r approximately 58,000 polypeptide present in both the partially purified HRK-A activity from cauliflower (15) and a similar activity partially purified from barley endosperm (manuscript in preparation) is recognised by two independent antibodies raised to a plant SNF1-related peptide and heterologously-expressed protein.

Proteins which interact with barley SNF1-related protein kinase (BKIN12)

In collaboration with Dr. J.R. Dickinson (University of Cardiff, UK), a number of cDNAs encoding proteins which interact with BKIN12 have been isolated using the 2-hybrid system. These include a putative *SNF4* homologue (*SNF4* encodes an activating subunit of the protein kinase in yeast), a small heat shock protein, a putative cadmium-induced protein and a protein with a ring finger motif (unpublished data).

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Inheritance and linkage relationships of morphological and isozyme loci in the A-genome diploid oat. D. L. HOFFMAN, USDA-ARS, P. O. Box 307, Aberdeen, Idaho, USA

Introduction. The genetic characterization of oat (*Avena spp.*) has historically lagged behind that of the other cereals. This may be due to polyploidy, large genome size, and the lack of well-defined cytogenetic stocks. The recent development of DNA marker technologies have led to development of a partial map for the hexaploid oat (O'Donoughue et al., 1995) and more complete maps for the A-genome diploid oat (Rayapati et al., 1994; O'Donoughue et al., 1995). The hexaploid oat map included only one morphological trait. The diploid maps did not include any morphological or isozyme loci. Such loci may be simpler alternatives to DNA-based markers for marker-assisted selection and could provide further information for comparative mapping. The objectives of this study were to 1) determine the inheritance of morphological traits and isozyme markers within diploid oats and 2) determine the linkage relationships of the traits and markers found to be monogenically expressed. This information will be a valuable addition to ongoing oat mapping efforts. This is the first report of this kind for the A-genome diploid oat.

Materials and Methods. Two *Avena strigosa* Schreb. and one *A. wiestii* Steud. accessions were chosen for study (Table 1.) These were screened for polymorphisms in 24 enzyme systems using methods described in Hoffman and Goates (1990). Twelve seedlings, tillering plants, and panicles of each accession were observed for morphological differences. Crosses were made unidirectionally between the accessions using the conventional method of Brown and Patterson (1992). Ninety-six F₂ plants and four plants of each parent were grown in a greenhouse. Leaf tissue of four-leaf seedlings was removed for isozyme analysis. Two mature panicles were removed from each F₂ and parental plant and seed traits were scored. Eight pregerminated F₃ seeds from each F₂ were planted and tillering plants were scored for leaf hairs and growth habit. All of the monogenic traits were then scored for a B/D F₆ recombinant inbred population that had been evaluated for crown rust resistance (Wise et al., 1996). Scores of the isozyme bands and morphological traits for the F₂ populations and F₃ families were initially tested for monogenic ratios and two-point linkage using the LINKAGE-1 program of Suiter et al. (1983). Linkages were verified and the most probable gene orders were determined with MAPMAKER Version 3.0 (Lander et al., 1987) program. Linkages and gene orders for the B/D recombinant inbred population were determined by MAP MANAGER Version 2.6.5 (Manly and Elliot, 1991).

Results and Discussion. Polymorphism among the three accessions was observed for five morphological traits and seven isozyme bands (Table 2.) The observed phenotypes of each trait fit the expectation for monogenic inheritance. All traits except for lemma color were tested in at least two different segregating populations. One linkage group of five loci was identified (Fig. 1) by compiling information from all three crosses. All of the other traits assorted independently. Corresponding EST and BGL markers loci have been found to be linked in hexaploid oat (Hoffman et al. 1990). Two similar markers also map to the distal end of the long arm of chromosome 3 in barley. This linkage group appears to be congruent between the oat and barley genomes. Three isozyme loci, *Acp-1*, *Nag-1*, and *Mdh-1*, mapped to a chromosome which has the *PcA* crown rust resistance gene cluster (data not shown). *Acp-1* was found to be 28 cM from *PcA*. Locations of the other morphological and isozyme loci studied here as molecular mapping of the B/D recombinant inbred population continues. This study of morphological and isozyme

genes of diploid oats supplements the molecular mapping effort of the oat genome. Polymorphic DNA markers have also been identified among these diploid accessions and these will be added to the continuing oat mapping effort.

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Table 1. Diploid oat accessions studied for the inheritance and linkage relationships of morphological and isozyme loci.

Accession No.	Avena Species	Original Source	Abbreviation
CI _{AV} 2630	strigosa	Dept. of Agri., Australia	A
CI _{AV} 3815	strigosa	Inst. Pl. Breed., Poland	B
CI _{AV} 1994	wiestii	Ministry of Agri., Egypt	D

Table 2. Monogenic analysis for morphological traits and isozyme bands in diploid oats.

Trait/Band	Proposed Symbol	Chi-Square Values		
		B/D	A/B	A/D
Leaf sheath pubescence	<i>Lsp</i>	1.50	3.06	-
Growth habit	<i>Gh</i>	0.17	1.20	-
Lemma pubescence	<i>Lp</i>	0.22	-	2.72
Lemma color	<i>Lc</i>	1.39	-	-
Rachilla pubescence	<i>Rp</i>	0.06	2.00	-
Seed-base pubescence	<i>Sbp</i>	2.00	-	2.72
Acid phosphatase-1	<i>Acp-1</i>	1.06	1.40	-
Esterase-1	<i>Est-1</i>	0.56	3.58	2.23
Esterase-2	<i>Est-2</i>	-	0.50	0.22
Esterase-5	<i>Est-5</i>	0.67	3.40	1.33
basic β -Galactosidase-1	<i>Bgl-1</i>	-	2.06	0.75
n-acetyl Glucosaminidase-1	<i>Nag-1</i>	2.23	2.06	-
Malate dehydrogenase-1	<i>Mdh-1</i>	1.06	-	1.06

Est-1 — *Est-2* — *Bgl-1* — *Lsp* — *Gh*
5 23 17 26

Figure 1. Linkage group identified by analysis with the LINKAGE-1 program. Distances are estimated in centimorgans.

The barley chromosome 5 linkage maps. J. Jensen, Environmental Science and Technology Department, Gene Technology and Population Biology. P.O. Box 49, DK-4000, Roskilde, Denmark.

INTRODUCTION. The introduction of molecular markers has increased the possibility for chromosomes mapping. This has also stimulated the development of different computer programs to facilitate mapping. Here different linkage maps of barley chromosome 5 will be compared. Some of the present knowledge of the barley chromosome 5 linkage maps and different methods used to map chromosomes are compared.

RESULT AND DISCUSSION. Maps of barley chromosome 5 from different crosses were considered. The maps were estimated with Gmendel (Holloway and Knapp 1994), Mapmaker (Lander et al. 1987) and with the method of 'Jensen' (Jensen and Jørgensen 1975, Jensen 1987).

The map estimates reported were compared to map estimates obtained by the method of 'Jensen' on the same data. This method estimated shorter map than Mapmaker and Gmendel. Furthermore, the order of some of the loci was also different.

The methods of 'Jensen' were basically developed to estimate map from samples of different two-point tests(crosses) (Jensen and Jørgensen 1975). Therefore, this method can join linkage maps based on original estimated recombination frequencies. The commercial mapping program JoinMap has also the possibility of joining linkage maps, a possibility that was 'inherited' from the methods of 'Jensen'. JoinMap has many more facilities than the method of 'Jensen', but it lacks some essential qualities (in the author's opinion).

The current map of barley chromosome 5 as given in Barley Genetics Newsletter (cf. Jensen 1995) consists of mainly classical morphological markers from two-point-tests. Examples of joining this map with maps based on molecular markers is presented on the poster.

Molecular markers may cause problems in identifying loci, because a given probe may map to different positions in different crosses. This makes it difficult to compare and join maps from different crosses. The likelihoods that two scored markers belong to the same locus increase if the marker allele has the same molecule size and/or if the parents of the crosses studied have common ancestors. Therefore, description of the marker's molecular size and of the pedigree of the parents used in the crosses is important information that should be listed in publications.

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Synchronization of cell division in oat root tip cells and isolation of metaphase chromosomes for generation of an oat flow karyotype. H.F. KAEPPLER¹, K. ARUMUGANATHAN², S.M. KAEPPLER¹, and J.H. LEE². ¹ Department of Agronomy, University of Wisconsin, 1575 Linden Drive, Madison, WI 53706. ² Center for Biotechnology and Department of Agronomy, University of Nebraska, Lincoln, NE 68583.

Introduction. The analysis and sorting of plant chromosomes is of great interest for use in genetic mapping, gene cloning, gene transfer, and deciphering the evolution of plant genomes. (Dolezel et al., 1994; Vega et al., 1994; Sawahel and Kiichi, 1995; and Heslop-Harrison, 1995). Sorted, specific chromosomes of oat would be useful in constructing chromosome-specific libraries and to develop chromosome painting probes. Chromosome specific libraries can be utilized in genetic mapping and gene cloning applications. Chromosome painting probes can be used to physically follow segments introgressed from wild species, to monitor translocation and substitution stocks, and assist in genetic mapping efforts. To date, there are only a limited number of reports of the successful isolation of specific plant chromosomes (Wang et al., 1992; Dolezel et al., 1993 Schondelmaier et al., 1993; Arumuganathan et al., 1994; Vega et al., 1994; Chen and Armstrong, 1995; Pich et al., 1995). Chromosomes were isolated in those studies by either of two methods: microdissection or flow cytometric sorting based on DNA content.

When chromosomes of a plant species are dissimilar in size and/or base pair content, flow cytometric separation and isolation of chromosomes is advantageous over microdissection because thousands of chromosomes can be isolated in a relatively short period of time, and PCR amplification steps required with microdissected chromosomes are not needed. Prerequisite to flow sorting is the synchronization of cell division. This is necessary to obtain samples with high enough mitotic indices to ensure a high yield of sortable chromosomes. Previously, high mitotic indices were obtained by synchronizing plant cell suspension cultures (Dolezel et al., 1994). Obtaining finely dispersed, fast growing suspension cultures is difficult in cereal monocots such as oat, and is usually genotype specific. Another drawback to using suspension cultures is that they are prone to karyological instability. Development of methods to synchronize cells and obtain high mitotic indices in the root tips of germinating seedlings would eliminate each of those disadvantages. To date, such methods have not been reported in oat. Recently, Kaeppeler et al., (1996) reported the successful synchronization of cell division in seedling root tips of maize, and the subsequent analysis and sorting of metaphase chromosomes from the synchronized cells. The objectives of this study were to test methods similar to those used in maize to synchronize cell division in seedling root tips of oat, and to develop a preliminary flow karyotype for oat using metaphase chromosomes isolated from the synchronized cells.

Materials and Methods. Unblemished seeds of the oat variety "Belle" were placed embryo side up on moistened paper towels in plastic containers. The seeds were overlayed with moist paper towels, and the plastic containers covered and placed in a dark incubator, at 26 °C for 24 hr. The containers were then incubated in the dark at 30 °C for 13 hr. Oat seedlings with root lengths of 0.5 cm were placed in 9 cm petri plates containing 1-2 Whatman # 1 filter papers and 10 ml 1.25 mM hydroxyurea (HU) solution. Seedlings were placed so that the root tips were immersed in the HU solution. The petri plates were sealed and placed in the dark in a 26 °C incubator. Cell division synchronization was monitored

hourly by flow analysis of isolated nuclei for up to 19 hours to determine when maximum cell cycle stages (G1, S, G2/M) were reached and the degree of synchronization at those peaks. Maximum synchronization at the G2/M stage of the cell cycle occurred after 14.5 hr. incubation in HU solution. At 14.5 hr. HU treatment, seedlings with root lengths of 1.0 -2.0 cm were washed 3 times with ddH₂O. Rinsed seedlings were placed in 9 cm petri plates containing 1-2 Whatman # 1 filter papers and 0.2mM trifluralin solution, 0.05% colchicine solution, or 0.4 mM amiprophos-methyl (APM) solution . Seedlings were placed with root tips immersed in the solutions. The petri plates were placed in a dark, 26 °C incubator. Trifluralin, colchicine and APM induced chromosome condensation was monitored hourly by preparing root tip squashes using modified carbo-fuschin stain, and observing chromosome spreads on a light microscope. When chromosome condensation was at an optimum, chromosomes were isolated from root tips for flow analysis and sorting.

Treated root tips approximately 0.5 mm in length (minus the root cap) were dissected from the seedling roots. Root tips were chopped in a 60x20 mm petri plate on ice with a scalpel in a 5-10 mL drop of fresh, ice-cold chromosome isolation hypotonic buffer (10 mM MgSO₄.7H₂O, 50 mM Kcl, 5 mM Hepes, 3 mM Dithiothreitol, and 0.25% Triton -X). Propidium iodide (PI) was added (final concentration = 100µg/ml for nuclei, 25 µg/ml for chromosomes) to the isolation buffer immediately before use. Following chopping, 0.5ml isolation buffer + PI was used to wash nuclei to the edge of the petri plate. The solution was collected with a pipettor and passed through a 30mm nylon mesh. Nuclei were collected in a sample tube beneath the mesh. Chromosomes were isolated by passing the solution through a 15mm mesh. Samples were stored on ice prior to flow cytometric analysis. Samples were analyzed for PI florescence intensity, which is correlated with DNA content.

A FACScan flow cytometer (Becton-Dickensen Immunocytometry Systems) and FACS Vantage particle sorter were used for flow analysis and sorting of isolated oat nuclei and chromosomes.

Results and Discussion. Treatment of oat root tips with hydroxyurea solution resulted in high levels of synchronization of cell division as determined by flow cytometric analysis (Fig.1). Division in untreated oat root tips was not synchronized (Fig.2).

Fig.1 Synchronization of cell division in oat root tips treated with 1.25 mM hydroxyurea (HU)

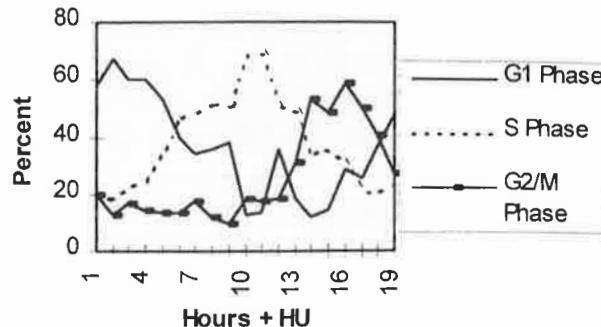
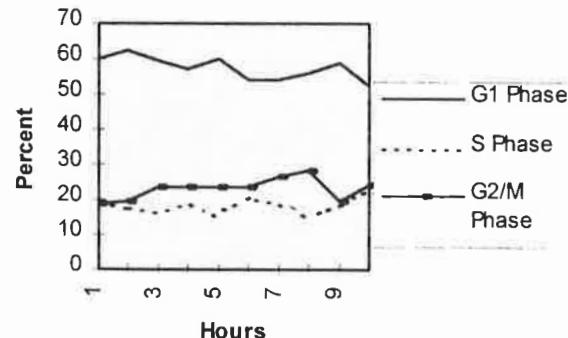
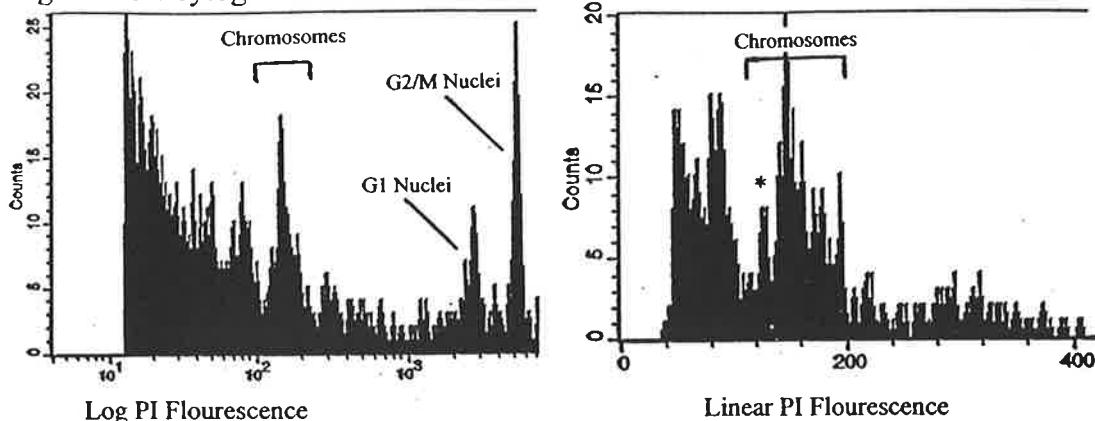


Fig. 2. Percent of cells in G1, S and G2/M phases of the cell cycle in nonsynchronized oat root tip cells.



Highest mean mitotic indices (70-80%) and optimum chromosome condensation was achieved by treatment of synchronized root tips in the G2/M phase of the cell cycle with trifluralin, colchicine or APM solutions for 4.0-4.5 hr. Placement of treated root tips in ice water for 24 hr. reduced clumping of chromosomes. Chromosomes isolated from synchronized, trifluralin-, colchicine- or APM-treated oat root tip meristematic cells were analyzed by flow cytometry and a flow karyotype generated (Fig.3).

Fig.3. Flow cytograms of oat chromosomes and nuclei.



Clear chromosome and nuclei peaks were observed following flow cytometric analyses. Within the chromosome peak cluster, a distinct peak (*) was observed in all experiments and is thought to contain oat chromosome 18, the smallest chromosome in the genome of *A. sativa*. Further analyses are required to confirm the exact content of the peak.

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GISH and RFLP facilitated identification of a barley chromosome carrying powdery mildew resistance from *Hordeum bulbosum*.

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INTRODUCTION. In addition to the utility of *Hordeum bulbosum* in haploid production (Kasha and Kao, 1970) and its associated advantages in plant breeding and genetic analysis, this wild progenitor also carries important abiotic attributes such as winter hardiness and drought tolerance and resistance to some important pests and diseases such as powdery mildew, stripe rust, leaf rust, scab, barley yellow mosaic virus and Russian wheat aphid. These attributes make *H. bulbosum* attractive for making introgressions to incorporate these desirable traits into cultivated barley. It is also known that there is genome homoeology between *H. bulbosum* and *H. vulgare* facilitating allosyndesis (Kasha and Sadasivaiah, 1971; Xu and Snape, 1988). Although stable transfers of *H. bulbosum* chromatin into the background of cultivated barley are rare, there are some reports of such transfers (Pickering, 1991; Xu and Kasha, 1992; Pickering et al. 1994). This poster reports investigations of chromosomal introgressions that are associated with powdery mildew resistance. Procedures involved Fluorescent genomic in-situ hybridization (GISH), RFLP analysis and powdery mildew resistance screening.

MATERIALS AND METHODS. The plant materials analyzed were derived as self-pollinated progeny from a BC₁ plant (BC_{1.2}) exhibiting mildew resistance. The plant BC_{1.2} came from a F₁ hybrid between autotetraploid *Hordeum bulbosum* (2n=4x=28) accession GBC- 141 and diploid *Hordeum vulgare* cv 'Su Pie', backcrossed with 'Su Pie' (Xu and Kasha, 1992). 'Su Pie' is a Chinese winter barley cultivar highly susceptible to powdery mildew. The segregation of BC₂ progeny confirmed a ratio of 1:1. indicating that the resistance derived from *H. bulbosum* is conditioned by a single dominant gene and can be inherited in a stable fashion (Xu and Kasha, 1992).

Screening for powdery mildew on BC_{1.2} selfed progeny was done using a mixture of several naturally prevalent isolates in Ontario, Canada. Plants were grown in a growth chamber in the presence of infected spreader plants and the parental lines.

Root-tips collected from selfed progenies of plant BC_{1.2} were placed in ice water for 12-16 hours, fixed in 3:1 ethanol:glacial acetic acid and macerated with cellulase and pectinase according to procedures of Leitch et al. (1994). Slides were stored at -70°C until frozen and coverslips removed and thereafter stored at -20°C until use in in-situ hybridizations. In-situ hybridizations were carried out with biotin labelled or fluorescein dCTP labelled genomic DNA of *H. bulbosum* used as the probe along with an excessive amount of *H. vulgare* DNA used to block the homoeologous DNA common to the two species. The procedures used were essentially similar to William and Mujeeb-Kazi (1995) or Reader et al. (1994).

Southern hybridizations and the RFLP analysis of the parental controls and the backcross derived progeny plants were according to the standard procedures.

RESULTS AND DISCUSSION. Using GISH analysis on the BC_{1.2} selfed progeny it was observed that up to four chromosomes in the 14 chromosome barley plants showed a hybridization signal in the terminal regions, indicating presence of *H. bulbosum* chromatin. This showed that there were two independent *H. bulbosum* introgressions involving two different barley chromosomes. Some plants had a hybridization signal indicating the presence of *H. bulbosum* chromatin on the smallest non-satellited *H. vulgare* chromosome which is probably chromosome 5 according to the barley karyotype (Noda and Kasha, 1978). Some plants carried *H. bulbosum* chromatin on a long barley chromosome. The plants carrying only the introgression on chromosome 5 were susceptible to powdery mildew whereas the lines that had the introgression on the long barley chromosome were resistant.

RFLP analysis using the probe MWG 949 (specific to chromosome 2L) showed complete association between *H. bulbosum* presence on the long chromosome and resistance to powdery mildew. Therefore, barley chromosome 2 carried the introgression associated with the powdery mildew resistance derived from *H. bulbosum*. Furthermore, the RFLP probe detected the presence of a fragment from *H. bulbosum* and a corresponding absence of a fragment from *H. vulgare*, thus enabling the differentiation of the plants homozygous and heterozygous for the mildew resistance.

Efforts will be made to transfer the *H. bulbosum* derived powdery mildew resistance into elite breeding lines through a procedure facilitated by molecular markers. Powdery mildew is a major disease of barley in parts of Canada and Europe.

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Evidence of a recombination between *v* locus and PCR-based cMWG699 marker on chromosome 2 of barley

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Introduction. Number of kernel row is an interesting morphological character for investigating the phylogeny of cultivated barley (Takahashi 1955). The character is predominantly controlled by the *V-v* alleles on the chromosome 2. The *vv* and *VV* genotypes have six- and two-rowed ears, respectively, and the heterozygote *Vv* genotype has a non-six-rowed ear showing partial fertility of lateral florets as modified by *l* series on chromosome 4 (Woodward 1949). The *v* locus is closely linked to cMWG699 locus, showing no recombination among a population of 71 *F*₁ anther-derived doubled haploid plants (Graner et al. 1991) or 144 *BC*₁*F*₁ plants (Komatsuda et al. 1995). We report here that recombination between cMWG699 and *v* was obtained by simply increasing the size of segregating population, even though recombination frequency was as low as 0.1%. The STS-approach (Tragoonrung et al. 1992) was applied for analyzing hundreds of segregating samples for linkage analysis.

Materials and Methods. Near-isogenic lines for *V-v* alleles were produced by a recurrent backcrossing method from the cross between Azumamugi (*vv*) and Kanto Nakate Gold (*VV*) using Azumamugi as a recurrent parent. Linkage analysis was performed using 282 *BC*₆*F*₂ and 377 *BC*₇*F*₁ plants segregating for the *V-v* alleles. All the *BC*₆*F*₂ and *BC*₇*F*₁ plants had *l*/*l* alleles so that lateral florets of *Vv* heterozygotes were partially fertile, whereas those of *VV* homozygotes completely sterile. Thus the phenotypic difference allowed to discriminate *Vv* genotype from *VV*, being co-dominant marker. End-sequencing of the inserted DNA of clones, primer design, PCR amplification, and detection of polymorphisms were performed according to the STS methods described by Tragoonrung et al. (1992).

Results and Discussion. Based on the terminal sequences of inserts of MWG clones located around the *v* locus, twenty-base primers were synthesized as follow ; MWG801 (5'-ATTCTTTGTGCTCCTTCAC-3' and 5'-TGCCAGTGTGCG CCATAAGCG-3'), cMWG699 (5'- AACTGTTTCTCATTG TGA -3' and 5'- AAGTGTCCCTGCCTTCCAAA -3'), MWG865 (5'-GGTTGTTG CCCTGTTGCTGT-3' and 5'-GACAGTAACATTCGGAACATC-3') and MWG503 (5'-GTCGTCAGAGCCCACGCCAC-3' and 5'-TCCAAGCGGCAACCATCCC G-3').

Each primer pair amplified a single, discrete DNA band with the size of 0.7-0.9 kb, indicating its high specificity to the genomic sequences. The DNA

amplification products of the two cultivars were the same in size, but after digestion (*AccI* for MWG801 and MWG865, *TaqI* for cMWG699, and *HhaI* for MWG503), DNA fragments showed polymorphisms which were useful as co-dominant genetic markers.

The linkage analysis arranged MWG801, cMWG699, *v*, MWG865 and MWG503 in this order from proximal to distal, with map distances between each two markers 3.5, 0.1, 1.3 and 10.0 centi-morgans, respectively. The order and map distances were in good agreement with that previously studies. Moreover, the recombination between cMWG699 and *v* locus was detected at the first time in this study.

Cytological observation on the meiosis revealed that no chromosomal inversion was evident for the isogenic lines. So, it is concluded that the tight genetic linkage between cMWG699 and *v* locus is because of either the closed physical distance or unknown mechanisms which strongly suppressed the recombination.

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Integrating visual markers with the Steptoe x Morex RFLP map. Dave Kudrna, Andris Kleinhofs, Andrzej Kilian and Jon Soule, Depts. Crop and Soil Sciences & Genetics and Cell Biology, Washington State University, Pullman, WA 99164-6420, USA.

The North American Barley Genome Mapping Project (NABGMP) has produced 3 RFLP maps with ca. 1000 markers. Additional 1000+ visual and physiological traits have been identified in barley and characterized to various degrees. About 200 of these have been mapped. We have initiated an effort to integrate the visual markers with the RFLP maps. The initial goal is to place 44 well characterized visual markers on the RFLP map with respect to RFLP probes. This will approximate the location of these markers on the RFLP map and relate all other markers mapped with respect to these standard markers. The visual markers used are: Chr.1 - br, wx, f8, gl5, msg10, f4, Xa, pmr; Chr.2 - f, msg2, yst4, fol-a, li, gp, f-sh; Chr.3 - yst2, msg5, wst, f2; Chr.4 - f9, K, f10, gl, br2, gl4, msg24; Chr.5 - msg1, nec1; Chr.6 - nar1, o, msg6, sex1, uc2, f11, mt,,f; Chr.7 - nld, f6, cud, mt2, cer-zj, cer-zp, va, nar2, msg19; Unknown - nar3, nar4, nar5, nar6, nar8, Az86, Az94, R11301; The Bulked Segregant Analysis method was used to establish approximate map locations with respect to RFLP markers. The homozygous visual marker plants were crossed to one of the RFLP map parents (Steptoe or Morex) and F2 seed produced. Approximately 15 mutant and 15 wild-type plants were bulked separately for DNA extraction. DNA was digested with the restriction enzymes EcoRI, HindIII and XbaI, blotted and hybridized with probes spaced uniformly throughout the barley chromosomes. Approximate linkages were deduced by estimating the ratio of the intensity of the recombinant and parental bands in the bulked mutant sample. Analyses of the data established the locations of the visual markers with respect to flanking RFLP markers and placed these markers in specific chromosome segments (bins) of the established RFLP map. Further refinement of the map position will be obtained using RFLP markers within each chromosome segment. The merged map will be presented.

Molecular mapping of loci controlling hydrolytic enzyme activity

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Introduction

The development of molecular markers, the mapping of known-function genes and QTLs will increase the flexibility and sophistication of our breeding programs. At the Waite Institute, three doubled haploid (DH) populations have been established and detailed molecular linkage maps constructed. The Adelaide populations were selected for the segregation of traits important for the Australian breeding programs but many of these traits, notable aspects of quality, coincide with international mapping objectives. One population consisted of 150 DH lines generated using the *Hordeum bulbosum* method from a cross of Clipper (Australian) x Sahara (African) while the other two populations were generated by anther culture and consisted of 114 DH lines from Haruna Nijo (Japanese) x Galleon (Australian, feed) and 120 DH lines from Chebec (Australian) x Harrington (Canadian). The mapping data from these populations has been combined with data from elsewhere to construct consensus linkage maps of each barley chromosome. The populations have also been used to map loci encoding 1,3- β -glucanase, 1,4- β -xylanase, limit dextrinase, genes induced by gibberellic acid and several QTLs controlling the activity of α -amylase, β -amylase, β -glucanase and limit dextrinase..

Seven genes encoding 1,3- β -glucanase are clustered on the long arm of chromosome 3.

1,3- β -Glucanase are widely distributed in higher plants, where they function in developmental processes such as microsporogenesis, pollen tube growth, senescence and in the removal of dormancy or wound callose. However, most attention has been focused on their role in plant-pathogen interactions. Specific probes or polymerase chain reaction primers were generated for the seven barley 1,3- β -glucanase genes for which cDNA or genomic clones are currently available. The seven genes all mapped to the long arm of chromosome 3 with the genes encoding isoenzymes GI, GII, GIII, GIV, GV and GVII clustered over a region of less than 20 cM. The region is franked by the RFLP marker MWG2099 on the proximal side and the BYMV resistance, ym4, at the distal end. The gene encoding isoenzyme GVI lies approximately 50 cM outside this cluster towards the centromere. There is no evidence to suggest that 1,3- β -glucanase genes represent plant resistance genes of the type envisaged by the gene-for-gene model of plant-pathogen interaction. It is more likely that the enzymes participate in a general, non-specific response to pathogen attack.

The four members of the 1,4- β -xylanases gene family are on chromosome 7

Cell walls act as a barrier to enzyme movement within the endosperm during seed germination and, if incompletely degraded, can cause filtration problems in brewing and instability problems in the finished beer. The major components of cell walls of barley endosperm are 70% 1,3 1,4- β -glucan and 20% arabinoxylan. The β -glucans have been studied extensively and their structure and functional properties are well understood (MacGregor and Fincher 1993). On the other hand, the extent of arabinoxylan degradation during germination has not been clearly demonstrated. Hydrolytic depolymerization of arabinoxylan is mediated by the concerted action of 1,4- β -D-xylan endohydrolase, 1,4- β -D-xylan exohydrolase, β -xylosidase and arabinofuranosidases (Preece 1958). Three 1,4- β -D-xylan endohydrolases have been purified and two cDNAs have been cloned from germinated barley grain (Slade et al., 1989; Banik and Fincher 1995). Using the full length cDNA of 1,4- β -xylan endohydrolase as probe, four members of the gene family have been mapped on chromosome 7 in the three doubled haploid populations. This gene family is located on the distal region of chromosome 7 franked by the RFLP markers AWBMA3 and CDO419. No recombination has been found between the members of the gene family in the three DH populations, but all members are highly polymorphic.

Single copy of limit dextrinase gene is on chromosome 1

Limit dextrinase hydrolyses the α -1,6-glycosidic bonds in starch and, in combination with the other starch degrading enzymes, degrades starch to fermentable sugars. High activity of limit dextrinase may lead to an improvement in fermentability. Recently, a partial cDNA of this gene has been cloned by the group of G. Fincher. Using this partial cDNA as probe, the gene encoding limit dextrinase has been mapped to the long arm of barley chromosome 1, very close to the centromere. The gene and its immediate surrounding sequences appear to be highly conserved since only low polymorphism was observed with the six parents although 17 different restriction endonucleases were tested. Only one copy of this gene exists in barley genome although it is considerably more complex in wheat.

Genes induced by gibberellic acid are located on chromosomes 2 and 7

The hormone gibberellic acid (GA3) is involved in many physiological processes in plants. It controls internode and leaf growth, alters leaf shape, induce abnormal sex expression and alters seed dormancy. It also leads to increased activity of α -amylase and β -glucanase. To help understand the relationship between the GA3 induced genes and the malting qualities in barley, two genes, Es1A and As2A induced by GA3, have been cloned from barley (Speulman 1995). Two sets of PCR primers have been designed to amplify those two genes with wheat-barley addition lines. Es1A was mapped to barley chromosome 2 and Es2A to chromosome 7. Those genes appear to be highly conserved in barley since no polymorphism was found between three sets of parents used in generating the mapping populations, although 17 different restriction endonucleases were tested against the PCR products. It is interesting to note that a single locus on chromosome 2 controls the activity of α -amylase, β -glucanase, limit dextrinase and plant height (see below); all these traits are regulated by GA3. Es1A may be the candidate for this locus.

Mapping the QTLs controlling some malting qualities

In barley many economically important characters are quantitative, requiring replicated field trials for accurate measurement. As this is time consuming and expensive, breeders are looking for more rapid alternatives to the conventional selection schemes. The use of molecular markers to identify and select individual loci controlling quantitative traits (QTL) offers one such possibility. Recently some agronomically important characters have been mapped (Backes et al., 1995; Thomas et al., 1995; Han et al., 1995). We have also been interested in mapping the QTLs controlling aspects of malting qualities. The loci controlling the activity of hydrolytic enzymes produced during grain germination and, consequently during malting, are major targets. In addition to loci controlling seed dormancy, seed weight and shape, loci controlling the activity of the enzymes α -amylase, β -amylase, β -glucanase and limit dextrinase were mapped. The major loci controlling these characters are listed on Table 1. Particularly interesting is the locus located on the chromosome 2 linked with RFLP marker ABC468. This appears to control the activity of the enzymes α -amylase, β -glucanase and limit dextrinase and may represent a gibberellic acid response locus that turn on a series of genes associated with germination (see above). This region is likely to be a particularly promising target for breeding and selection.

Acknowledgements

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Table 1 Loci controlling the activity of hydrolytic enzymes in barley

Trait	Chromosome	Closest RFLP marker
Hydrolytic enzyme activity		
α-amylase	2 7 5 6	ABC468 WG364 AWBMA27 BCD269
β-amylase	2 3 4 4 7 1	BCD339(A) BCD512 CDO1406 BMY ABC254 WG789
β-glucanase	2 7 1	ABC468 WG364 ABC465
Limit dextrinase	2 7 6 1	ABC468 WG364 PSR154 MWG503(B)
Coding Loci		
1,3 -β-glucanase		
GI	3	cMG693
GII	3	BCD131
GIII	3	BCD131
GIV	3	MWG41
GV	3	MWG838
GVI	3	CDO178
GVII	3	CDO105
Xylanase	7	AWBMA3
Limit dextrinase	1	ABG380

The genomes of *A.lusitanica*, *A.hispanica* and *A.matritensis* confirmed using GISH. G.S. Markhand and J.M. Leggett, Cytogenetics Group, Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, Dyfed SY23 3EB, UK.

INTRODUCTION. The genus *Avena* contains fifteen diploid ($2n=2x=14$), seven tetraploid ($2n=4x=28$) and eight hexaploid ($2n=6x=42$) species/taxa. Historically, genomic designations have been allocated to the various species/taxa based on the chromosome homology or lack of it in intra and interspecific hybrids, and on the detailed analyses of their respective karyotypes. Depending on the ploidy level, one or more of four genomes AA BB CC or DD have been assigned to the taxa of the genus. The AA genome is known to occur at all ploidy levels as is the CC genome, but the DD genome is known only in the hexaploid taxa, and the BB genome was assigned to the tetraploid *A.barbata* complex only.

At the diploid level, only two CC genome species (three taxa) are known which have distinct karyotypes, quite different to those of the AA genome species. They are also different to one another and have been designated CvCv (*A.ventricosa*) and CpCp (*A.eriantha* and *A.clauda*).

The AA genome diploids are divided into the AsAs genome of the *A.strigosa* complex (which includes the taxa *A.hirtula*, *A.wiestii*, *A.nuda*, *A.brevis* and *A.atlantica*) and the modified AA genome species *A.canariensis* (AcAc), *A.damascena* (AdAd), *A.longiglumis* (AlAl) and *A.prostrata* (ApAp). The remaining AA genome species/taxa *A.lusitanica* *A.hispanica* and *A.matritensis* have not had their genomic constitutions or karyotypes verified although Baum (1977) presumed these to be AA genome taxa.

In order to establish the genomic identity of these taxa, we employed genomic *in situ* hybridisation.

MATERIALS AND METHODS. Seeds of *A.lusitanica*, *A.hispanica* and *A.hirsuta* were obtained from Plant Gene Resources of Canada. The *A.hirsuta* accession is presumed to be *A.matritensis* (see Baum 1977).

The protocol followed for seedling germination, root tip fixation, enzyme treatment of root tips, chromosome squash slide preparations, probe production and *in situ* hybridisation and detection were as described by Leggett and Markhand (1995a) except that the total DNA extracted, sheared, labelled and hybridised was from the AsAs genome species *A.strigosa* cv. Saia, and the rDNA probe was the cloned sequence 18S.26S (pTa71) (Gerlach and Bedbrook, 1979).

RESULTS. Probing the diploid species *A.lusitanica*, *A.hispanica* and *A.matritensis* with labelled total AsAs genomic DNA extracted from *A.strigosa*, resulted in all of the chromosomes of the three species being labelled over their entire length, indicating that they are homologous to *A.strigosa* chromosomes at the DNA level. The *A.lusitanica* accession was found to contain both diploid and tetraploid plants, which must be

either a contaminant, or a tetraploid form of this oat. However, a tetraploid form has not been recorded for this species. In any event, the tetraploid forms also had twenty eight labelled chromosomes. The rDNA probe was only hybridised to *A.matritensis*, and four sites of hybridization were observed on SAT chromosomes, as in the other AA genome diploid species.

DISCUSSION. The GISH results clearly indicate that *A.lusitanica*, *A.hispanica* and *A.matritensis* contain a large number of DNA sequences which are homologous to those of the AsAs genome diploid *A.strigosa*. However, it is clear from previous GISH results that genomic DNA sequence homology does not necessarily reflect homology at the whole chromosome level. For example, when the tetraploid species *A.agadiriana* was probed with labelled total genomic DNA from *A.strigosa*, all twenty eight chromosomes were uniformly labelled (Leggett and Markhand 1995b), yet chromosome pairing in the triploid hybrid between *A.strigosa* and *A.agadiriana*, demonstrated that chromosome pairing was incomplete (Leggett, 1989).

Similarly, and perhaps more importantly, when the bivalent forming hexaploid cultivated oat *A.sativa* (genomes AACCD) is probed with labelled total genomic DNA from *A.strigosa*, all the AA and DD genome chromosomes are labelled uniformly (Leggett, 1994). In haploid plants of *A.sativa* (in which none of the chromosomes have true homologous partners), intergenomic pairing is extremely poor and does not reflect the apparent homology of the AA and DD genomes at the DNA level as demonstrated using GISH.

This situation is in complete contrast to the situation in some herbage grasses, where for example, chromosome pairing is complete and regular in hybrids between diploid *Lolium* and *Festuca* (Peto, 1933) yet their respective genomes can be readily distinguished by using GISH (Thomas et al 1994).

In *Avena*, it is of course possible that the chromosome pairing control mechanism is capable of discriminating between the chromosomes of *A.strigosa* and *A.agadiriana* and between the AA and DD genomes of *A.sativa* thus preventing them from pairing, despite their apparent homology at the DNA level.

From the evidence we present above, it is clear that *A.lusitanica*, *A.hispanica* and *A.matritensis* contain AA genome DNA. However, in the light of the apparent discrepancies between chromosome and genome homologies as mentioned above, the true biological relationship between *A.lusitanica*, *A.hispanica*, *A.matritensis* and the other AA genome diploid oat species can only be confirmed by examining the chromosome pairing of their respective inter- and intra-specific hybrids.

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Comparison of grain and malt quality QTL in barley mapping populations.

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Introduction. The North American Barley Genome Mapping Project has mapped markers and quantitative trait loci (QTL) in a six-row barley cross, Steptoe/Morex, and a two-row barley cross, Harrington/TR306. Morex and Harrington are malting barley cultivars, Steptoe is a feed barley cultivar, and TR306 is a breeding line. Detection of QTL for grain and malt quality traits was one objective in these mapping experiments. Results on QTL for grain and malt quality traits in Steptoe/Morex have been reported by Hayes et al. (1993) and by Han et al. (1995). Results on QTL for malting quality traits in Harrington/TR306 have been reported by Mather et al. (submitted). The two crosses represent distinct germplasm groups and one of them (Steptoe/Morex) is a much wider cross than the other (Harrington/TR306). We did not expect to detect many of the same QTL in both crosses. Nevertheless, we wanted to carefully compare the QTL maps, to investigate whether any genome regions were important for malting quality in both crosses. As the same traits are mapped in additional crosses, this comparative work can be extended, to determine whether there are any genome regions that affect malting quality across different germplasm groups.

Methods. The initial report (Hayes et al. 1993) on QTL locations in Steptoe/Morex employed a skeletal map of 129 markers, chosen from among 295 markers that had been mapped at the time of analysis. Since then, many additional markers have been mapped in Steptoe/Morex. From North American Barley Genome Mapping Project marker data available in January 1995 (A. Kleinhofs, personal communication), we derived a 223-marker 'base map' of Steptoe/Morex (<http://gnome.agrenv.mcgill.ca/info/basemaps.htm>), with markers chosen to provide 2- to 5-cM intervals between markers (where possible) and to provide a stable marker order in Monte Carlo evaluations with a simulated annealing mapping algorithm (Holloway and Knapp, 1994). For Harrington/TR306, we followed the same procedure, and derived a 127-marker base map. This is the same map used in our reports on QTL detection in Harrington/TR306 (Tinker et al., 1996; Mather et al., submitted). Phenotypic data for nine grain and malt quality traits (kernel plumpness, kernel weight, grain protein, fine-grind extract, fine-coarse difference, soluble protein, extract β -glucan, diastatic power and α -amylase activity) were analysed by simple and composite interval mapping of QTL main effects and QTL \times environment interactions, using MQTL software (Tinker and Mather, 1995) as described by Tinker et al. (1996). Phenotypic data for Steptoe/Morex came from samples of grain grown in nine environments (four sites in 1991, five sites in 1992). Phenotypic data for Harrington/TR306 came from samples of grain grown in six environments (four sites in 1992, two sites in 1993). The marker maps were not merged; we used the Steptoe/Morex base map for Steptoe/Morex QTL analyses and the Harrington/TR306 base map for Harrington/TR306 QTL analyses.

For comparison of QTL positions, we aligned the two marker maps based on the positions of common markers, and displayed the simple interval mapping QTL scans on a common scale. This was possible because the two base maps had many markers in common, and the order of these common markers was the same on both maps. The common scale for displaying QTL scans was obtained by aligning the positions of markers that occurred on both maps, and by adjusting the lengths of the intervals between these common markers to the means of the interval lengths from the two crosses.

Results. For all nine traits, there were obvious differences in the QTL positions between the two crosses. In most genome regions where there were significant QTL effects for one or more traits in one cross, there were no corresponding effects for the same traits in the other cross. In Harrington/TR306, most traits were strongly affected by a QTL at the "minus" end of chromosome 7, but no significant QTL were detected in that region of the Steptoe/Morex map. (There was, however, a peak for extract β -glucan that almost reached the significance threshold, suggesting the possibility of a QTL at which the Steptoe allele increases extract β -glucan.) Steptoe/Morex had QTL for several traits on the "minus" arm of chromosome 7, but these were nearer the centromere, and clearly did not correspond to the region that was important in Harrington/TR306. Elsewhere in the genome, QTL for malting quality traits were usually in different positions in the two crosses. There were, however, a few genome regions where QTL were detected for the same trait(s) in both crosses:

Chromosome 1. In the *Brz-Amy2* region of chromosome 1 on the Steptoe/Morex map, Morex contributed alleles that increased α -amylase activity and reduced extract β -glucan concentration. In the same chromosome region of the Harrington/TR306 map, there were minor effects for the same traits, with Harrington contributing alleles for higher α -amylase activity and for higher extract β -glucan concentration.

Chromosome 4. In a broad region of chromosome 4, Steptoe contributed alleles for plumper kernels and lower grain protein, while Morex contributed alleles for higher soluble protein and lower fine-coarse difference. At corresponding positions on the Harrington/TR306 map, TR306 contributed alleles for plumper kernels but higher grain protein, and there were minor QTL \times environment interactions for soluble protein and fine-coarse difference.

Chromosome 5. In the hordein region on the "plus" end of chromosome 5, both Morex and Harrington contributed alleles with a positive effect on diastatic power. This QTL was the clearest case of similar effects at a similar or identical position in the two crosses. Other α -amylase and diastatic power QTL (those on chromosomes 1, 2 and 4 in Steptoe/Morex and on chromosome 6 in Harrington/TR306) correspond with known α - and β -amylase loci (*Amy1*, *Amy2*, *Bmy1*, *Bmy2*) but the diastatic power QTL on chromosome 5 does not correspond to any known amylase locus.

Discussion. For the two crosses examined here, alignment of the marker maps was quite straightforward, and provided a useful common scale on which to display and compare QTL scans. This was partly because the two maps had many markers in common (34 on the base maps, more on the complete maps) and because the intervals between common markers were of similar length in the two crosses. For other pairs of crosses, map

alignment may be more difficult, and comparisons of QTL positions may be less reliable. Comparisons among more than two mapping populations will be more complicated.

The dissimilarity between Steptoe/Morex and Harrington/TR306 QTL positions for grain and malt quality traits is not surprising, given that Morex and Harrington represent two different categories of North American malting barley, and that Morex was crossed with an unrelated feed barley of different adaptation and poor malting quality, whereas Harrington was crossed with a related breeding line of intermediate quality. The differences in QTL positions between the two crosses suggests the possibility of combining favourable genes for malting quality from two-row and six-row parents, possibly using marker-assisted selection. The similarities of QTL position observed on chromosomes 1, 4 and 5 may warrant further investigation, and mapping of grain and malt quality QTL in Harrington/Morex may help in the interpretation of the differences and similarities observed here. Extension of this comparative approach to investigate other crosses should allow for systematic evaluation of whether certain genome regions affect grain and malt quality across different germplasm groups.

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Characterization of a YAC-clone from barley chromosome 1HS

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Introduction. In order to understand the genome structure or specific genomic regions of important crops, physical maps and moreover large insert libraries are essential. Yeast artificial chromosomes (Burke et al. 1987) are effective tools for the construction of libraries with large inserts. Until now, YAC libraries were developed from the genomic DNA of different organisms including barley (Michalek et al. 1994). One of the problems during analysis of YAC clones consisting of barley DNA is the content of repetitive DNA, which makes it difficult to use the terminal insertion sequences as probes in RFLP experiments for the confirmation of the localisation of the YAC clone or for repeated screening attempts in a chromosome walking strategy. Here we show the initial analysis of a 1300 kb YAC clone which was detected with RFLP marker MWG036 which maps 0.7 cM distal from the *Mla* locus (Schüller et al. 1992).

Materials and Methods. The YAC yWHS136G1 was cloned with the vector pYAC4 into the yeast host AB1380 and consists of genomic DNA of barley cultivar 'Igri'. For restriction analysis a southern blot containing the fragments generated by partial digestion of DNA was sequentially hybridized to a left arm probe and a right arm probe, consisting of vector DNA. For the construction of subclones the band representing the YAC clone was cut out of a preparative PFGE gel prepared with low melting point agarose. The extraction of the DNA with an agarase treatment was followed by an EcoRI digestion. Fragments were ligated into the vector pBluescript SKII.

Results and Discussion. For the restriction analysis the rare-cutting enzymes *Mlu*I, *Not*I, *Sfi*I and *Sma* I were used. It was possible to establish a restriction map for *Not*I (Fig. 1). For the other enzymes too many fragments were generated resulting in a banding pattern which was too complex for exact mapping. Furthermore, it turned out that it is very time consuming to establish the appropriate separation conditions for the mapping of a clone which is 1300 kb in size. For further mapping activities it is suggested to use only restriction enzymes with an 8-base pair recognition sequence.

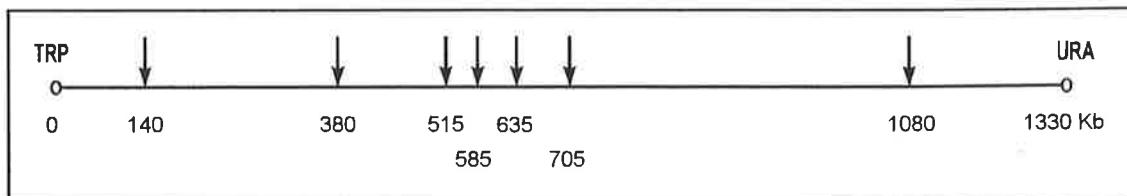


Fig. 1: *Not*I restriction map of yWHS136G1

One difficulty in the analysis of YAC clones from barley DNA is the mostly repetitive nature of the terminal insertion sequences. This was the case also with yWHS136G1. Therefore, we decided to subclone the YAC and to screen for RFLP markers which could be mapped on an appropriate mapping population e.g. the Igri/ Franka population.

Tab. 1: Hybridization patterns of plasmid subclones

hybridization pattern	number of probes	%
highly repetitive	43	49
multi copy	30	34
low copy	8	9
single copy	7	8
Σ	88	100

The subcloning set resulted in 88 clones which were analysed in subsequent steps. After an initial screening with the dot blot technique to differentiate clones with highly repetitive sequences 45 probes were analysed on small membranes with DNA of the parents of the mapping population which was treated with the restriction enzymes BamHI, EcoRI, EcoRV, HindIII and XbaI. The distribution of the hybridisation patterns is shown in Tab. 1. Surprisingly, 31 of these clones formed five groups with similar or identical hybridisation patterns and none of the clones showed polymorphism between 'Igri' and 'Franka'. Because of the large proportion of similar clones we decided to sequence four clones from four different groups. After sequence comparisons at the EMBL and Gene Bank sequence databases it turned out that two clones had 100 % and two clones 76 % identity with chloroplast sequences from barley, maize or rye. After the initial screening of filters with YAC clones consisting of barley DNA with a chloroplast specific probe it was estimated that less than 1 % of the clones carry chloroplast-DNA (Kleine et al. 1993) concluding that it is unlikely to pick a chloroplast DNA containing clone. Since the barley chloroplast genome is approximately 132 kb in size (O'Brien 1993) the detection of such sequences was not expected. The results indicate, that at least a part of the chloroplast genome was integrated into the YAC clone, forming a chimeric clone with genomic barley DNA and barley chloroplast DNA. The exact amount and distribution of plastid DNA has to be determined in further studies.

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Towards construction of a molecular map for Japanese barley cultivars.

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Introduction. Recently, extensive RFLP maps of the barley genome have been reported from three groups in North America and Germany. The objectives of our research were to (i) construct a molecular map for Japanese barley cultivars, especially two-rowed malting cultivars, (ii) develop the molecular markers tightly linked to agronomically important traits such as environmental stress tolerance (acid soil tolerance, aluminum tolerance, etc.) and pathogen resistance for barley improvement, and (iii) integrate our barley map with other ones, so that all linking information derived from different genetic studies could provide the international research network. In this report, we describe the results of screening of RFLP using characterized markers as probes against three parental cultivars for our barley genome mapping project.

Materials and Methods *Plant materials* Three barley (*Hordeum vulgare* L.) cultivars, Ishukushirazu, Ko A (Japanese two-rowed malting spring barley) and Mokusekko 3 (Chinese six-rowed winter barley) were used as parents for RFLP mapping.

RFLP probes Three sources of barley RFLP probes were used. NABGMP (North American Barley Genome Mapping Group) probes were supplied from Dr. A. Kleinhofs. MWG (Munich Mapping Program) probes were obtained from Dr. A. Graner. Cornell probes were from Dr. M.E. Sorrells.

DNA isolation and Southern analysis Barley genomic DNA was isolated

by CTAB method. Six restriction enzymes *Bam*HI, *Bgl*II, *Dra*I, *Eco*RV, *Hind*III, and *Xba*I were chosen for detection of polymorphic DNA fragments. Hybridization was done in 50 % formamide, 4X SSPE, 0.1X BLOTO, 1% SDS, and fragmented herring sperm DNA (500 µg/ml) at 42°C. Membranes were washed in 2X SSC and 1% SDS at room temperature for 5 min., followed by one more wash under the same conditions but for 15 min., then in 0.5 X SSC and 0.1 % SDS at 42°C twice for 30 min.

Results and Discussion One hundred and thirty NABGMP probes were used for the detection of polymorphism in three parental cultivars. Among these, 100 probes (77%) showed polymorphic DNA fragments between Ko A and Mokusekko 3 with at least one of the six restriction enzymes. On the other hand, only 55 probes (42 %) were polymorphic between Ishukushirazu and Ko A. Similarly, 49 MWG probes and 37 Cornell probes were tested. Thirty-six MWG probes (73 %) and 30 Cornell probes (81 %) were polymorphic between Ko A and Mokusekko 3. Twenty-five MWG probes (51%) and 18 Cornell probes (49%) were polymorphic between Ishukushirazu and Ko A. As a result, a total of 216 probes were screened against three parental cultivars. Seventy-seven % of the probes tested were polymorphic between Ko A and Mokusekko 3, but only 44 % were polymorphic between Ishukushirazu and Ko A. This suggested that these polymorphic markers could be used as joint points to other barley genome maps. The lesser extent of polymorphism detected among the two-rowed malting cultivars was almost same using barley, *T. tauschii*, and wheat genomic DNA or barley and oat cDNA as probes. These data correlated with their genetic similarity based on the pedigrees reported. Polymorphisms between Ishukushirazu and Mokusekko 3 were also detected at a high frequency (71 %). As Mokusekko 3 has diverse genetic backgrounds derived from China, it was a suitable parent to construct a molecular map for Japanese two-rowed malting barley. Furthermore, there was no significant difference in the ability to generate polymorphic DNA fragments using six different restriction enzymes.

At present, we have started to develop new molecular markers for our barley map and we would like to joint these molecular markers to other barley genome maps.

Probable location of de-epoxydase gene catalyzing xanthophyll cycle on the seventh chromosome based on trisomic barley (*Hordeum spontaneum*). J. MURAI, J. FUJIGAKI¹, A. WADANO and T. TAIRA, College of Agriculture, Osaka Prefecture University, Sakai, Osaka 593, ¹Junior College, Tokyo University of Agriculture, Sakuragaoka, Setagaya-ku, Tokyo 156, Japan.

Introduction. When the distribution of the genes concerned with light and dark reactions in photosynthesis are taken into consideration based on the analyses of chloroplast genomes in rice (Hiratsuka et al., 1989) and tobacco (Sugiura, 1992), the two-third of genes as to light reaction and all the genes except a gene for large subunit of RuBisCo as to dark reaction are anticipated to locate on nuclear genome. The location of loci for such genes, however, is not identified yet so far.

In the present study, we attempted to examine the variations of content in pigments related to the light reaction of photosynthesis and also to search which chromosome is responsible to the localization of gene(s) concerned with xanthophyll cycle using a trisomic series of barley.

Materials and Methods. The disomic (2n=14) and a trisomic series (2n=15) of barley (*Hordeum spontaneum* C.Koch var. *transcaspicum* Vav.) were used. Each of the trisomic plants is named, based on the extra chromosome and their morphology, as Bush, Slender, Pale, Robust, Pseudo-normal, Purple and Semi-erect in order of chromosome (Tsuchiya, 1960).

The mixture of chlorophyll and carotenoid was analyzed by HPLC (JASCO, Japan) with C18 column (YMC AL-313, YMC Ltd., Japan) according to the method of Masamoto et al. (1993). The measurement of violaxanthin de-epoxidase activity was performed according to the method of Hager and Holocher (1994). Leaves were homogenized by a mortar and pestle with buffer (pH 7.8, containing 50 mM potassium phosphate/0.4 M sucrose/15 mM sodium chloride/5 mM magnesium chloride/1 mM EDTA). The suspension of thylakoids was treated by twenty times of freeze-thaw cycle and then the supernatant was obtained by centrifugation at 43,000 g for 10 min at 20°C. The supernatant was used as a crude enzyme solution. On the other hand, after disomic plants were stood at dark for 24 h, leaves were collected and homogenized with 100% cold acetone. The supernatant was collected by centrifugation at 3,500 g for 8 min at 4°C and dried out by a vacuum pump. The substrate solution was obtained by adding 100% ethanol to the dried supernatant. The enzyme solution containing 100 µg of chlorophyll was mixed with 15 ml of McIlvaine buffer (pH 5.2, containing 100 mM citric acid/200 mM potassium phosphate), 100 mM of ascorbic acid and 8 nmol of violaxanthin. The content of substrate in mixture was measured at the reaction time of 5, 10, 20, 40 and 80 min. The de-epoxidase activity was calculated from the decrease of substrate content.

Results and Discussion. Carotenoid contents per 1 mol of

chlorophyll in leaves of disomic and trisomic plants are shown in Table 1. In comparison between disomic and

Table 1. The contents of carotenoid pigments per chlorophyll in disomic and trisomic barley (*H. spontaneum*).

Types of plants	Carotenoids ¹⁾ (mmol mol ⁻¹ chlorophyll a+b)						
	Neo	Viol	Anth	Lut	Zea	β-Car	V+A+Z
Disomic	31.7	46.6	14.6	126.4	18.8	99.7	80.0
Trisomics							
Bush	29.5	53.9	11.9	117.5	13.8	95.1	79.5
Slender	29.8	39.5	14.5	122.4	27.6	92.7	81.6
Pale	26.4	37.8	15.9	122.1	25.0	102.3	78.7
Robust	31.7	42.6	16.4	121.9	27.5	95.6	86.5
Pseudo-normal	31.3	41.3	17.3	128.9	27.3	100.7	85.9
Purple	28.3	47.9	12.9	117.6	27.0	95.0	87.8
Semi-erect	31.3	33.5	18.6	140.8	49.3	89.7	101.4

1) Neo, Neoxanthin; Viol, Violaxanthin; Anth, Antheraxanthin; Lut, Lutein; Zea, Zeaxanthin; β-Car, β-Carotene.

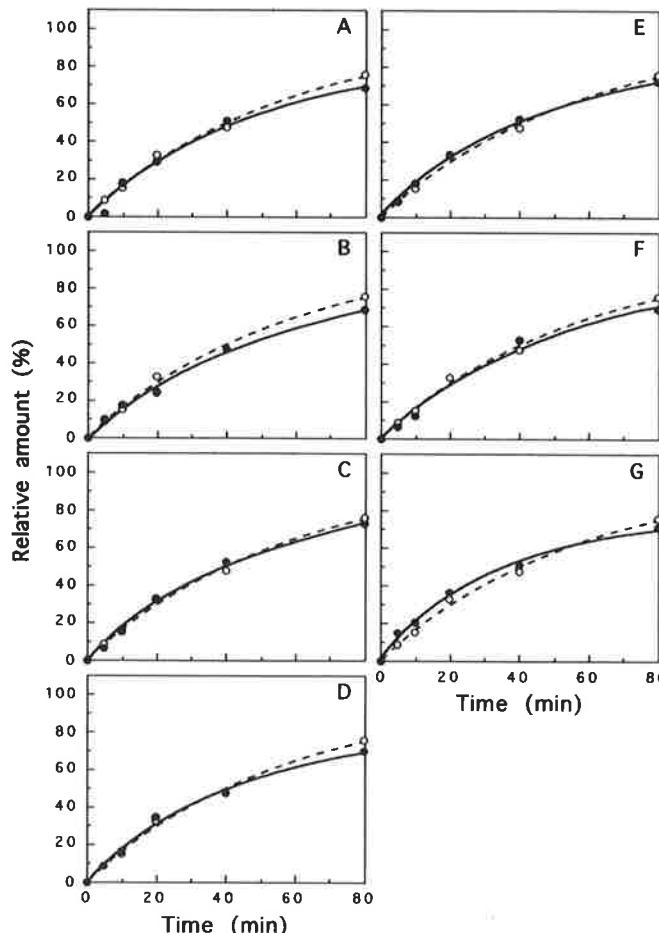


Fig. 1. Time course of violaxanthin de-epoxidase activities in disomic and trisomic barley. The relative amount represents the proportion of antheraxanthin and zeaxanthin in the total amount of violaxanthin, antheraxanthin and zeaxanthin. Solid and broken lines indicate trisomics and disomics, respectively. A, Bush; B, Slender; C, Pale; D, Robust; E, Pseudo-normal; F, Purple; G, Semi-erect.

trisomic plants, the total contents of neoxanthin, lutein and β -carotene were almost same, but there were some variations in content of violaxanthin, antheraxanthin and zeaxanthin, all of which consist of xanthophyll cycle. Of seven trisomic plants, Semi-erect which bears three chromosomes of number 7 exhibited the lowest content of violaxanthin, but on the contrary, the highest content in antheraxanthin and zeaxanthin. In the latter case, Semi-erect showed almost three times higher content than disomic plant.

The activities of violaxanthin de-epoxidase extracted from both disomic and trisomic leaves are shown in Figure 1. All trisomic plants except Semi-erect showed similar to or getting lower activities than disomic plants with progressing reaction time. In contrast, Semi-erect persisted a higher activity for 57 min than disomic plant. This comprises that the initial rate of the enzyme from Semi-erect was 1.2 to 1.6 times higher than those in disomic and other six trisomic plants.

In comparison between disomic and trisomic plants, the contents of carotenoid per chlorophyll kept up nearly constant, suggesting that there is little variation in the pigment formation despite the increase of chromosome number except Semi-erect which showed the highest total content of pigments involved in xanthophyll cycle. The results obtained here indicates that the addition of chromosome 7 to the disomic condition affects the epoxidation or de-epoxidation in xanthophyll cycle reactions, particularly the enhancement of de-epoxidation results in the higher content of zeaxanthin. Thus, we temporarily concluded that a gene coding for violaxanthin de-epoxidase would be located in the chromosome 7 of barley.

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Comparison of different high resolution banding patterns in barley chromosomes. O.V. MURAVENKO, A.R. FEDOTOV, N.L.BOLSHEVA, L.I. FEDOROVA and A.V. ZELENIN, Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Vavilov st. 32, Moscow, 117984, Russia.

Introduction. Investigation of chromosome C-banding pattern in different varieties of *Hordeum vulare* L. and related wild species has shown that this method may be used successfully for precise identification of barley chromosomes, their mapping, and establishing of evolution relationships in genus *Hordeum*. C-banding pattern polymorphism allowed investigators to distinguish different barley cultivars and construct their chromosome passports. It has been shown that C-banding pattern polymorphism can serve as a definite criterion of barley genome variability as well as genetic divergence and the ecological plasticity of different cultivars (Linde-Laursen, 1978; Muravenko et al., 1991; Von Bothmer et al., 1991).

Nevertheless, C-banding pattern is often not enough for precise localization of translocation breakpoints, chromosome physical mapping and study of chromosome functional and structural organization.

We modified standard high resolution banding methods (Cortes et al., 1980; Yang & Zang, 1988; Kakeda et al., 1990) for barley chromosomes and carried out a comparative study of the obtained G-like banding patterns for chromosome investigation.

Materials and Methods. The seedlings of *Hordeum vulgare* L. cv Djaukabutag were used in our study.

Barley chromosome preparation and C-banding staining were carried out according to the technique previously developed in our group.

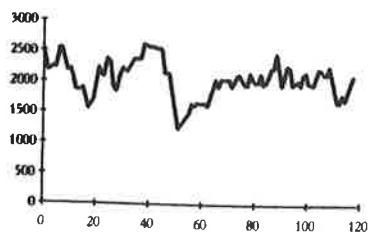
To obtain chromosome preparations for high resolution staining we followed the technique developed by Amosova et al.(1989). The FPG-banding patterns were obtained by a modified Cortes et al.(1980) technique and G-like banding - according to common trypsin method (Yang & Zang, 1988; Kakeda et al., 1990) with modifications including root pretreatment with ethidium bromide (10 mkg/ml) and fixation in 70% methanol.

Chromosome images in the bright-field light with Leitz 63x, 1.4 Planapo objective were detected using a Peltier cooled slow scan CCD camera 512 x 512 pixcels (pixel size 16 mkm) and proceeded with a specially designed software.

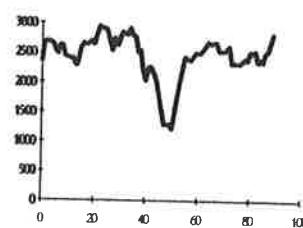
Results and Discussion. The replicative banding method was the first high resolution banding technique successfully applied for the investigation of plant`chromosomes. A modifications of high resolution FPG technique, used in this work, revealed slightly and intensively stained bands corresponding, respectively, to the early replicating DNA and that, replicating in the mid and late S-period.

This technique allowed us to obtain complicated contrast banding patterns, reproducible for a chromosome at a definite stage of its contraction and BrdU incorporation in early S-period. Comparison of barley chromosome C- and FPG-banding patterns has revealed that DNA of most of C-bands replicates in middle and late parts of the S-period (Fig. 1a and 1b). Thus, similar to other

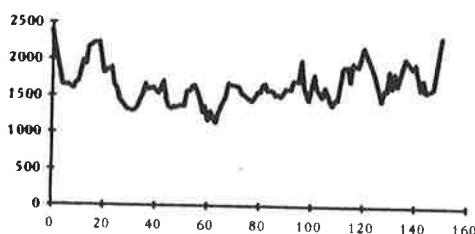
Chromosome 5 (7)



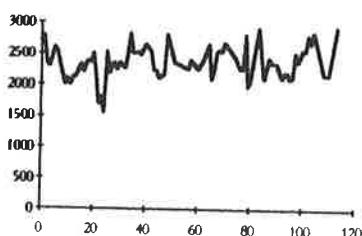
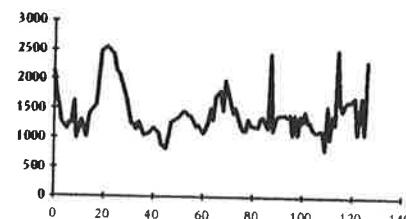
Chromosome 6 (6)



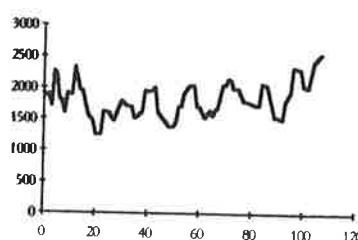
a



b



c



Barley chromosomes with densitograms after C-(a), FPG-(b) and G-trypsin(c) banding respectively. Arrows show centromeres.
X-axis, pixels; Y-axis, intensity
1 pixel = 0.08 micrometers

Fig.1

plants, there is not a complete correspondence between late replicating DNA and constitutive heterochromatin detected as C-bands in barley chromosomes (Cortes et al., 1980; Taniguchi & Tanaka, 1991).

A great number of intercalar replicative Giemsa-positive bands and, in some cases, asymmetric banding pattern in homologous or in chromatids makes difficult chromosome identification without image analysis. The image analysis of at least ten prometaphase plates revealed marker FPG bands in homologous and made possible chromosome identification.

A modification of trypsin banding technique, developed by us, revealed in barley chromosomes sharp G-band-like patterns comparable to those in mammals (Yang & Zang, 1988; Kakeda et al., 1990). This high resolution technique, similar to FPG, revealed more complicated G-banding patterns than those detected by C-banding method (Fig. 1a and 1c). Comparison of both banding patterns has shown that there is only a partial coincidence between C- and G-bands.

Visual and image comparative analyses of FPG and trypsin banding patterns of homologous chromosomes showed both patterns to be mostly similar in the band distribution along chromosomes and sometimes differing in the size of bands and in the intensity of their staining (Fig. 1b and 1c). Similarity of both banding patterns suggests that G-like bands in plant chromosomes are comparable to G-bands detected in mammals and are in favour of assumption of the similarity of chromosome organization in plants.

The trypsin G-like banding technique developed in this work does not include DNA damaging procedures, such as long acid treatment and UV-irradiation, and may be thus proposed for chromosome microdissection without using of deleted and addition lines.

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Intraspecific C-banding chromosome polymorphism of *Hordeum spontaneum* C.Koch. O.V. MURAVENKO, M.A. NAZAROVA, E.D. BADAeva and A.V.ZELENIN, Institute of Botany, Turkmen Academy of Sciences, Magtymguly av.79, Ashgabat, 744000, Turkmenistan; Engelhardt Institut of Molecular Biology, Russian Academy of Sciences, Vavilov st. 32, Moscow, 117984, Russia.

Introduction. C-banding patterns of cultivated barley are associated with taxonomic position and also with environmental conditions and the area of distribution (Vosa, 1976; Linde-Laursen, 1978; Muravenko et al., 1991; Von Bothmer et al., 1991). C-banding polymorphism makes it possible to characterize particular barley forms by constructing "chromosomal passports" which simplify their comparison (Badaeva et al., 1990; Muravenko et al., 1990).

Wild barley *Hordeum spontaneum* C.Koch has an I-genome as well as cultivated *H. vulgare* L. *H. spontaneum* was used as a donor of agriculturally valuable traits in introgressive *H. vulgare* x *H. spontaneum* or *Triticum aestivum* L. x *H. spontaneum* hybrids (Von Bothmer et al., 1991). However, intraspecific C-banding polymorphism of *H. spontaneum* has not been studied enough.

In this paper we analyzed intraspecific C-banding polymorphism of *H. spontaneum* from Turkmenistan, which is the northern border of the area of its distribution and from the center of origin - Israel (Nevo, 1992).

Materials and Methods. 28 accessions from five localities of Turkmenistan and from six localities of Israel were studied.

Table 1. Exact location of the studied accessions of *H. spontaneum*

# number	I	Collection site
1,2,3,4		South-Western Kopetdag near Daina toward to Orekhovo
5,6,7		South-Western Kopetdag, dry gorge toward to Makhtum-Kala
15,16,17,		Central Kopetdag, the plain below mountains
18		near Ashgabat
8,9,10,11		South-Eastern Kopetdag, near Kaakhka
12,13,14		South-Eastern Turkmenistan, near Takhta-Bazar
71284-48		Damon, Israel
71284-23		Damon, Israel
77145-44		Tel Hay, Israel
77145-2		Tel Hay, Israel
77130-20		Evzariya, Israel
77147-5		Yeroham, Israel
77131-7		Bet Shean, Israel
77131-48b		Bet Shean, Israel
71283-10		Bar Giyyora, Israel

The seeds from Israel were supplied by Dr. E.Nevo. The seeds from Turkmenistan were collected by authors.

Chromosome slides and C-banding technique were done according to Bolsheva et al. (1984). Barley chromosomes were classified according to the genetic nomenclature of wheat chromosomes (Muravenko et al, 1986).

Results and Discussion. Analysis of C-banding patterns of wild barley *H. spontaneum* chromosomes revealed significant intra- and interpopulation variation. The number and position of polymorphic C-bands on chromosomes of different accessions were specific for each geographical region. All seven I-genome chromosomes contains polymorphic C-bands. "Chromosomal passports" of barley from eleven localities were constructed basing on the results of visual C-banding analysis. Comparison of karyotypes of accessions from the same geographical areas allowed us to construct two generalized chromosomal passports" Turkmen and Israeli population of *H.spontaneum* presented as Tables 2 and 3.

Table 2. "Chromosomal passport" of *H.spontaneum* from Turkmenistan.

ArmlRegionlSeg- l	I No	Chromosomes						
		lment	1	2	3	4	5	6
S	2	7	1	2-0			1-0	
		5	1	1-0		2-1	1-0	
		3	2-1	2-0		2-1	3-0	
		1	3-1	3-0		4-0	4-0	
1	9	2-0	-	-	2-0	-	-	2-0
	7	1-0	-	-	1-0	-	-	1-0
	5	1-0	6-2	5-2	4-2	2-0	2-0	1-0
	3	2-0	5-2	7-3	6-3	5-1	5-2	2-0
	1	3-2	3-2	5-2	8-4	7-4	5-2	6-2
C								
L	1	1	3-2	3-1	2-0	2-1	5-3	6-2
		3	4-2	6-1	3-0	7-3	5-2	5-2
		5	2-0	5-1	5-2	6-2	3-1	1-0
		7	2-0	-	-	-	-	-
		9	2-0	-	-	-	2-0	2-0
2	1	3-1	1-0	3-0				1-0
	3	3-1	-	2-0				2-1
	5	3-1	3-0	2-0				2-0
	7		2-0					

Comparative study of accessions of *H. spontaneum* rom five localities from Turkmenistan revealed differences in the level of C-banding polymorphism. Barley collected in South-Western Kopetdag, near Orehovo, was the most polymorphic, and population from Central Kopetdag - the less variable. C-banding patterns of barley karyotypes from the same locality were more similar, han those from different regions of Turkmenistan. Comparison of "chromosome passports" of *H. spontaneum* with those of cultivated local *H. vulgare* variety "Altyndan" showed that they have similar heterochromatin distribution.

Table 3. "Chromosomal passport" of *H.spontaneum* from Israel.

Arms	Region	Segment	Chromosomes							
			No	1	2	3	4	5	6	7
I										
S	2	7		2-0	2-0			2-0		
		5		2-0	1-0		3-1	-		
		3		2-0	1-0		-	-		
		1		4-0	3-0		7-1	8-0		
1	9	4-1	-	-	2-0	-	-	2-1		
	7	2-0	-	-	1-0	-	-	1-0		
	5	1-0	6-1	9-3	4-0	-	-	1-0		
	3	2-0	4-2	10-6	9-2	2-0	8-3	2-0		
	1	1-7	2-1	2-1	12-8	9-4	5-1	8-3		
C										
L	1	1	1-5	2-1	1	8-2	7-2	4-1	5-2	
		3	1-5	6-2	9-1	8-3	4-1	8-5	6-1	
		5	2-0	7-3	6-1	8-3	2-0	1-0	2-0	
		7	2-0	-	-	1-0	2-0	-		
		9	2-1	-	-	-	2-1	3-0	-	
2	1	2-0	3-0	5-0				2-0		
	3		3-0	3-1	2-0			2-0		
	5		2-1	2-0	2-0			2-1		
	7			2-0						

Four Israeli accessions of *H. spontaneum* showed heteromorphism of homologous chromosomes which can be explained by their hybrid origin.

Nine of ten analysed accessions of *H. spontaneum* from Israel contain intercalary C-bands in either 3l or 6l long arms or in both positions (Table 2). These bands were absent in Turkmen accessions (Table 2 and 3). At the same time, most accessions from Turkmenistan contain intercalary C-bands in 5l and 6l short arms which has not been detected in Israeli accessions, thus, being specific for Turkmen population of *H. spontaneum*.

The obtained data confirmed dependence of C-banding pattern formation in barley karyotype on the area of its growth, which has been revealed earlier on cultivated barley *H. vulgare* (Linde-Laursen, 1978; Muravenko et al., 1991).

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Linkage analysis and distribution of RAPD markers in barley. E. NOLI, M.C. SANGUINETI, R. TUBEROSA, S. SALVI, and E. SCHILIRO', Department of Agronomy, University of Bologna, Via Filippo Re 6, 40126 Bologna, Italy.

Introduction. The availability of linkage maps from different mapping populations of the same species is useful to analyse the localization and effects of agronomically important QTLs in different genetic backgrounds. In barley, several linkage maps have been published based on RFLP markers (reviewed in Kleinhofs and Kilian, 1994). When homozygous lines are used for mapping purposes, as is the case in barley, RAPDs could represent an interesting alternative and/or integration to RFLPs. Until now, the use of RAPDs for mapping purposes in barley has been marginal. Twenty-three RAPD markers were mapped in a barley cross and were found to be distributed throughout the genome (Giese et al., 1994). However, coincidence of four RAPD bands together with a higher than expected frequency of tightly linked RAPD markers was observed. Tinker et al. (1993) have also reported a higher than expected frequency of clustered RAPDs in barley. Our objective has been to verify the possibility of constructing a barley linkage map prevalently based on RAPD markers and investigate their distribution on the map.

Materials and methods.. Seventy-six doubled-haploid (DH) lines were obtained from anthers of F₁ plants of the cross 'Arda x Opale' (Noli et al., 1994). For each line, ten plants were grown in the greenhouse in disease-free conditions. DNA was isolated from freeze-dried leaf tissue of 8 plants/line. RAPD analysis was performed according to the protocol of Williams et al. (1990) with some modifications. Reaction volumes of 18 µL contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25 °C), 0.1% Triton X-100, 2 mM MgCl₂, 15 ng DNA, 400 nM primer (Operon, USA), 200 µM of each dNTP, 1.2 units of *Taq* DNA polymerase (Promega). Amplification was programmed for 40 cycles of 1 min at 94 °C, 1 min at 36 °C, 2 min at 72 °C, with an initial melt at 94 °C for 1 min, and a final extension at 72 °C for 8 min. DNA for RFLP analysis was digested with *Bam*H, *Hind*III, and *Eco*RI. The procedures utilized to reveal polymorphisms were those described in Sharp et al. (1988). STS analysis was conducted according to the protocol of Tragoorung et al. (1992). Aliquots of amplified products were digested with *Alu*I, *Hae*III, *Hha*I, *Hinf*I, *Msp*I, and *Taq*I and separated on 3% agarose. Each chromosome was represented by one or more of the RFLP probes and STS markers. The linkage map was obtained analysing the polymorphisms of 60 RAPDs, 7 STSs, and 25 RFLPs. Linkage analysis was performed with the program JoinMap vers.1.4 (Stam, 1993) using the Kosambi function.

Results and discussion. Based on the amplification pattern and its reproducibility in separate experiments, we considered 60 RAPD bands originated from 35 primers which evidenced polymorphisms between Arda and Opale. In total, 12 linkage groups were identified using a LOD score of 2.0. Considering the information available for the RFLP and STS markers, 11 linkage groups were assigned to the seven barley chromosomes. For one linkage group identified by one RAPD and one STS marker and representing 5 cM, it was not possible to determine the chromosomal localization. Further work is in progress to add more RAPD and RFLP markers to the existing map.

The linkage map (Fig. 1) covers 528 cM which represent 35-40% of the genetic length of the barley genome estimated in previous maps (Kleinhofs and Kilian, 1994). In our case, the average distance among sets of linked RFLP markers was 30% less than that reported in the published maps. Segregation analysis of the DH lines for each of the 92 markers herein considered showed that 7 RAPD, 4 RFLP, and 1 STS markers did not fit the expected 1:1 segregation ratio (Fig. 1). In our case, the skewed ratios should not be related to partial inconsistency of allelic expression of RAPD loci, but instead to uniparental inheritance due to linked genetic factors controlling anther culturability in barley. This is supported by the fact that skewness in favour of the same type of parental allele characterized clusters of linked RAPD loci and, more importantly, also linked RFLP loci (Fig. 1). In most cases (8/12) the favoured parental alleles were those of Arda. In barley, distorted segregation of molecular marker clusters has been reported also for other DH mapping populations (Heun et al., 1991; Graner et al., 1991; Giese et al., 1994).

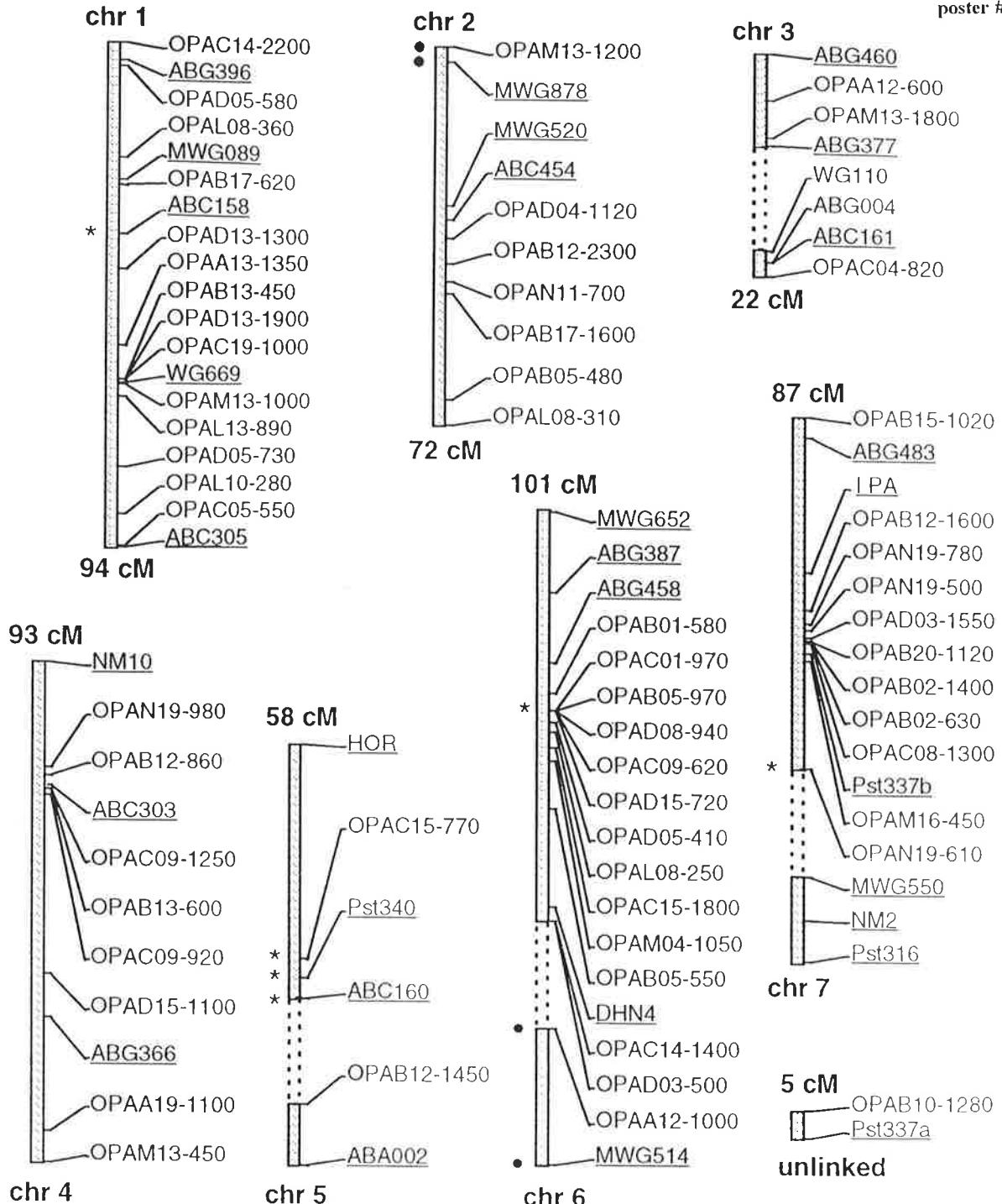


Figure 1. Linkage map of the cross 'Arda x Opale', based on RFLP and STS (underlined) and RAPD markers. Markers with a segregation ratio significantly distorted towards Arda and Opale alleles are indicated by * and •, respectively.

For 14 independently-scored RAPD bands, there was coincidence of map position in groups of two or more markers (Fig. 1). Only in one case, two coincident markers were amplified by the same primer; such bands could represent the two allelic forms of a codominant RAPD. For the other 12 bands, the size of the fragments and the 3' sequence of the primers differed within each group of coincident bands, thus indicating unrelated priming sites. Interestingly, when the DNA of seven other unrelated barley cultivars was assayed with the primers showing map coincidence, the presence or absence of the coincident bands occurred concurrently, suggesting that the DNA templates of such bands are consistently

present as closely linked loci in a wide spectrum of barley germplasm. Coincidence of RAPD bands in barley was also reported by Tinker et al. (1993) and by Giese et al. (1994) who suggested that it may be caused by the presence of polymorphic middle repetitive inverted sequences.

The distribution of RAPDs along the map was not at random as indicated by the frequency distribution of the map distances between pairs of RAPD markers (data not reported). Clustering of RAPDs could indicate regions characterized by low recombination frequencies, such as regions of repetitive DNA which represents a large portion of the barley genome. The nature of the template DNA of six RAPD bands produced by different primers was investigated with Southern analysis using the amplified bands as probes. Three bands showed an hybridization pattern typical of a single and/or low copy sequence, while the remainder showed a pattern more typical of a repeated sequence (data not reported).

Conclusions. Our results indicate that RAPD markers i) can be successfully mapped in barley, ii) are not randomly distributed in the genome, and iii) often show coincidence of map position. RAPDs could be a useful tool to integrate existing maps, especially for targeting chromosomal regions under-represented in terms of RFLP markers such as repetitive sequences.

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Mapping QTL for *Pyrenophora graminea* resistance in barley. N. PECCHIONI, G. VALE', E. TORRIGIANI and V. TERZI, Experimental Institute for Cereal Research, Section of Fiorenzuola d'Arda, I-29017, Via S.Protaso, 302, Fiorenzuola d'Arda (PC), Italy

Pyrenophora graminea Ito and Kuribayashi [anamorph *Drechslera graminea* (Rabenh. ex. Schlech.) Schoemaker] is a seed-borne pathogen and the causal agent of leaf stripe of barley (*Hordeum vulgare* L.). The brown stripes on the barley leaves drastically reduce the photosynthetic area and causes reductions in yield.

It is well known that genetic differences exist between barley cultivars, in a continuous way ranging from highly susceptible to highly resistant (Tekauz, 1983; Delogu et al., 1989). Variability in the infectiveness of different *Pyrenophora* isolates and in host response to different fungus strains has been recognized (Gatti et al., 1992) too. Resistance reactions can be explained either governed by polygenic systems or the presence of a single dominant gene, by two genes with epistatic effects or by two recessive genes with additive effects. It was postulated that a single genetic factor controlling the complete resistance to *Pyrenophora graminea*, was introduced in many North European barley cultivars from *Hordeum laevigatum* via cv. Vada. Giese et al. (1993) mapped the MiLa locus as the Vada-resistance factor on the M arm of barley chromosome 2. However, little is known about either the leaf stripe quantitative resistance, widespread in barley European germplasm, or the genetic basis of other complete resistances found in non-European cultivars.

Since the parents of a published barley RFLP map (Heun et al., 1991) are different in respect to their leaf stripe resistance, we used this doubled haploid population to study the genetic basis of barley leaf stripe quantitative resistance.

From obtained results, a major gene underlying this quantitative resistance was mapped with molecular markers in a barley doubled haploid (DH) population derived from the cross Proctor x Nudinka. This QTL accounts for $r^2 = 58.5\%$ and was mapped on barley chromosome 1, tightly linked to the "naked" gene. A second resistance QTL accounting for 29.3% of the variation in the trait was identified on the P arm of barley chromosome 2. Another two minor QTLs were detected by further analysis. None of the QTLs was found in the barley chromosome 2 "Vada" region studied by Giese et al. (1993).

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CYTOGENETIC CHARACTERISTICS OF INTERGENERIC HYBRIDS BETWEEN WILD HORDEUM SPECIES AND WHEAT. G.I. Pendinen, Vavilov Institute of Plant Industry, Bolshaya Morskaya st., 42, 190000, S.-Petersburg, Russia.

Introduction. Species of the genus Hordeum often are used in interspecific and intergeneric crosses to species possessing resistance to drought, salt and low temperature stresses. Together with solving practical tasks, studies on genomic compatibility and interactions of genomes of parental species in a synthesized hybrid genome are an important aspect of distant hybridization. The aim of this investigation was to conduct a cytogenetic study of intergeneric hybrids between several wild barley species and wheat and to reveal peculiarities of chromosome pairing in MI of meiosis and degree of stability of chromosome number in PMCs.

Materials and Methods. Several intergeneric hybrids between wheat and wild Hordeum species were used in our investigation: H. pubiflorum (2x), H. jubatum (4x), H. procerum (6x), H. lechlery (6x) with T. aestivum cv. Chinese Spring; H. geniculatum (4x) and H. murinum (2x) with T. aestivum cv. Chinese Spring, H. murinum (4x) with T. durum cv. Cocorit 71 (4x). One plant was obtained and then studied in every combination. H. geniculatum was the tetraploid cytotype of Hordeum murinum. In this paper the name of the specimen as registered in the collection of the Vavilov Institute of Plant Industry is used. Chromosome pairing in MI of meiosis and chromosome numbers in PMCs were investigated. The spikes were fixed according to Newcomer and PMCs were squashed in 1% acetoorcein.

Results and Discussion. Analysis of chromosome number in PMCs did not reveal any significant variability in sporogenic tissue (Table 1). However, variability of chromosome numbers in sporogenic tissue has been reported by several investigators for the hybrids of H. vulgare and other Triticum species (Fedak, 1980; Mujeeb-Kazi and Rodrigues, 1983; Surikov and Pendinen, 1994; and others). This variability may prevent the isolation of cytogenetically stable and fertile forms. This variability may be associated with the presence of the H. vulgare genome in the synthesized hybrid genome because variability of chromosome number in microsporocytes has been also shown for the hybrids of H. vulgare and wild barley species such as H. procerum, H. jubatum, H. lechlery (von Bothmer, Flink, Jacobsen et al., 1983).

Evolutional peculiarities of different species of the genus Hordeum could be a reason for differences in cytogenetic stability of the intergeneric hybrids of H. vulgare and wild Hordeum species with Triticum. Distant hybridization occurred during speciation of allopoloid Critesion species (H. procerum, H. lechlery, H. jubatum) and Triticum species. Probably H. murinum (4x) has an autoploid origin. However, this species is allopoloid on the modern stage of evolution (von Bothmer, Jacobsen, Baden et al., 1991).

Table 1. Variability of chromosome numbers at MI of intergeneric hybrids between Hordeum and Triticum.

Hybrids	No. of Euploid cells	Aneuploid cells			
	cells	No.	%	No.	%
<u>H. pubiflorum</u> x <u>T. aestivum</u> ($2n=4x=35$)	75	70	93.3	5 (27,32,36)	6.7
<u>H. jubatum</u> x <u>T. aestivum</u> ($2n=5x=35$)	73	72	98.6	1	1.4
<u>H. procerum</u> x <u>T. aestivum</u> ($2n=6x=42$)	37	37	100	0	0
<u>H. lechleriy</u> x <u>T. aestivum</u> ($2n=6x=42$)	42	42	100	0	0
<u>H. geniculatum</u> x <u>T. aestivum</u> ($n=5x=35$)	38	38	100	0	0
<u>H. murinum</u> x <u>T. durum</u> ($2n=4x=28$)	51	51	100	0	0
<u>H. murinum</u> x <u>T. aestivum</u> ($2n=4x=28$)	84	84	100	0	0

The genome of H. vulgare developed at the diploid level. Participation in speciation by distant hybridization and allopolloidy is not characteristic of H. vulgare. It is possible that functioning of the genome of H. vulgare was altered in the presence of an alien genome in the synthesized hybrid genome. In our studies a higher degree of stability of hybrids could be explained by evolutionally stipulating the possibility of wild barley genomes functioning in complex allopolloid genomes. Meiosis is univalent in all hybrids between wheat and Hordeum species of Critesion section (Table 2). The frequency of chromosome pairing in MI does not depend on the ploidy of parental forms of Hordeum. Rare chromosome associations are represented by rod bivalents.

The frequency of chromosome pairing is significantly higher in the hybrids H. murinum ($4x$) x T. durum and H. geniculatum x T. aestivum. Chromosome associations are represented by rod and ring bivalents. Microsporocytes containing 7 bivalents were observed in both hybrids. The rare chromosome association in MI of meiosis of H. murinum ($2x$) x T. aestivum hybrid was represented by a rod bivalent only. It is possible that the higher degree of homoeology between the chromosomes of 2 barley genomes may stipulate a high degree of pairing in the hybrids of tetraploid cytotype H. murinum and wheat.

Frequency of pairing is significantly higher in the hybrids of tetraploid H. murinum with wheat if compared with this parameter in the hybrids of Triticum with polyploid species from Critesion section. These differences could be connected with different evolution of these species. The tetraploid cytotype of H. murinum could be of autopolyploid origin and, therefore, could exhibit a higher homoeology of genomes than allopolyploid Critesion species.

Table 2. Chromosome pairing at metaphase I in intergeneric hybrids between *Hordeum* and *Triticum*.

Hybrids	Mean configurations				III
	I	II	rods	rings	
<u><i>H. pubiflorum</i></u> x <u><i>T. aestivum</i></u> ($2x=4x=28$)	26.66	0.63 (0;1)	0	0.63 (0;1)	0.03 (0;1)
<u><i>H. jubatum</i></u> x <u><i>T. aestivum</i></u> ($2n=5x=35$)	33.94	0.53 (0-3)	0	0.53 (0-3)	0
<u><i>H. procerum</i></u> x <u><i>T. aestivum</i></u> ($2n=6x=42$)	41.90	0.05 (0;1)	0	0.05 (0;1)	0
<u><i>H. lechlerii</i></u> x <u><i>T. aestivum</i></u> ($2n=6x=42$)	40.04	0.96 (0-3)	0	0.96 (0-3)	0.2 (0;1)
<u><i>H. geniculatum</i></u> x <u><i>T. aestivum</i></u> ($2n=5x=35$)	27.89	2.94 (0-7)	0.61 (0-3)	3.55 (0-7)	0
<u><i>H. murinum</i></u> x <u><i>T. durum</i></u> ($2n=4x=28$)	20.39	3.41 (0-6)	0.40 (0-3)	3.81 (0-7)	0
<u><i>H. murinum</i></u> x <u><i>T. aestivum</i></u> ($2n=4x=28$)	26.45	0.78 (0-2)	0	0.78 (0-2)	0

All hybrids studied were sterile. Partial autofertility was noted only in the hybrid *H. murinum* ($2x$) x *T. aestivum*, possibly due to an unreduced gamete. Reductive division was absent in several microsporocytes of this hybrid. At the end of the MI chromosomes divide into chromatids which move to the poles, resulting in the production of microspore diads in 66% of the microsporocytes. These diads possess an unreduced chromosome number. The progeny of this hybrid were 56-chromosome plants, possibly amphiploids. The lack of reductive division might take place also in the maternal meiosis. Amphiploid progeny of this hybrid may be the result of sperm with unreduced chromosome number fertilizing an unreduced egg-cell.

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An Amplified Fragment Length Polymorphism (AFLP*) map of the barley (*Hordeum vulgare*) genome.

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Introduction: Net blotch caused by the pathogenic fungus (*Pyrenophora teres*) causes substantial yield losses to barley grown in Canada every year. Perhaps more importantly, barley plants infected with this disease exhibit reduced seed filling and hence cannot be marketed as a premium priced, malting quality grain. Certain barley cultivars exhibit isolate specific resistance, however, this resistance has proved difficult to work with due to its complex inheritance. The majority of the resistance genes identified to have been recessive in their expression making them extremely difficult or impossible to select for in early generation material (Afanasenko et al., 1995). Certain *Hordeum vulgare* accessions derived from Ethiopia possess excellent net blotch resistance (Legge and Tekauz, person. commun.). Analysis of a population derived from a cross between one such line, MEH486 and Harrington, suggested the presence of three recessive resistance genes. The incorporation of all the resistance found in this line into elite germplasm would be greatly facilitated through the use of marker-assisted selection. Unfortunately, for maximum expression of resistance, all three resistance loci must be homozygous for the recessive allele. This reduces the feasibility of the use of bulk-segregant analysis for marker identification.

Keygene Inc. has developed a technique termed Amplified Fragment Length Polymorphism (AFLP*), that can be used to generate a molecular map of a plant genome in a fraction of the time required for RFLP or RAPD analysis. The objective of this study was to use this technique to construct a genomic map based on the population derived from the cross between Harrington and MEH486. This map would then be used through quantitative trait loci analysis to identify chromosome regions containing net blotch resistance genes.

Materials and Methods:

An F₇ population was produced through single seed descent selection from a cross between Harrington and MEH486. Resistance to net blotch in this population was evaluated through application of the net form of the pathogen using isolate WRS858. Net blotch inoculum was increased as described by Tekauz (1990). Five clumps of five seedlings were planted in six inch pots. Each clump consisted of five seedlings from a different single F₇ line. After two weeks, when the plants had reached the three leaf stage, an aqueous spore suspension was applied at a rate of 10 ml per pot. Inoculum concentration was adjusted to 10⁴ conidia per ml. Inoculated plants were kept in the dark for 24 hr at as high a humidity level as possible. Reaction types were rated a week after inoculation on a numerical scale (1 to 10) based on lesion type.

DNA was extracted from a bulk sample of leaves harvested from five seedlings of each line using the protocol described by Lu et al., (1994). AFLP* analysis was performed as described by van Eck et al., (1995) using a Gibco BRL AFLP* kit. Genomic DNA was restricted

with Eco RI and Mse I, and ligated to restriction site specific adapters.(Gibco, BRL). Aliquots of this ligated mixture were subjected to pre-amplification using primers that were specific to the ligated adapter, the remaining portion of the restriction recognition site, and a single base that anneals on the 3' side of this known sequence. An aliquot was diluted 10:1 from this round of amplification and subjected to a further amplification round, termed selective amplification. This round was identical to the first, except that the primers extended three bases into the unknown sequence rather than just one. Products were separated on sequencing gels (Bio-Rad), using standard sequencing electrophoresis protocols. Following electrophoresis gels were silver-stained using the Promega silver sequence, silver-staining kit.

Results and Discussion:

Resistance to isolate WRS858 (representing the net form of net blotch, *P. teres f. teres*) was evaluated in an F₇ segregating population derived from Harrington/MEH486 (Table 1). If we score reaction phenotypes 1 to 3 as resistant, then the data appears to fit a three recessive resistance gene model. Given the difficulty associated with confidently ascribing moderate resistance versus moderate resistance a qualitative approach to correlating phenotypic variation with potential molecular markers was abandoned in favour of a quantitative approach.

The parents were screened with 64 AFLP primer combinations resulting in the identification of at least 500 polymorphic fragments. Ten primer combinations that amplified 15 or more polymorphisms were selected for use in constructing a genomic map. Approximately 90 % of the polymorphisms identified between the parents segregated in a reproducible Mendelian manner on the segregants. Linkage groups were anchored to known chromosome locations through the use of previously mapped allele specific amplicons and RFLPs.

Analysis has not been completed at time of submission for this poster. A complete discussion of results will be presented in the poster itself.

Table 1. Evaluation of net blotch resistance in an F₇ population derived from the cross between the resistant parent, MEH486 and the susceptible parent, Harrington.

	R (1 - 4)	MR (5 - 7)	MS (8 - 9)	S (10)	χ^2
First rating	17	62	60	12	3.39
Second rating	18	67	42	24	7.07
Expected (1:3:3:1 ¹)	19	57	57	19	

¹ Ratio based on the following genotypes

- 1: aabbcc
- 3: aabbCC, aaBBcc, AAbbcc
- 3: AABBcc, AAbbcc, aaBBCC
- 1: AABBCC

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*AFLP is a registered trademark of KeyGene Inc.

The introgression of genes from *Hordeum bulbosum* L. into barley (*H. vulgare* L.). R. PICKERING, A.M. HILL, G.M. TIMMERMAN-VAUGHAN, M.J. GILPIN, M.G. CROMEY and E.M. FORBES, New Zealand Institute for Crop & Food Research Limited, Private Bag 4704, Christchurch, New Zealand.

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In this paper we describe the progress, achievements and future directions in *Hordeum vulgare* - *H. bulbosum* gene introgression programmes. The aim of the research has centred chiefly on the transfer of desirable traits from *H. bulbosum* into *H. vulgare*. These traits include resistance or tolerance to pests, diseases and stress. The first successful hybridization was reported by Kuckuck (1934), and many hybrids (diploid, triploid and tetraploid) were subsequently obtained during the 1950s and 1960s. However, none of these attempts achieved the aim of gene transfer. In 1970 Kasha & Kao described the formation of haploid barley plants from crosses between diploid *H. vulgare* and diploid *H. bulbosum*, and this discovery resulted in the establishment of doubled haploid breeding programmes around the world (see Devaux, this symposium). Meanwhile, research into gene transfer continued and a recombinant involving the transfer of hairy leaf sheaths from the wild species was reported (Pohler & Szigat 1982). A powdery mildew-resistant plant was subsequently described (Pickering et al., 1987) but molecular confirmation of its identity had to wait (Pickering et al., 1995). The first proof of *H. vulgare* - *H. bulbosum* introgressions was reported by Xu & Kasha (1992) who obtained a powdery mildew-resistant plant from the backcross of a partially fertile triploid hybrid (*H. vulgare* 2n = 14 x *H. bulbosum* 2n = 28) to barley. A consistent supply of similar, partially fertile, triploid hybrids had previously been produced (Pickering, 1988) from crosses between *H. vulgare* and tetraploid *H. bulbosum* derived from chromosome-doubled diploid genotypes. The hybrids were backcrossed to barley and a series of monosomic substitution plants was obtained involving the substitution of *H. vulgare* chromosomes by their *H. bulbosum* homoeologues (Pickering, 1992). Double monosomic substitutions, often involving barley chromosomes 1 (7I) and 4 (4I) were also identified as well as recombinants. The more fertile of the single monosomic plants have been selfed, and disomic chromosome 1 (7I) substitution plants were found among the progeny. These disomic substitutions were all derived from shrivelled seeds. Research in Germany has concentrated on screening progeny from fertile tetraploid *H. vulgare* - *H. bulbosum* hybrids and has led to the selection of plants with resistance to powdery mildew and barley mild mosaic virus (Michel, 1995). A list of the known recombinants derived from several programmes is

The introgression of genes from *Hordeum bulbosum* L. into barley (*H. vulgare* L.). R. PICKERING, A.M. HILL, G.M. TIMMERMAN-VAUGHAN, M.J. GILPIN, M.G. CROMEY and E.M. FORBES, New Zealand Institute for Crop & Food Research Limited, Private Bag 4704, Christchurch, New Zealand.

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presented in Table 1.

Table 1 Current list of *H. vulgare* - *H. bulbosum* recombinants; * = unpublished

Code number	Transferred trait	Chromosome location	Reference
K301	Hairy leaf sheath	4M (4IM)	Pohler & Szigat (1982) Hill *
30X2	Hairy leaf sheath	4M (4IM)	Pickering et al. (1994)
81882	Powdery mildew resistance	2P (2IP)	Pickering et al. (1995)
36L5	Stem rust/spot blotch resistance	3M (3IM)	Steffenson * Hill et al. *
17A6	GISH/FISH positive	3M (3IM)	Pickering *
38U4	Leaf rust resistance	6P (6IP) +unknown	Steffenson * Hill *
886Z3	Leaf rust resistance	2M (2IM)	Steffenson * Hill *
916J2/2	Narrow leaf	6P + 6M (6IM+6IP)	Thiele et al. (1992)
BC ₁ -2/RP6	Powdery mildew resistance	2M (2IM) +unknown	Xu & Kasha (1992) Hill *
WB5/117	BaMMV resistance	6P (6IP) +unknown	Michel (1995)
14/1/30	Powdery mildew resistance	2P (2IP) +unknown	Michel (1995)

To circumvent the problems associated with sexual fertilization (eg incompatibility and certation) anther culture from triploid and tetraploid hybrids was carried out. Three novel chromosome substitution plants were regenerated (Pickering & Fautrier, 1993). The technique has since been developed further although response in culture and plant regeneration is largely genotype-dependent (Gilpin et al., unpublished).

Characterization of progeny from hybrids. At Crop & Food Research, morphological, pathological and cytological analyses are initially carried out, and plants with anomalous characteristics are investigated more critically. Methods used comprise C-banding and RFLP analyses on Southern or dot blots with rye repetitive sequence probes (pSc119.1 and pSc119.2, McIntyre et al., 1990). These probes hybridize preferentially to centromeric and interstitial (pSc119.1) or sub-telomeric (pSc119.2) sites on all (pSc119.1) or several (pSc119.2) *H. bulbosum* chromosomes

(Xu et al., 1990). The signals on *H. vulgare* are either weak or absent. AFLP is also valuable for confirming the presence of *H. bulbosum*-introgressed DNA when the preliminary detection methods fail (Pickering et al., 1995). To locate introgressions, RFLP analysis is adopted using chromosome-specific probes. More recently, we have performed FISH and GISH to establish the number and physical location of introgressions. Sometimes more than one recombination event has occurred in substitution plants and recombinants. For example, 16R1, which was characterised by C-banding as a chromosome 1 (7I) monosomic substitution, also contains an *H. bulbosum* introgression on chromosome 6 (6I).

Future prospects. Now that recombinants are becoming easier to obtain and characterize, we hope to improve our preliminary identifications by extensive use of dot blots with genomic probes. We also plan to use GISH to estimate the frequencies of crossing-over events during meiosis. Furthermore, by combining GISH with microsatellite sequences (Pedersen & Linde Laursen 1995), we hope to identify the chromosome on which the introgressed DNA is located. It should also be possible to compare the physical and genetic maps and collinearity of genes between *H. vulgare* and *H. bulbosum*. Nevertheless, our main aim is still to supply plant breeders with basic barley breeding material possessing durable resistance genes that are different from those now available.

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Molecular mapping of leaf rust genes in barley using RFLP's. S. A. RADI and L. S. DAHLEEN. USDA-ARS, Northern Crop Science Lab. P. O. Box 5677, State University Station, Fargo ND 58105

Introduction. The goal of this project is to characterize new and known genes in barley for resistance to leaf rust, and to identify linked molecular markers which will be useful their use in barley breeding programs. Leaf rust, incited by *Puccinia hordei* Otth., is one of the limiting factors in many barley production areas. Although the disease can be controlled by the use of foliar fungicides, the most common and effective control practice is disease resistant cultivars. Genetic resistance is an economical and environmentally-friendly means of reducing disease losses. Sources of genetic resistance to *P. hordei* have been identified in several different barley genotypes. To date, about fourteen genes for resistance to leaf rust have been described in barley, designated as *Rph1* to *Rph14* (Jin and Steffenson, 1994). However, only a few of these genes for resistance have been used widely by North American, European, and Australian barley breeding programs. The currently grown cultivars in the Upper Midwest are susceptible to leaf rust. This disease is likely to remain a significant production problem because new pathotypes of *P. hordei* occur rather frequently and the known genes conferring resistance are not effective worldwide (Jin et al., 1995). Virulent isolates of the pathogens have been found on genotypes carrying each of the known resistance genes, which makes it necessary to search for new resistant sources.

DNA markers have been used in studies to locate genes controlling specific traits. Because DNA markers are environmentally insensitive, these markers can be used to monitor the introgression of desirable genes into adapted germplasm grown in both the greenhouse and field.

Backcross populations of leaf rust resistance genes have been developed from *H. vulgare*, sources from North Africa, *H. spontaneum*, and from known sources of resistance genes using Bowman barley as the recurrent parent. Ten homozygous leaf rust resistance lines, each having different resistance genes, have been selected for evaluation. Bowman was chosen because it is adapted to the Upper Midwest barley producing region.

This research is part of an extensive genome mapping effort by the North American Barley Genome Mapping Project (NABGMP). The project objectives are as follows: 1) utilize ten backcrossed-derived homozygous resistant lines to identify RFLP markers associated with both new and currently known resistance genes, and 2) map the chromosomal locations of genes conferring resistance to leaf rust using ten backcross derived segregating populations and selected RFLP markers, to facilitate their use in breeding resistant barley cultivars.

Materials and Methods. Ten barley introductions were used as donors of *Rph* genes in crosses to Bowman barley (Table 1) and for study of RFLP markers. These ten genotypes represent different *Rph* genes, four with known *Rph* genes and six with unknown *Rph* genes. Seed for this study was provided by barley breeder Dr. J. D. Franckowiak, NDSU,

Fargo, ND. The 15 to 20 seeds of each homozygous backcrossed line were sown in 15 cm pots in potting mix with a slow-release fertilizer (14-14-14) added. Plants were grown in a greenhouse with a photoperiod of 16 h at 18-25 C. Leaf tissue was collected from each genotype at the 3-5 leaf stage and dehydrated. DNA extraction was done using proteinase K and CTAB according to the methods of Kleinhofs et al. (1993). This method recovers large quantities of clean and high quality DNA. Using the restriction enzymes *Bam* H1, *Eco* RV, and *Hind* III, 20-30ug DNA was digested according to the manufacturer's protocol. The DNA fragments were separated in 0.9% agarose and transferred to nylon membranes by Southern blotting. Seven of the ten parental lines were evaluated for polymorphisms between Bowman and the specific line using genomic and cDNA clone sequences supplied by NABGMP, and the membranes were hybridized to detect RFLP's between the genotypes (Kleinhofs et al., 1993). The remaining three lines are currently being screened for polymorphisms.

A local isolate of race 8 of *P. hordei* (ND 8702), which is predominant in the Northern Great Plains, was used for leaf rust inoculations. Fifty F₂ plants from each backcross line were inoculated seven to ten days after planting when the primary leaf was fully expanded. Urediniospores were obtained from Dr. B. J. Steffenson, NDSU, Fargo, ND. After inoculation, plants were allowed to dry and placed in mist chambers at 20 C, with a 16/8 h light-dark photoperiod. The chambers were misted for one out of every five minutes to keep humidity near saturation. After a 24 h wet period, plants were placed in the greenhouse. Infection ratings and segregation ratios were evaluated in progeny from all ten lines, twelve to fourteen days after inoculation. The lesion type rating was based on the system developed by Levine and Cherewick (1952) using the scale of 0-4. DNA extracted from leaf tissue from the same F₂ plants has been blotted and is currently being tested with clones that detected polymorphisms in that specific line. The chi-square values were calculated on lesion data to determine the number of genes and type of gene action. Linkage relationships between molecular markers and lesion types will be evaluated using the MAPMAKER computer package (Landers et al., 1987).

Results and Discussion. The inoculation data showed that nine of the ten lines had one dominant gene (3:1) for resistance to leaf rust and one line showed a recessive segregation ratio (1:3). These segregation ratios were expected, except the one line. The unexpected results of the line Bowman*3/Hor2596, indicated that the seed lot was mixed, because previous studies by B. J. Steffenson, (pers. comm.), concluded that genotypes with the Hor2596 background in them carried one dominant resistance gene .

Seven of ten parental lines have been screened with 120 clone sequences to detect RFLP's. Sixty-two genotype-enzyme-clone sequence combinations identified polymorphisms. Currently, the 62 clone sequences detecting polymorphism are being hybridized to membranes containing the F₂ progeny DNA. The expected results of this project are to position the four known and six unknown leaf rust resistance genes on the barley linkage map using RFLP's.

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Table 1. Sources of resistance to *Puccinia hordei* used for mapping of resistance genes.

<u>Genotype</u>	<u>Resistance Gene</u>
Known Sources	
Bowman*7//Estate/3.2 uz als	<i>Rph</i> 3
Bowman*8/3/CI1243//Cebada Capa/MT81195	<i>Rph</i> 7
Bowman*3/Hor2596	<i>Rph</i> 9
Triumph/4*Bowman	<i>Rph</i> 12
North Africa Sources	
Bowman*3/Tunisia 17	<i>Rph</i> ?
Bowman*3/Tunisia 29	<i>Rph</i> ?
Bowman*3/Tunisia 34	<i>Rph</i> ?
Bowman*3/PI531990	<i>Rph</i> ?
Bowman*3/PI531849	<i>Rph</i> ?
<i>H. spontaneum</i> Source	
Bowman*3/PI466373	<i>Rph</i> ?

Characterization of Oat x Maize Partial Hybrids and Their Derivatives. O. RIERA-LIZARAZU, H.W. RINES^a, and R.L. PHILLIPS, Dept. of Agronomy and Plant Genetics, University of Minnesota, ^aUSDA.-ARS, 411 Borlaug Hall, 1991 Buford Circle, St. Paul, MN 55108, USA.

Introduction. Interspecific and intergeneric hybridization between crop plants and between crops and their wild relatives has been termed "wide crosses". In cereals, where wide crosses have been particularly useful, karyotypically stable crosses which produce hybrid plants have been used as starting points to widen the genetic base of a crop (Mujeeb-Kazi and Kimber 1985; Knott 1987) or to construct stocks for genetic analysis (Islam et al. 1981). Also, uniparental genome elimination in karyotypically unstable hybrids has been utilized for cereal haploid production (Kasha and Kao 1970; Barclay 1975).

Recently, sexual hybridization between distantly related species of two subfamilies (Pooideae and Panicoideae according to Clayton and Renvoize 1986) of the Gramineae (Poaceae) family has attracted research interest for potential gene transfer between such divergent gene pools and as an alternate means for the production of haploid plants (for review see Laurie et al. 1990). Examples of hybridization between species of such divergent gene pools include hexaploid wheat (*Triticum aestivum* L.) x maize (*Zea mays* L.) (Zenkteler and Nitzsche 1984; Laurie and Bennett 1986), wheat x sorghum (*Sorghum bicolor* L. Moench) (Laurie and Bennett 1988b), wheat x pearl millet (*Pennisetum glaucum* R. Br.) (Laurie 1989), barley (*Hordeum vulgare* L.) x maize (Laurie and Bennett 1988a), rye (*Secale cereale* L.) x maize (Laurie et al. 1990), and tetraploid wheat (*Triticum turgidum* L.) x maize (O'Donoughue and Bennett 1994) crosses. These wide hybrids were karyotypically unstable where uniparental chromosome loss resulted in the production of haploid embryos of various Triticeae species (Laurie et al. 1990).

The production of haploids from different Triticeae species and a near total lack of oat (*Avena sativa* L.) haploid plant recovery from anther culture (Rines 1983) motivated researchers in our laboratory to try oat x maize crosses as an alternate means to produce oat haploids. In 1990, Rines and Dahleen reported the production of oat haploid plants from oat x maize crosses. Oat haploid embryo formation in oat x maize hybridization likely involves a process of maize chromosome elimination (Rines and Dahleen 1990) similar to that described for wheat haploid embryo formation in wheat x maize crosses (Laurie and Bennett 1989). Oat haploid plants produced from oat x maize crosses do differ, however, from wheat haploids in that oat haploids are partially self-fertile. A process of meiotic restitution that produces viable gametes results in seed set in up to 40% of primary and secondary florets of main tillers in these haploids (Rines and Dahleen 1990; Davis 1992).

Another dramatic way in which progenies from oat x maize hybridization differ from those from wheat x maize hybridization was our discovery that oat x maize crosses not only result in the production of oat haploids but also result in the production of partial hybrids with 21 oat chromosomes plus one to four maize chromosomes (Riera-Lizarazu et al. 1996). This report constituted the first report of the production of karyotypically stable partial hybrids involving highly unrelated species from two subfamilies of the Gramineae (Pooideae - oat and Panicoideae - maize). Here we present on the characterization of these oat x maize partial hybrids and their progenies.

Results and discussion. From a series of seven crossing experiments involving cultivated hexaploid oat and maize, we recovered a total of 90 plants through embryo rescue. Their production involved the emasculation and maize pollination of about 15,000 oat florets and the excision and plating of over 1,000 embryos, with between 0.2 to 1.0% of florets pollinated resulting in a plant.

We used root-tip cell chromosome preparations (Mujeeb-Kazi and Miranda 1985), chromosome *in situ* hybridization experiments using total maize genomic DNA as the probe (genomic *in situ* hybridization or GISH; Schwarzacher et al. 1992), and Southern analysis using the 185 bp maize chromosome knob-specific repetitive sequence (Peacock et al.

1981) to detect maize chromosomes in oat x maize progenies. Fifty-two plants (58%) produced from oat x maize hybridization were oat haploids ($2n=3x=21$) following maize chromosome elimination. Twenty-eight plants (31%) were found to be karyotypically stable partial hybrids with one to four maize chromosomes in addition to a haploid set of 21 oat chromosomes ($2n=21+1$ to $2n=21+4$). One individual had an oat-maize chromosome translocation. The remaining nine of the 90 plants (10%) produced were found to be apparent chromosomal chimeras, where some tissues in a given plant contained maize chromosomes while other tissues did not or different tissues contained a different number of maize chromosomes.

Maize DNA restriction fragment length polymorphism (RFLP) probes (Burr et al. 1988; Coe et al. 1990; Gardiner et al. 1993) were used to identify the maize chromosome(s) present in the various oat-maize progenies. Southern analyses using maize RFLP markers revealed that maize chromosomes 2, 3, 4, 5, 6, 7, 8, and 9 were present in partial hybrids and chromosomal chimeras. Maize chromosomes 1 and 10 were not detected in the plants analyzed to date.

Partial self-fertility, which is common in oat haploids, also was observed in some oat-maize hybrids. Upon selfing, partial hybrids with one or two maize chromosomes showed nearly complete transmission of the maize chromosome to give self-fertile maize chromosome addition oat plants. Fertile lines were recovered that contained an added maize chromosome or chromosome pair representing six of the ten maize chromosomes. Four independently-derived disomic maize chromosome addition lines contained chromosome 4, one line carried chromosome 7, two lines had chromosome 9, one had chromosome 2, and one had chromosome 3. One maize chromosome 8 monosomic addition line was also identified. In addition, we identified a double disomic addition line containing both maize chromosomes 4 and 7. Phenotypic effects in these plants range from slight variations from normal oat growth rate and morphology in the maize chromosome 2, 4, 8, and 9 addition lines to much reduced growth in the maize chromosome 7 addition and abnormal stem and panicle morphology in the maize chromosome 3 addition line. Fertility in general remains high with full transmission of the added maize chromosome pair to the next generation.

Karyotypically stable oat-maize derivatives including self-fertile maize chromosome addition oat plants represent unique materials which could be a source for gene transfer from maize to oat. These addition lines, which are grown isolated from our standard genetic and breeding materials to prevent uncharacterized transfers, are being currently evaluated for disease response reactions as well as changes in morphology and growth parameters. Also, efforts are underway where maize lines carrying *Mu* transposons have been crossed to oat as an attempt to transfer maize transposons to oat (Walker et al. 1996).

The availability of these unique oat lines with added maize chromosomes creates novel opportunities to also exploit them in gene mapping. For example, any previously unmapped sequence from maize could presumably be located to its chromosome by simply hybridizing the sequence to a blot made with DNA from a series of chromosome addition lines if that sequence is either maize-specific or shows a banding pattern distinguishable from oat. In addition, DNA libraries for specific maize chromosomes might be produced from these addition lines by physically separating maize from oat chromosomes by size using flow cytometric chromosome sorting, chromosome microdissection, or genomic subtraction techniques. Undoubtedly, these materials will be of use in a wide array of other studies.

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Verification of quantitative trait loci for grain yield in the 'Steptoe'/'Morex' barley cross. I. ROMAGOSA, Centre UDL-IRTA, A. Rovira Roure 177, 25196 Lleida, Spain. F. HAN, S.E. ULLRICH, Dep. of Crop and Soil Sciences. Washington State Univ., Pullman, WA 99164-6420, USA. P.M. HAYES, Dep. of Crop and Soil Science, Oregon State Univ., Corvallis, OR 97331, USA. D.M. WESENBERG, USDA-ARS, P.O.Box 307, Aberdeen, ID 83210, USA. The NORTH AMERICAN BARLEY GENOME MAPPING PROJECT.

Introduction. In a recent study (Romagosa et al., 1996), grain yield data of 150 barley doubled haploid lines (DHL) derived from the 'Steptoe' x 'Morex' (S/M) cross, grown at 16 environments was analyzed for quantitative trait locus (QTL) x environment interaction. Four regions of the genome, on chromosomes 2, 3, 6 and 7, were found responsible for most of the differential genotypic expression across environments. The QTLs were identified using additive main effect and multiplicative interaction genotypic scores (Gauch, 1992) as phenotypic data. They were resolved at chromosomal regions that were also identified using MQTL (Tinker and Mather, 1995a, b). They accounted for approximately 50% of the genotypic main effect and 30% of the genotype x environment interaction (GE) sums of squares. The parallel use of classification (cluster analysis of environments) and ordination (principal component analysis of GE matrix) allowed summarization of most variation in the GE matrix in just a few dimensions, specifically four QTLs showing differential adaptation to two main clusters of environments, each with two subgroups. Using an independent set of DHLs produced from the S/M cross, not used in the mapping process, the next objective of this work was to carry out a preliminary verification of these QTLs for yield.

The S/M cross was made as a compromise between the need for adequate DNA-level polymorphism for linkage mapping and the need to generate meaningful QTL information. However, some DHLs produced showed wide adaptation and outyielded Steptoe, the high yielding parent. Thus, molecular marker assisted selection (MMAS) could be of direct use in breeding within this germplasm pool. Multilocation testing of lines selected based on their genotype for the markers, should render the highest probability of recovering superior genotypes, and would limit the number of entries in the trials. Thus, a related objective was to assess the value of these four QTLs for MMAS, following this testing scheme.

Materials and Methods. 92 DHLs derived from the S/M cross that were not used in the original mapping efforts and the two parents were planted and yield data collected in 1995 in Pullman, WA (WA-95) and Klamath Falls, OR (OR-95), according to a randomized block design with three replications. Based on GE analysis, using grain yield data from the original mapping population, these two sites were expected to be quite different, since they belonged to different clusters of environments. A third site in Aberdeen, ID could not be harvested. The genotype of these 92 DHLs was determined for the eight flanking markers of the four yield QTLs (Table 1) defined in a previous paper (Romagosa et al., 1996). A subset of 70 entries in which there were no crossovers between any of the flanking markers were used to validate the four yield QTLs in all subsequent analyses.

Results and Discussion. The average effects of the four proposed QTLs were statistically significant (Table 1) and together explained around 75 % of the differences among DHLs

(data not shown) at WA-95. At QTL1, the *S* allele was significantly superior to the *M* allele. On the contrary, the *M* allele was superior for the other three loci. These results fully confirm the role of these QTLs in determining grain yield. Results at OR-95 were not that clear, the QTLs explained 43 % of the genotypic differences. Only two QTLs produced significant differences. Surprisingly, that was not so for QTL1 (Table 1), which corresponded to the only genetic effect that was found for average grain yield across environments in the original mapping population. However, although not significant, DHLs carrying the *S* allele had a higher yield mean than those carrying the *M* allele. A significant crossover QTL x E interaction was found for QTL4 only. The *M* allele was superior at WA-95, whereas lines with the *S* allele significantly outyielded the others at OR-95.

Table 1. Average grain yield of DHLs carrying the Steptoe (S) or Morex (M) allele at each of four QTL. Yields for each QTL at each site followed by different letters are different at $p \leq 0.05$, according to the analyses of variance.

SITE	ALLEL	GRAIN YIELD (g/m^2)			
		QTL1‡ Chromosome 3 ABG399- BCD828	QTL2 Chromosome 2 ABC156A- ABG358	QTL3 Chromosome 6 CDO497- BCD340E	QTL4 Chromosome 7 ABC324- ABC302
WA-95	<i>M</i>	439 b	514 a	490 a	520 a
	<i>S</i>	528 a	452 b	477 b	447 b
OR-95	<i>M</i>	586 a	648 a	605 a	542 b
	<i>S</i>	596 a	535 b	578 a	641 a

‡ Each QTL is followed by the chromosome on which it was located and its flanking markers.

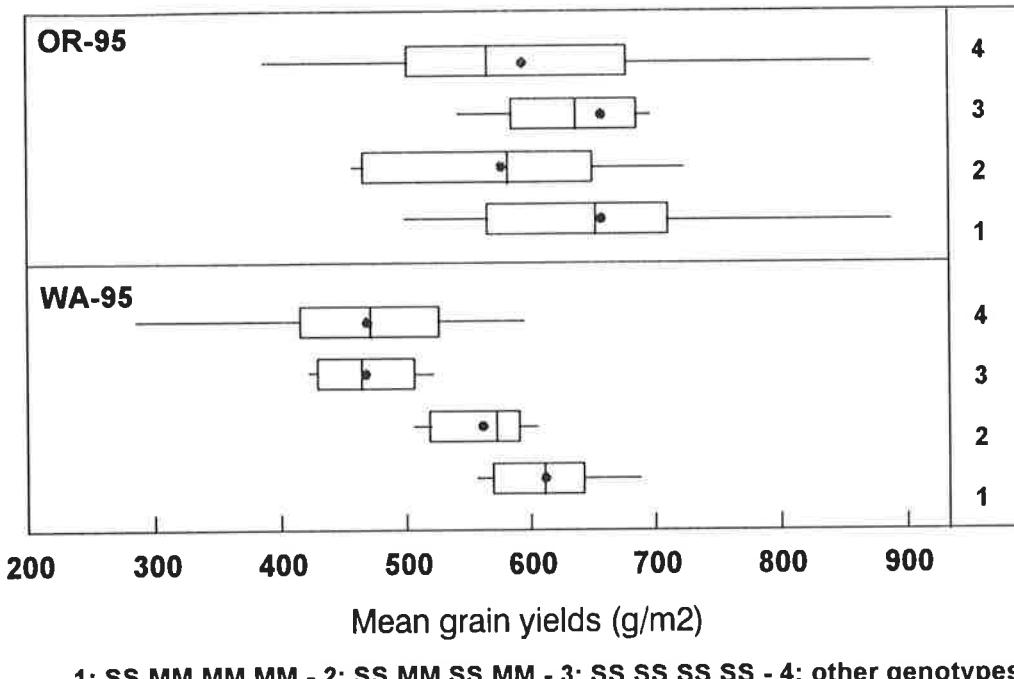
The analysis of grain yields in the original mapping population at 16 sites suggested that most superior lines could be identified within certain genotypes. The simplest strategy would be to genotype the flanking markers of the four identified QTL regions, then to select genotypes with the *SSMMMMMM*, *SSMMSSMM* or *SSSSSSSS* constitution (Romagosa et al. 1996). To determine if the probability of recovering superior genotypes was higher for these genetic constitutions, a relative comparison of grain yield of lines with these genotypes, versus the others in the trial was undertaken. Such comparison is shown in Figure 1, which shows independent box plots (Tukey, 1977) for each genetic combination independently for the two trials. DHLs with the *SS MM MM MM* genotype were clearly superior at WA-95, where most lines with this genotype were the top entries in the trial (bottom of Figure 1). The superiority of this genotype was not so clear at OR-95, as expected from Table 1. However, at this site the top yielding line did belong to this genotype and lines with this genotype also showed the largest grain yield mean and median (top of Figure 1).

Overall, a preliminary verification of these four QTLs was successfully carried out. At present, a more thorough assessment of adaptation of this second set of S/M DHLs, using a wider range of environments is underway. A final validation of the use of these yield QTLs

in MMAS should wait until direct selection within a segregating generation from this cross proves successful.

Figure 1. Relative performance of DHLs with the *SSMMMMMM*, *SSMMSSMM* and *SSSSSSSS* genotypes at two sites. Left and right edges of boxes represent the 25% and 75% percentiles, respectively; the central vertical line shows the median; the central dot the mean; the horizontal lines extend to the minimum and maximum yield values.

Molecular Marker Assisted Selection for Yield



1: SS MM MM MM - 2: SS MM SS MM - 3: SS SS SS SS - 4: other genotypes

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Comparison of heading date QTLs on chromosome 7 across 4 barley crosses. W.T.B. THOMAS, W. POWELL, R. WAUGH, E. BAIRD, A. BOOTH, P. LAWRENCE, B. HARROWER and N. BONAR, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5 DA, UK and P.M. HAYES, Dept of Crop & Soil Science, Oregon State University, Corvallis, OR 97331, USA.

Introduction. With the advent of molecular markers, loci controlling a number of economically important traits have been located in several different barley crosses - Steptoe x Morex (Hayes *et al.* 1993b), Igri x Triumph (Laurie *et al.* 1994), Dicktoo x Morex (Pan *et al.* 1994), Igri x Danilo (Backes *et al.* (1995), Blenheim x E224/3 (Thomas *et al.* 1995), Tystofte Prentice x Vogelsanger Gold (Kjaer *et al.* 1995) and Harrington x TR306 (Tinker *et al.* 1996). Heading date (HD) has been the most widely reported trait to date and is a suitable trait to compare across different studies as it has generally been measured on the same scale. HD is also an important character in barley as it is a major factor influencing adaptation to environment.

Whilst QTLs for HD have been reported by all the above studies, comparison of chromosomal locations across all the crosses is difficult due to the absence of common segregating markers. Most of the markers used to construct the above maps were RFLPs which, whilst they make comparison relatively easy, are time consuming and generally less informative than PCR based methods. Waugh *et al* (1996) have outlined the advantages of AFLPs in rapidly constructing genetic maps and demonstrated their transferability across different crosses. In this report, we demonstrate that this transferability can be used to facilitate QTL comparisons, using HD as an example.

Materials and Methods. AFLP markers were scored on the Blenheim x E224/3, Dicktoo x Morex and Igri x Franka DH populations (Waugh *et al.* 1996) and were added to existing information (Thomas *et al.* 1995; Pan *et al.* 1994; Graner *et al.* 1994) to construct maps using Joinmap 2.0 (Stam & van Ooijen, 1995). Map data for Steptoe x Morex were obtained from graingenes (<http://wheat.pw.usda.gov>), for Harrington x TR306 from Tinker *et al.* (1996), for Igri x Triumph from Laurie *et al.* (1994). HD loci were obtained from Laurie *et al.* (1994), Hayes *et al.* (1993), Tinker *et al.* (1996), Backes *et al* (1995) and Kjaer *et al.* (1995). New data, obtained from all 69 DH's grown together with parents and a control in a glasshouse experiment in the spring of 1993, was used for Blenheim x E224/3 in this study. The data was. Two treatments were applied to the experiment. The germinated seeds were vernalised at 4°C for 5 weeks in one but not in the other. There were 4 replicates, grown in a row and column design. HD was measured in the standard manner and the means of each treatment were scanned for QTLs using MQTL (Tinker and Mather, 1995) with 1000 permutations to determine thresholds for a genome wide 5% error rate. Both simple interval mapping (SIM) and simplified composite interval mapping (sCIM) (Tinker and Mather, 1995) were carried out. In addition MQTL was used to reanalyse the field HD data from the Dicktoo x Morex DH's of Pan *et al.* (1994) using the additional AFLP markers reported by Waugh *et al.* (1996).

Results and Discussion. MQTL analysis of the data revealed 3 primary QTLs, one on chromosome 2 in the region of PSR687, one on chromosome 3 in the region of *denso* and one on chromosome 7 in the region of OPA-19. In addition, 3 secondary QTLs were revealed by the sCIM analysis. One was at the end of the long arm of chromosome 1, 1 on

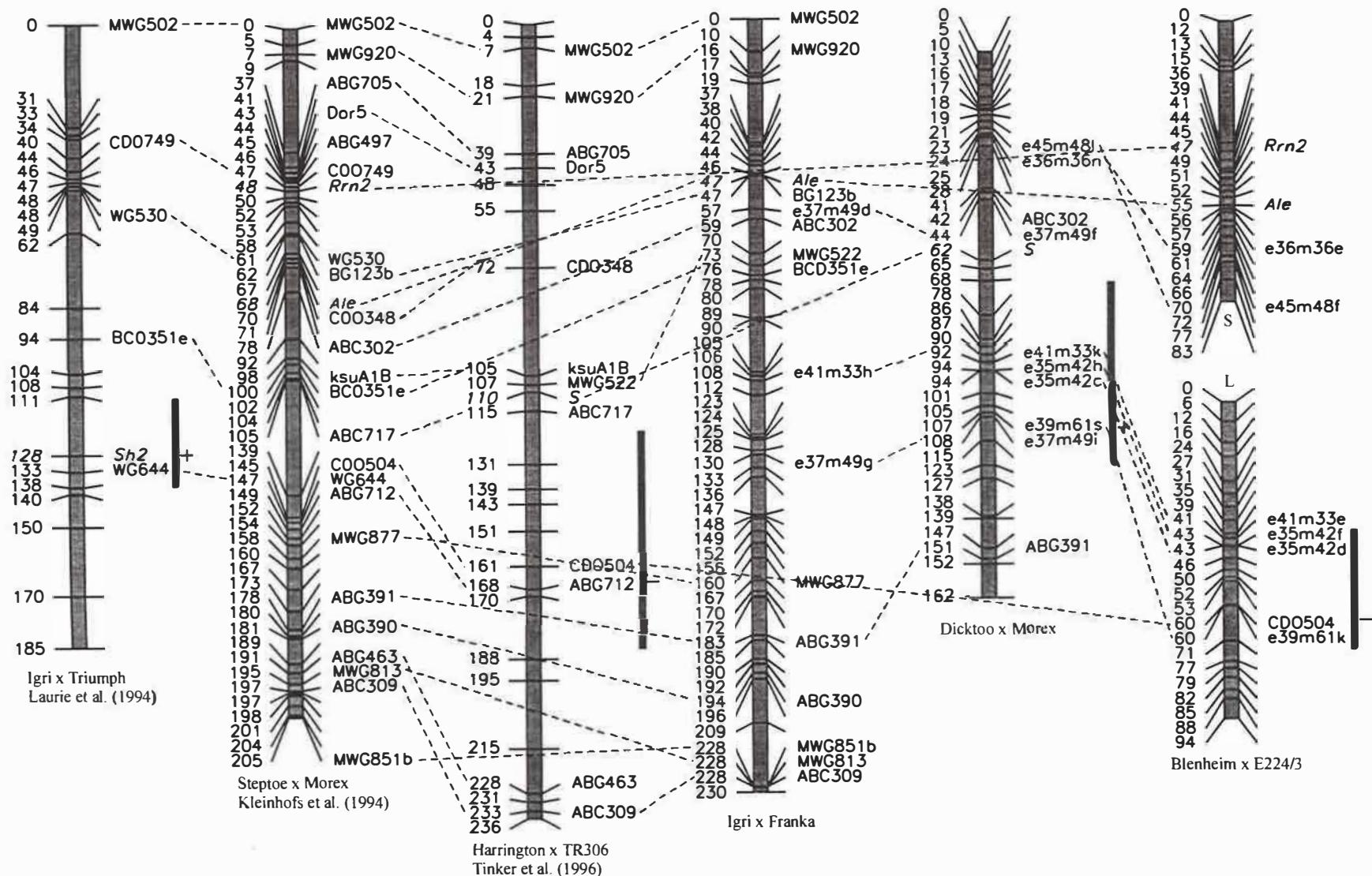


Figure 1. Chromosome 7 maps of 6 barley crosses. Dashed lines indicate the positions of markers segregating in more than 1 cross. Black bars show the position of HD QTLs with + and - indicating the effect of alleles from the first named parent

the short arm of chromosome 2 and the other in the region of rubisco activase on chromosome 4. The QTLs accounted for 68% of the phenotypic variation in HD with no evidence of QTL x Environment interactions, although vernalisation increased the magnitude of the primary QTLs on chromosome 2 and 7. The Blenheim alleles on chromosome 2 and 7 had the largest effect on HD of +1.8 and -1.7 days respectively. However, the *denso* locus accounted for the greatest individual portion of phenotypic variation (23%) but had a slightly lesser mean effect of +1.6 days. The QTLs on chromosomes 2 and 7 accounted for 20 and 18% respectively of the phenotypic variation.

Apart from Steptoe x Morex and Tysofte Prentice x Vogelsanger Gold, previous QTL studies of HD in barley crosses revealed effects on chromosome 7, although the Igri x Danilo study finding was not confirmed by Mapmaker/QTL analysis (Backes *et al.* 1995). The effect in Igri x Triumph was ascribed to the vernalisation locus *Sh2* which acted with the *Sh* locus on chromosome 4 to confer a vernalisation requirement for Igri. Dicktoo is a winter barley but lacks a vernalisation requirement whereas Morex is a spring barley but Pan *et al.* (1994) consider that the HD QTL on chromosome 7 may correspond to an allele at *Sh2* as Takahashi and Yasuda reported *Sh2* as a multi-allelic locus associated with variation in winter growth habit. There are also HD QTLs from Harrington x TR306 and Blenheim x E224/3 located on the long arm of chromosome 7. That from Harrington x TR306 would appear to be in the same region as *Sh2* as its peak encompasses the region marked by CDO504 and ABG712 which, in Steptoe x Morex, are located close to WG644 (Figure 1). Laurie *et al.* (1994) report the most likely location of *Sh2* as being ~5cM from WG644. In addition, CDO504 is close to the QTL peak in Blenheim x E224/3 (Figure 1). However, there are no common RFLP markers to compare the locations of the QTL in Dicktoo x Morex with the other crosses.

A block of 4 AFLP markers appears to be common to both Dicktoo x Morex and Blenheim x E224/3 with e39m61s being at the peak for Dicktoo x Morex. This corresponds to e39m61k in Blenheim x E224/3, which covers the interval spanned by the QTL peak (Figure 1) so it would appear that HD effects in four different barley crosses are due to variation at the same or closely linked loci. AFLP's have been invaluable in reinforcing the findings for Blenheim x E224/3 and revealing the similarity for Dicktoo x Morex.

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Searching for QTL Epistasis. N.A. TINKER AND D.E. MATHER, Dept. of Plant Sci., McGill University, 21,111 Lakeshore, Ste-Anne-de-Bellevue, QC, H9X 3V9, CA

Introduction.

The effects of alleles at one quantitative trait locus (QTL) may depend on alleles at a second QTL. Awareness of this "QTL epistasis" could increase the efficiency of marker-assisted plant breeding. Epistasis among QTL has been detected in soybean^{2,3,6} and barley⁴ but little is known about power, precision, or accuracy of inferences about QTL epistasis. Here, we describe a simple procedure to search for QTL epistasis and we evaluate inferences about QTL epistasis in simulated barley data.

Methods.

Simulation. A hypothetical genome was simulated to emulate existing barley maps: 154 markers spaced at 10-cM intervals on seven 210-cM chromosomes. A QTL was located at cM position 105 (midway between two markers) on each of chromosomes 1 and 2. The QTL on chromosome 1 always had a strong additive effect. The other QTL had no net additive effect, but could influence the effect of the first QTL. All scenarios were designed to give the same amount of genetic variance, but effects were chosen (see Fig. 1) so that the proportion of genetic variance attributable to epistasis was 0% (absent), 2% (weak), 10% (moderate), or 50% (strong). Populations of $N = 150$ or 300 F_1 -derived doubled haploid (DH) progeny were generated. Random, normally distributed residual effects were added to quantitative trait phenotypes to give broad-sense heritabilities of 50%.

QTL Interval Mapping. The position of an "anchor locus" (QTL-A) was estimated using simple interval mapping by regression¹. Then, "QTL epistasis mapping" was performed: the estimated position of QTL-A was held constant and regression models were tested at other map positions to detect an interacting locus (QTL-B). A full model (F) contained coefficients for an intercept, additive effects of A and B , and their interaction (AB). A reduced model (R) did not contain AB . A test statistic (TS) = $N \cdot \ln(RSS_R / RSS_F)$ was computed from residual sums of squares (RSS) of these models. A threshold value of TS giving a 5% type-I error rate was derived by analyzing 1000 permutations of one simulated data set. This threshold was applied to the detection of significant AB interactions in 1000 additional simulations. The position of QTL-B was estimated as the map location on chromosome 2 that maximized TS . Effects of genotypes $aabb$, AAb , $aaBB$, and $AABB$ were estimated using linear combinations of the regression coefficients.

Results and Discussion.

The Method. The problem with searching for QTL epistasis is that any two genomic positions could conceivably interact, presenting an unreasonable number of possibilities to consider. The solution employed here was to limit the search to interactions where one QTL has a significant additive effect. This is similar to the solution employed by

Lark *et al.*^{2,3} except that it does not require visual inspection of cumulative frequency histograms. Instead, interactions are detected based on linear regression models.

Interpretation. Because of its large additive effect, the QTL on chromosome 1 (QTL-A) was always detected. Using QTL-A as an anchor locus, the qualities of statistical inferences about QTL-B were explored (Fig. 1). Power indicates how often QTL-B was detected in repeated experiments. When epistasis was present, power for detecting QTL-B ranged from 0.046 to 1, increasing with larger populations and stronger interactions. Accuracy is gauged by how close an average estimate was to the value of the simulated parameter. Both the position of QTL-B and the effects of the genotypes were estimated accurately. Precision is measured by lack of variation among estimates of the same parameter. The position of QTL-B was estimated more precisely with stronger epistasis or with larger populations. The effects of genotypes were estimated more precisely in larger populations but the precision of estimating genotypes was not affected by different strengths of epistasis.

Error Control. Threshold values of TS derived by permutation were approximately 13. With no epistasis, the frequency of QTL detection on chromosome 2 was 0.003 (Fig. 1), equivalent to a genome-wide type-I error rate of 2.1%. Thus, error control was slightly better than intended. A TS value of 13 is approximately equal to an LOD score of 2.8¹. Thus, error control was achieved using thresholds that are typical for interval mapping in a genome of this size. For new data, thresholds should be derived through permutation whenever it is uncertain that assumptions for regression are met. If many scans are performed (e.g. for other anchor loci or for additional traits) the over-all type-I error rate will increase.

Implications. In all the scenarios simulated here (Fig. 1), QTL-B had no net additive effect. With traditional interval mapping, QTL-B would not be detected, so the superior genotype *AAbb* would not be distinguished from the less optimal *AABB*. With QTL epistasis mapping, weak epistasis was seldom detected and strong epistasis was almost always detected. For moderate epistasis, the power of detection was high (90%) in populations of 300 DH lines, but comparatively low (50%) in populations of 150 DH lines.

Failure to detect weak epistasis may not be a serious problem. Weak interactions are probably common, but they may be too small to offer any meaningful interpretation and they would be unlikely to alter decisions made in marker-assisted breeding. Moderate and strong interactions are of greater interest. It would be valuable to know about loci with little or no additive effect that are involved in moderate to strong interactions. If such loci exist, information about their positions and effects could influence our understanding of the genetic control of quantitative traits. This information could also affect decisions made in marker-assisted breeding. The results presented here demonstrate that such loci can be readily detected when their epistatic effects are strong, but that detection of loci with moderate epistasis may require large mapping populations.

Applications. MQTL⁵, the software used to perform these analyses, is available by internet at "<http://gnome.agrenv.mcgill.ca/tinker/mqtl.htm>". Using MQTL, we have

searched for loci that interact with primary QTL in two well known barley DH populations: Harrington/TR306 and Steptoe/Morex. Although these data provide evidence for a large number of QTL with additive effects, few instances of QTL epistasis were detected. As suggested by the simulations, such populations may be inadequate for detecting weak to moderate epistasis. However, these methods may be useful for detecting QTL epistasis for other traits or other populations.

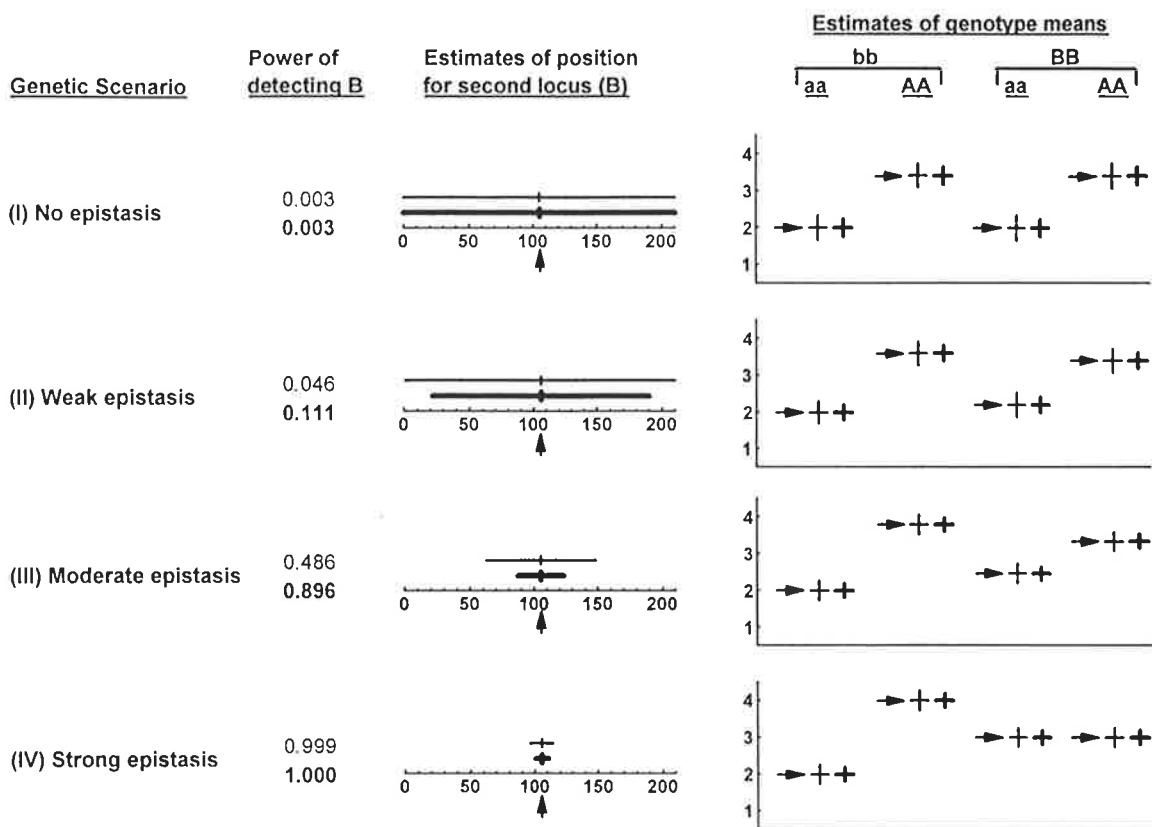


Figure 1. Analysis of QTL epistasis in 1000 simulated doubled-haploid data-sets for each of four genetic scenarios (I to IV) and two sizes of population: $N=150$ (normal type) and $N=300$ (bold). Simulated positions of QTL-B and simulated effects of genotypes $aabb$, $AAbb$, $bbBB$, and $AABB$ are indicated by arrows. The position of QTL-A was in the centre of another linkage group (not shown). Power of detecting B was estimated as the frequency of significant peaks on the chromosome containing B. Error bars show means \pm two standard deviations of estimated parameters.

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The actively transcribed barley DNA repeat similar to the *Arabidopsis thaliana* (L.) retrotransposon-like element. O.U. URBANOVICH and N.A. KARTEL, Institute of Genetics and Cytology BAS, 27, F.Skaryna St., Minsk, 220072, Belarus.

Introduction. Repetitive DNA represents a large proportion of most of the higher plant genomes (Flavel 1980) and may constitute about 90% of the total DNA in species with large genomes including crops. Repetitive DNA sequences constitute up to 70% of barley genome (Rimpau et al. 1980). They are of great importance for organization and functioning of higher plant genomes (Kreis et al. 1985). Besides, quite a number of repetitive sequences of eucaryotes is attributed to mobile elements, in particular to such as transposons and retrotransposons being of particular importance for species evolution. Recently retroelements were revealed in genome of many higher plants including cereals (Monte et al. 1995; Moore et al. 1991). In our experiments we cloned the actively transcribed repetitive element of barley genome by hybridization with cDNA obtained from poly(A)+ RNA. This repeat was found to be similar to the retrotransposone-like element of arabidopsis.

Material and methods. Seeds of species and varieties of barley, rye and wheat were obtained from the collection of our Institute and John Innes Centre (Norwich, UK). Plant DNA was prepared from nuclear pellet and purified by the modified method (Ermak et al. 1989). Repetitive sequence of barley was produced by cDNA library and inserted into pBR322 plasmid along EcoRI site (Prosnyak et al. 1985). DNA sequences were determined by the chemical degradation method (Maxam and Gilbert 1980) after subcloning in pUS 18. Isolation of plasmid DNA, separation of DNA in agarose gel and Southern hybridization were carried out using conventional methods (Maniatis et al. 1982). The following primers were synthesized for the PCR analysis : CTTCAT-TGAGCTTGCGATATG (3785-3407 bp) and GGAAGCCACAAAGCTCTTAGA (4191-4211 bp). Amplification reaction was conducted according to the scheme: 45 cycles - 94°C - 1min, 55°C - 2 min, 72°C - 2 min.

Results. We have isolated a highly repetitive DNA sequence from barley genome and have studied its molecular organization, nucleotide sequence and distribution in genomes of some representatives of Triticeae tribe. This sequence was called BRS1 (barley repetitive sequence). Its total length makes up 5052 bp. After sequencing BRS1 was analysed by the package of programs DNASIS and PC/GENE. An open reading frame for amino acid sequences in the range of 3604-4615 bp was detected inside of this sequence (Fig. 1). The open reading frame encodes sequence for 337 amino acids. The rest of BRS1 contains a set of terminating codons that makes coding of any important amino acid sequence practically impossible. BRS1 was divided into smaller fragments by HindIII. These fragments were hybridized with restrictionase-treated DNA of barley. Southern hybridization has shown that the site incorporating the reading frame is highly repetitive. The other part BRS1 displays a weak signal of hybridization. The comparison of the sequenced repeat with EMBL database has revealed that the studied barley sequence in the open reading frame is identical by 30% in 289 aa with retrotransposon-like element of *Arabidopsis thaliana* (L.). Taking into account such a high degree of homology the cloned repetitive sequence of barley DNA was assumed to be attributed to retrotransposon-like elements. The method of

polymerase chain reaction was used for getting information about the structure of BRS1 part, homologous to retrotransposon-like element of arabidopsis. It was shown that the amplified fragment 428 bp in genome of different barley varieties didn't undergo changes of the insertion or deletion type. The region located in the open reading frame is stable inherited in all investigated varieties of different origin. The Southern hybridization method has revealed that there are DNA sequences homologous to BRS1, in genome of rye, wheat and barley wild species. The observed hybridized bands are characteristic of every species considered displaying a high degree of interspecific polymorphism.

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MNENTKLCDFSNTNNNDFISTPIAPATNAESYEINAALLNLVM  
KEQFSGLPSEDVASHLNTFIELCDMQKKKDVDNDVIKLKLFPF  
SLRDRAKTWFSSLPKSSIDSWDKCKDAYISKYFPPAKIISLRN  
DIMNFKQLDHEHVAQAWERMKLMIRNCPANGLSLWMIIQIFYA  
GLNFASRNILDSATGGTFMEITLGEATKLLDNIMTNYSQWHTE  
RSPTSKKVHVIEEINSLSKMDELMNLVASRSAPLDPNMDPLS  
SLIESSNASLEVNFGRRNNFGNNNAFRGNYVPRPFPSSNNF  
GNSYNNTHGYNRLPSDLESNIKKSSSTLKRFMSMRP
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Figure 1. BRS1 open reading frame for amino acid sequences .

Discussion. At present quite a number of transposable elements including such as retrotransposon and retropotransposon-like elements was detected in genome of higher plants. Some of them such as BIS 1 and BARE-1 elements (Moore et.al. 1991; Manninen and Schulman 1993) were found in the repetitive sequences of barley DNA. Thanks to their properties such elements can be accumulated in a great number of copies and contain a fraction of host genome DNA, for instance, LINE1 elements (Hutchison et. al. 1989). And even in genomes where active transposable elements were not revealed, traces of ancient transposition in the form of direct target repeats flanking dispersed repetitive sequences are sometimes observed. Thus, transposable elements exert a great influence on evolution of repetitive sequences. Available results confirm the assumption in many cases that most of repetitive sequences are transposable elements or their degenerated descendants. Probably an ancestor of the DNA sequence cloned by us was retrotransposon that in course of evolutionary processes had undergone mutations and/or other changes, had lost its ability for transposition and is inherited by the genome as a part of repetitive sequences. The fact that BRS1 is represented by the same number of copies in genome of barley varieties of different origin, is stable inherited and doesn't show intraspecific polymorphism points to that it is not an active transposable element. BRS1 conservatism revealed within a species can be accounted for by the fact that transcription active sequences are under stricter control of natural selection than noncoding DNA sequences. On the other side BRS1 displays a high degree of interspecific polymorphism among species of Triticeae tribe. These results suppose that BRS1 amplification in genome has happened before divergence of rye, wheat and barley during evolution. Probably, retrotransposon from which BRS1 had descended was a part of ancestor genome of these species. It is very likely that BRS1 evolution is similar to WIS2-1A evolution

of wheat retrotransposon (Monte et.al. 1995). Conservatism of BRS1 structure makes it possible to use it for establishment of evolutionary relations between species of cereals family.

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Conservation of AFLP marker order in different barley populations.
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Introduction. The significance of Polymerase Chain Reaction (PCR) technology in the field of genome science is reflected in the development of numerous new approaches for the detection of polymorphism at the DNA level (RAPD – Williams *et al.*, 1990; AP-PCR – Welsh *et al.*, 1991; RAMP – Zietkiewicz *et al.*, 1994; DAF – Caetano-Anolles, 1993; SSR – Tautz *et al.*, 1988; CAPS – Konieczny & Ausubel, 1993 etc). The most recent and potentially one of the most powerful techniques described to date is that termed Amplified Fragment Length Polymorphism (AFLP – Zabeau & Vos, 1993; Vos *et al.*, 1995) developed by the company 'Keygene'. Recently, Becker *et al.* (1995) integrated 118 AFLP markers into a linkage map of barley obtained from a doubled haploid population derived from Procter x Nudinka. Their data demonstrated that AFLPs mapped to all regions of the barley genome (although they did exhibit some clustering) and were therefore useful as genome wide genetic markers in this species. AFLP is a particularly attractive technique because it is a generic technology which allows a large volume of information to be generated in a short space of time. However, one of the major criteria which has limited the application of other generic technologies is the dominant nature of the markers and this has resulted in an apparent lack of transferability between different populations. In this report we have addressed the transferability of AFLPs in three previously mapped doubled haploid barley populations. Based on the results from this study we have then used homologous AFLP amplicons as 'framework' markers in a previously unmapped barley population. The results suggest that AFLP markers can be used effectively to align linkage maps from different crosses.

Materials and Methods. Four doubled haploid barley populations extracted from the F1s of crosses between Blenheim x E224/3 (BxE; Thomas *et al.*, 1995), Igri x Franka (IxF; Graner *et al.*, 1991), Dictoo x Morex (DxM; Hayes *et al.*, 1993) and Lina x *H. spontaneum* (LxS; Waugh *et al.*, in prep) were used in this study. DNA was isolated from seedlings according to the method of Saghai-Marcoof *et al.* (1984). The protocol adopted for the generation of AFLPs was essentially the same as that described by Vos *et al.* (1995) except that 'zero base extension' primers were used for the generation of preamplified template DNA. Linkage analysis was performed using Joinmap V. 1.4 (Stam *et al.*, 1993).

Results and Discussion. AFLP markers were generated from all DH individuals and parental lines initially using *Eco*RI/*Mse*I and latterly *Pst*I/*Mse*I digested template DNA. The level of polymorphism ranged from 8% for the BxE population to 27% for LxS. Segregation data for c. 360, 300, 202 and 650 markers was scored for the BxE, DxM, IxF and LxS populations respectively. The AFLP segregation data was then added to the existing RFLP and RAPD data for the BxE, DxM and IxF populations to determine map location and the effect of adding these markers to map length. Figure 1a shows the AFLP markers integrated into the RFLP linkage data of Chr.1H for the four populations. Marker order was maintained after the addition of the AFLPs with one exception: the order of [Ris 13, MWG506] and MWG800 was inverted in the IxF population with the map interval increasing from 1.6–2.6 cM.

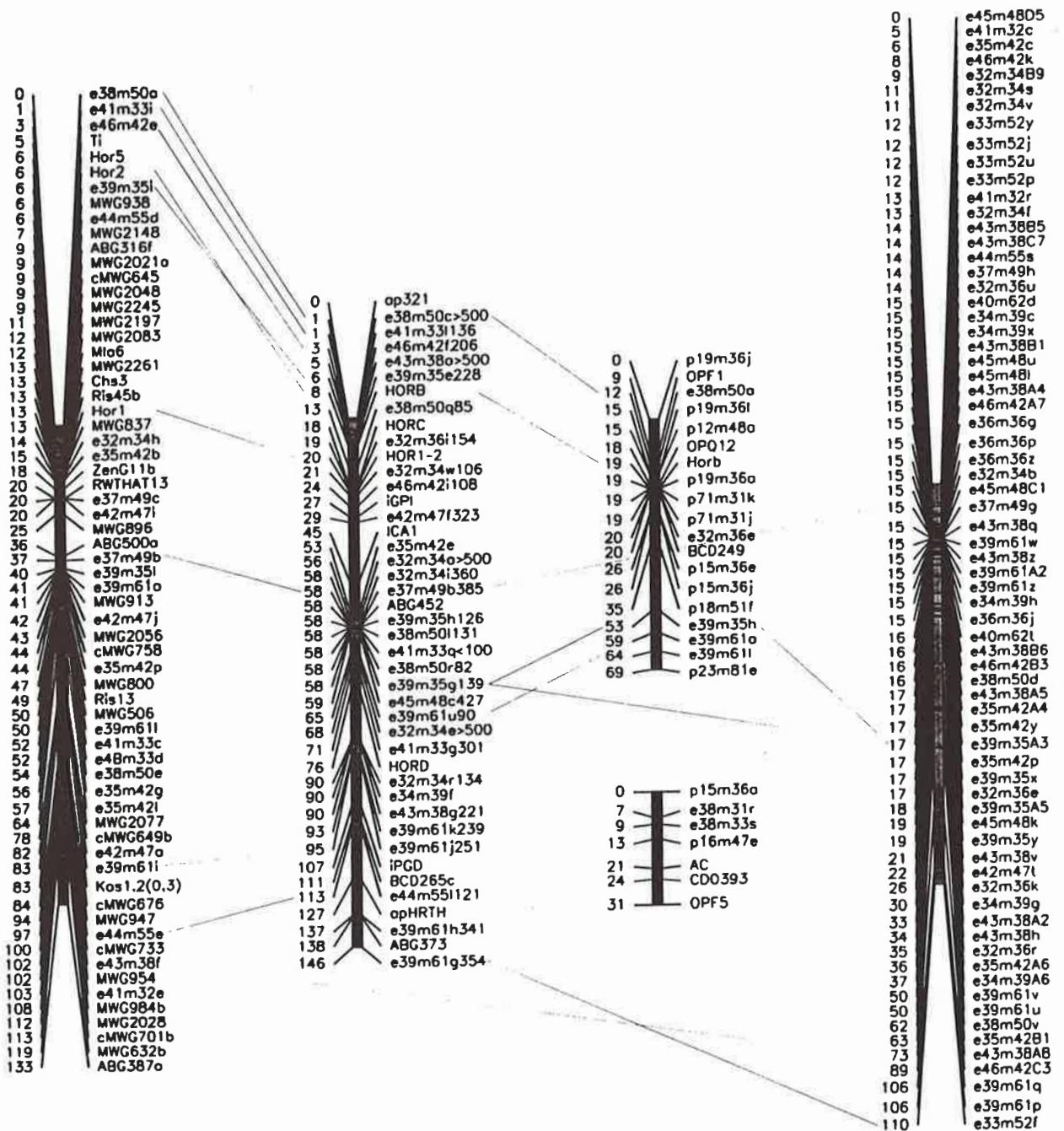


Figure 1. Alignment of AFLPs mapping to chromosome 1H between different barley populations.

These markers flank the centromere in 1H. The AFLPs were distributed throughout the linkage groups but did exhibit some apparent clustering outside the regions previously heavily mapped with RFLPs. In addition, they are largely centromeric in the LxS population. No significant increases in map length were detected except when markers were added to the ends of existing linkage groups. These results contrast to those of Becker *et al.* (1995) who found significant increases in map length after adding a relatively small number of AFLPs.

To determine whether apparently homologous AFLP fragments mapped to the same region of the barley genome, the size of segregating bands was determined and homologous segregating markers identified in the three populations. In each case the relative map position of homologous fragments was conserved (see Figure 1). This suggests that accurately sized, scored and documented AFLPs could be used as anchor markers in previously unmapped populations (as opposed to RFLPs). To test this hypothesis AFLP segregation data for the LxS population was examined. First, markers which were common to LxS and at least one of the other three populations were identified and these were examined for linkage. The same linkage order was obtained with these as would have been inferred from the other maps. These markers were then used to form a framework upon which other linked markers in the LxS population could be assembled (Figure 1). Seventy-one of the 650 AFLPs scored in LxS grouped with these other markers (at LOD = 9). Further examination suggested that this group corresponds to the long arm, centromere and centromeric portion of the short arm of 1H. No robust linkages were found with what could be assumed to be the distal portion of 1HS at this stringency.

The implications of these experiments are that AFLP amplicons can be used within cultivated barley as 'framework markers' in previously unmapped populations and may in many cases replace the need to map known RFLPs for assigning linkage groups to chromosomes. This will have a major effect on the speed with which new populations can be mapped and expedite a wide range of studies in barley genetics. It is however imperative that different labs exploiting this new and powerful technology accurately document the size and primer combination origin of mapped AFLP amplicons.

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