V International Oat Conference

&

VII International Barley Genetics Symposium

Proceedings

Dr. Vern D. BURROWS
AGRICULTURE & AGRI-FOOD CANADA
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Editors:
Al Slinkard
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POSTER SESSIONS Volume 2

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Through the generous support of these organizations these invaluable reference texts are provided to all conference registrants as part of their registration package and will be available to others to purchase at minimal cost. The collaborative support of the Quaker Oats Company of Canada and the Brewing and Malting Barley Research Institute in assisting the conference in this specific effort and for many years of long-term support of oat and barley research and development activity at the University of Saskatchewan and elsewhere in Canada is gratefully acknowledged.

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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
Program Committee
& Proceedings Editors

Contents

Section IX: Biotechnology (c) – Transformation

Transformation of barley with a stilbene synthase gene in order to produce plants with an increased fungal resistance

G. Brauer, A. Jähne-Gärtner, D. Becker, R. Brettschneider, H. Lörz

Universität Hamburg, Institut für Allgemeine Botanik, Angewandte Molekularbiologie der Pflanzen II, Ohnhorststr.18, D-22609 Hamburg

Introduction:

For plant breeding the transfer of resistance against fungal deseases and also against bacterial and viral infections of major importance. Diseases of crop plants, especially those of cereals, cause worldwide dramatic yield losses. In order to prevent these massive inset of pesticides or fungicides is necessary. Therefore, transgenic plants with an increased disease resistance are very attractive not only for breeding purposes but also because of environmental reasons.

The aim of this project is the transformation of barley and wheat with a gene from *Vitis vinifera* coding for a **stilbene synthase** in order to produce plants with an increased resistance against fungal attack. The stilbene synthase genes code for enzymes which synthesize stilbenes e.g. **resveratrol**. The phenolic substance resveratrol is a phytoalexin and was detected in several unrelated plant species such as grapevine, peanut or pines (Liswidowat et al. 1991). The synthesis of resveratrol and other hydroxystilbenes with an antifungal activity can also be induced by UV-light, wounding and other stress-factors. The precursor molecules for the formation of resveratrol are **malonyl-CoA** and **p-coumaroyl-CoA**. Both molecules are present in all plants and used as substrates by the chalcone synthase. Therefore, the stilbene synthase gene can be used in transformation experiments without any restrictions due to substrate availability.

Recently, it has been shown that the transformation of tobacco with a stilbene synthase gene enhances disease resistance in transgenic tobacco plants (Hain et al. 1993).

Material and Methods

GUS activity was determined histochemically as described by McCabe et al. (1988).

For the isolation, transformation and culture of the microspores the system of Jähne et al. (1994) was used.

For Southern-blot analysis the protocol from Becker et al. (1994) was used.

Plasmid construction:

For stable transformation experiments the constructs pGBI and pGBII were used.

The **pGBI**-construct contains the *bar* gene under control of the 35S promoter and the *stilbene synthase* gene under control of the stilbene synthase promoter with the 4-fold enhancer of the 35S promoter (Fig.1.). The **pGBII**-construct contains only the stilbene synthase gene under control of the stilbene synthase promoter (Fig.1.) and was used for co-transformation with the 35S bar-plasmid.

For transient transformation studies the construct pVst-EPG was used and compared with the plasmid pVstPG. The plasmid **pVstEPG** contains the VstI-promoter with the 4 times enhancer of the 35S promoter and the *gus* gene with the nos-terminator (Fig.1.).

The construct **pVstPG** contains the *gus* gene under control of the VstI-promoter and the nos-terminator (Fig.1.).

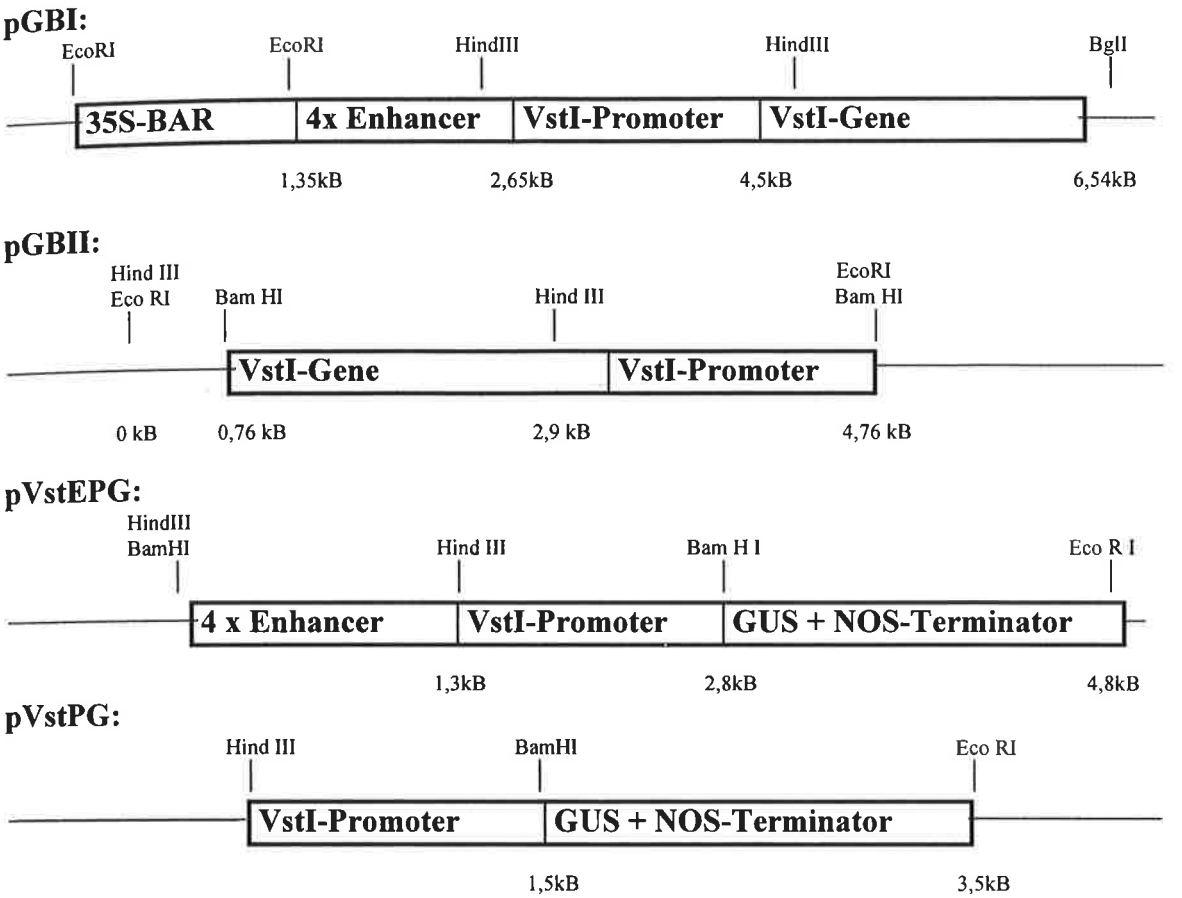

Fig 1.: Plasmid constructs for stable and transient transformation experiments

Results and Discussion:

The two promoter constructs pVstEPG and pVstPG were used for the first biolistic transformation experiments.Transient expression of the *gus* gene was analysed histochemically. For these experiments immature embryos were bombarded using a helium gas pressure of 1550 psi and 116µg of particles per bombardment. In these experiments it could be demonstrated, that both promoter-constructs are expressed in barley.For comparison of the two constructs pVstEPG and pVstPG the experiments were repeated 4 times and in each experiment 4 independent bombardments per construct with 10 embryos per petri dish were performed.

Table 1 presents the average number of transient transformation events per embryo of the different promoter constructs.

Average number of spots per embryo	
pVst-PG	**pVst-EPG**
4,8	27,3
7,0	28,4
0,6	34,3
7,25	19,35

Table 1.: Average number of transient transformation events by biolistic transformation of immature embryos with the contructs pVstPG and pVstEPG.

These results show clearly that the promoter with the enhancer-fragment (pVstEPG) gives higher number of transient GUS expression signals than the promoter without the enhancer-fragment.

Additionally, some immature embryos were bombarded with a promoter fusion containing the enhancer-fragment and the *gus* gene and were analysed histochemically, as well. With this construct no transient signals were detected. This result indicates that the enhancer element of the 35S-promoter has no promoter function, but the expression of the *gus* gene was much higher with the chimeric promoter fusion of the VstI promoter plus 35S enhancer elements.

For stable transformation experiments two plasmids were used: the chimeric plasmid pGBI and the plasmid pGBII in cotransformation with the 35S-bar plasmid. As target tissue barley microspores were used. This tissue has the advantage that homozygous plants can be produced in one step.
Putative transformants were screened for enzyme activity by spraying the whole plants with an aqueous solution of the herbicide Basta. Up to now five plants survived Basta spraying.
Three of this plants are containing the plasmid pGBI. Southern-blot analysis indicates the presence of the *bar* gene and the *Vst I* gene in all plants. Northern-blot analysis indicates the presence of the Vst I mRNA in two of this plants without previous induction of the promoter.
Furthermore, in two plants the integration of the transferred pGBII plasmid into the genome was determined by Southern blot analysis.

The next step will be the biochemical analysis of the transgenic plants and their progeny. The biological effects of the synthesis of resveratrol in barley on fungal pathogenesis will be analysed.

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A transient expression assay to evaluate the ability of introduced host response genes to confer disease resistance in cereals. W.R. BUSHNELL, R.W. GIROUX, A.J. NELSON, and L.J. SZABO, U.S. Dept. of Agriculture/Agricultural Research Service, Cereal Rust Laboratory, 1551 Lindig St., University of Minnesota, St. Paul, MN. 55108, U.S.A.

Introduction. Host response genes cloned from diseased plants often code for products that have antifungal activity or are involved in synthesis of antifungal compounds. Consequently, host response genes are candidates for introduction into transgenic cereals as a way to confer disease resistance. However, the generation of stably transformed cereals, to test effectiveness of introduced response genes, is a lengthy and costly process with the techniques currently available. To evaluate host response genes rapidly, we have developed a transient expression assay using barley coleoptile epidermis inoculated with *Blumeria (Erysiphe) graminis* f. sp. *hordei* (Fig.1; Nelson & Bushnell 1996, Transgene Res. in press). Individual response genes in combination with genes for anthocyanin are shot into the epidermis using microparticle bombardment. Red cells expressing the anthocyanin genes are then inoculated with spores of *B. graminis* to determine if the introduced response gene confers resistance to the pathogen. The method assumes that the introduced response gene has a high probability of being co-expressed with the anthocyanin genes. Presented here are positive results obtained in the assay for three host response genes: chitinase, ß-1,3-glucanase and thaumatin-like protein (*tlp1*).

Methods: Each experiment was done with 25 coleoptiles split to expose the inner adaxial epidermis and bombarded five at a time with DNA-coated gold microparticles using a high pressure particle delivery system (Dupont PDS-1000). Two plasmids were introduced simultaneously: pPHI687 with two genes (*C1* and *R'*) controlling anthocyanin production, and a second plasmid carrying the response gene to be tested. Both used a CaMV-35S/*Adh1*-intron promoter which gives constitutive expression. The response genes tested included: a rice chitinase gene (*RCH10*) supplied by C.Lamb; an alfalfa ß-1,3-glucanase gene(*Aglu1*) supplied by K. Orzech; and an oat thaumatin-like protein gene (*tlp1*) (from K .C. Lin). Coleoptiles were inoculated with the fungus two days after bombardment when 100-200 cells/experiment were expressing anthocyanin (Fig. 2). Two days later, infection rates were determined by counting the number of red cells challenged by one or more fungal appressoria and the number of challenged cells in which haustoria had been produced (Fig. 3). Data are presented for combined results of three or more experiments for each treatment.

Results. Infection rates of nonbombarded epidermal cells were about 60% as were infection rates for cells bombarded with gold particles without DNA or with only anthocyanin genes (data not shown). Thus, neither the bombardment procedures or the anthocyanin produced affected infection rates. In experiments with chitinase, ß-1,3-glucanse, and thaumatin-like protein genes, on the other hand, infection rates in red cells were 1/3 to 1/2 rates in neighboring non-red cells (Fig. 4). With the chitinase gene, protoplasts sometimes retracted from the appressorial wall, a phenomenon not seen with other treatments.

Coleoptiles are dissected from 4 day old seedlings and prepared for bombardment

↓

Coleoptiles are pre-incubated on 0.3M osmoticum 4-6h prior to bombardment

↓

Epidermal cells are bombarded with the anthocyanin reporter and host response gene constructs simultaneously

↓

Coleoptiles are incubated for 24h to allow constitutive expression of the anthocyanin reporter and host response gene

↓

Coleoptiles are incubated on a Ca^{2+}/MOPS buffer for 24h prior to inoculation with spores of the powdery mildew fungus

↓

Coleoptiles are inoculated in a settling tower to a density of 40-60 spores/ mm^2

↓

Coleoptiles are incubated for 24h to allow for fungal development

↓

Red cells and control cells are assayed for successful infections.

Fig. 1. Coleoptile transient expression assay

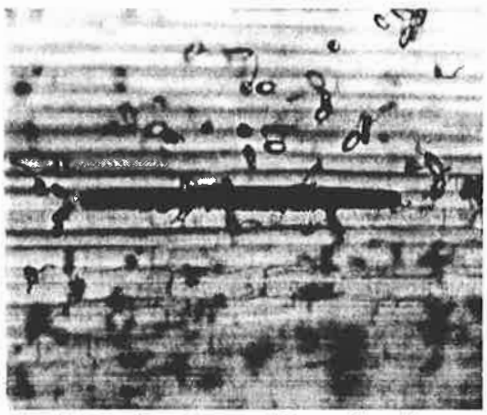

Fig.2. Red barley epidermal cell expressing anthocyanin, three days after particle bombardment and one day after tissue was inoculated with spores of *Blumeria (Erysiphe). graminis* f.sp. *hordei* .

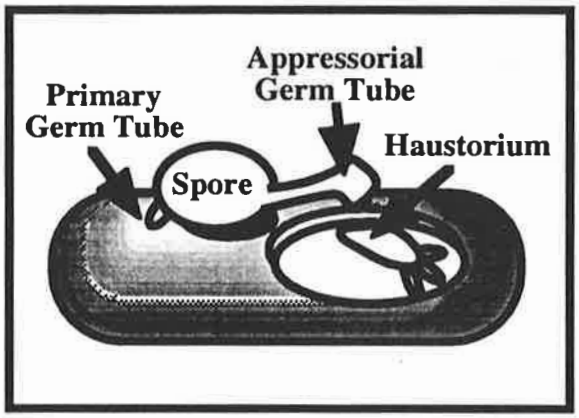

Fig. 3. Host response genes affect the development of haustoria in transiently-expressing coleoptile cells.

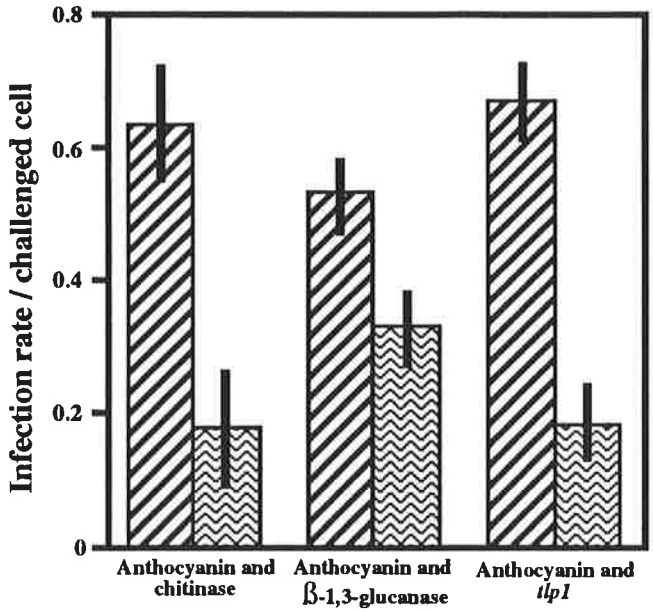

Fig. 4. Effects of transient gene expression of three host response genes on the rate of successful infection of barley coleoptile cells by the *Blumeria graminis* f.sp. *hordei* fungus. In all three treatments, the coleoptile cells were cotransfromed with both the anthocyanin construct and a host response gene (chitinase, ß-1,3-glucanase, or thaumatin-like protein (*tlp1*). The first bar in each pair represent the rates of infection in non-red cells which were considered as non-expressing control cells. The next bar in each pair represent the rates of infection in challenged red cells which were expressing the anthocyanin construct and had a high likelihood of also expressing the host response gene. Cells were considered to be challenged if one or more fungal appressoria were present on a particular cell. An infection was considered successful if the fungus formed a haustorium in the challenged cell.

Discussion. The results indicate that over-expression of the three genes tested, chitinase, ß-1,3-glucanase,and thaumatin-like protein, each significantly reduced the ability of the powdery mildew fungus to produce haustoria in plant cells. Thus, the transient expression assay indicates that all three genes have the potential for enhancing resistance to powdery mildew and probably also other diseases in transgenic cereals. Anthocyanin genes were useful as visible markers for transiently expressing epidermal cells in which rates of co-expression of the response genes were high enough to give markedly reduced rates of haustorium formation. The transient assay system will be useful in the following ways to evaluate genes for conferring disease resistance in transgenic plants: **1.** Host response genes, singly or in combination, can be tested rapidly for effectiveness .**2.** Genes for antifungal substances other than response genes can also be tested. **3.** Promoters for introduced genes can be evaluated. **4.** Signal sequences that target gene products for the vacuole or for excretion can be tested. **5.**Deletions or other mutations can be tested to determine location of sequences essential for activity against pathogens.

Transient expression of the *uidA* gene in barley microspores by Particle Inflow Gun (PIG) bombardment. A.M. CASTILLO, L. CISTUÉ, A. GALÁN and M.P. VALLÉS. Departamento de Genética y Producción Vegetal, Estación Experimental de Aula Dei, C.S.I.C., Apartado 202, Zaragoza, Spain.

Introduction. Anther and isolated microspore culture technology has been developed in our department to the stage where it is being used routinely for dihaploid production in a barley breeding program. The main objective in the breeding program is to release new varieties with improved yield potential, good grain quality and well adapted to our Spanish environments. Besides this routinely DH production for plant breeding purpose were are studying the induction of androgenesis in barley in order to improve the different steps of the anther and isolated microspore culture process. We are also interested in other potential benefit of these biotechnical procedures for the agronomic improvement of barley such the use of the microspores as targets for genetic transformation.

The transformation of microspores presents an alternative to solve the problems associated with current procedures. Microspores are unicellular explants with a high regeneration capacity via microspore-derived embryos and due to spontaneous chromosome doubling the regenerated plants are homozygous (Jähne et al., 1994).

In order to establish a simple method allowing the recovery of transgenic microspore-derived barley plants, we describe the optimization of physical and biological parameters for the efficient microprojectile-mediated DNA transfer and expression of GUS in microspore of barley (*Hordeum vulgare.* cv.Igri).

Materials and Methods. Anther and microspore culture were performed as described by Cistué et al. (1994, 1995). We are using the plasmid pAHC25 (Christensen et al., 1992). This plasmid contains the *uidA* gene and the selectable bar gene, both of them driven by the maize ubiquitin promoter, first exon and first intron.

For particle bombardment a flowing helium gun has been constructed based on the description of Finer et al. (1992). All plant material used for bombardment was suspended in culture medium and placed on a petri dish with a solid support. Plasmid was adsorbed onto gold particles according to Sanford et al. (1993). Different bombardment parameters were evaluated and fixed for the standard protocol: Baffle distance: 10 cm. Target-bombardment distance: 20 cm. Volume for shooting: 10 µl. For histochemical *in situ* assays the material was collected 48 hours of culture after bombardment.

Results and Discussion. Since factors as pressure, number of particles, osmotic pre-treatment, must be adapted to the properties of the bombarded tissue, first it was necessary to study which step of microspore culture procedure was the best to use as target for bombardment. Transient expression was obtained with microspore-derived embryos, freshly isolated microspores and pre-treated anthers.

When microspore-derived embryos were used, in some cases the whole structure showed GUS expression but mainly only one part of the embryo expressed the gene, that could give rise to quimeric plants. When the microspores were still inside the anther, we got transient expression in anther wall as well as in the microspores, but about 50 to 100% of the blue spots were localize on the anther wall (depending of the pressure used). The highest level of GUS expression was obtained when isolated microspores were used as target for bombardment.

From the different explants tested, we thought that isolated microspores could be the best target for bombardment, since the transient GUS expression was the highest and the proportion of viable microspores exposed in each bombardment was also higher than in anthers due to the microspore isolation procedure. In order to establish a standard protocol for microspores bombardment, the particle number and the bombardment pressure parameters were evaluated. Finally it was necessary to adopt a compromise between high transient expression and microspore damage produced using 6 to 7 bares of pressure and 200 mg of gold per shot.

The bombardment support was also evaluated. We tested combinations of four different solid supports (culture medium solidified with two concentrations of agarose and gelrite) an three media for suspension of microspores. Significant differences in the frequency of GUS-expressing microspores were founded. The combination Ficoll 200-FHG (Cistué et al., 1995) as suspension medium, and agarose 0,4% as solid support was shown to be the most efficient for GUS-expression.

As in the microspore culture technology there is a strong genotype effect, we are planning to check more varieties with different culture response in order to develop a standard protocol for the transformation of barley varieties cultivated in Spain.

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Development of a highly efficient in vitro regeneration system from leaf bases of oat and barley as a prerequisite for transformation experiments. C. Gless, H. Lörz & A. Jähne-Gärtner, Universität Hamburg, Institut für Allgemeine Botanik, AMP II, Ohnhorststr. 18, D-22609 Hamburg, Germany

Introduction. A prerequisite for the production of transgenic cereals has been the development of efficient *in vitro* culture systems from which fertile plants can be regenerated in a high frequency. Transgenic plants from all major cereals have been produced in the last years using various regeneration systems (for review see Jähne et al. 1995). However, there is only one report on transgenic oat plants, which have been regenerated after particle bombardment of suspension culture cells or immature-embryo-derived calli (Somers et al. 1992). The establishment of suspension cultures remains difficult as it is a very time-consuming and genotype-dependent process. Immature embryos are highly embryogenic explants but the growth of donor plants is labour-intensive and needs special conditions. Recently, efficient plant regeneration systems from leaf bases of oat have been developed (H. Chen et al. 1995, Z. Chen et al. 1995). Among the explants used for induction of regenerable cultures seedlings are the most easily available donor material, since the time- and space-consuming growth of mature and special growth conditions are not required. Here we report the establishment of an significantly improved plant regeneration system from oat and barley leaf bases. In several genotypes of oat more than 40 plants could be easily regenerated from one explant. This high regeneration potential makes oat leaf bases to a very attractive target for transformation experiments. In barley, regeneration from leaf bases could be achieved as well, but the regeneration frequencies were much lower.

Material and Methods.

Plant material: Different cultivars of oat (cv. Fuchs, Jumbo, Gramena, Bonus and Alfred) and barley (cv. Igri, Grit, Koral, Golden Promise and Dissa) were surface-sterilized with 2 % NaOCl, washed three times with sterile water and germinated on solid MS medium in the light. Hormonfree, as well as hormone-supplemented germination media were tested.

Preparation and culture of explants: Leaf bases of young seedlings were cut into 1-2 mm segments and transferred to callus induction medium and incubated in the dark at 26 °C. After about 4 weeks calli were transferred to shooting medium. Small plantlets were cultured on regeneration medium before they were transferred to the greenhouse and grown to maturity. Several media with varying concentrations of different hormones were tested for callus induction, shooting and regeneration of plants.

Biolistic gene transfer: Freshly isolated or osmotically treated explants (4h prior bombardment, 18 - 20 h after bombardment, 400 to 600 mOsm) were used as target tissue for particle bombardment. The particle gun employed in these experiments was a PDS 1000/He gun (BioRad, Germany). Gold particles were coated with the plasmid pDB1, which contains the *bar* and the *uid*A gene, and shot onto the cultures as described previously (Jähne et al. 1994). Cultures were screened histochemically 40 h after bombardment for GUS activity or transferred to glutamine-free callus induction medium supplemented with 2 mg/l PPT.

Results and Discussion. On all tested callus induction media it was possible to induce embryogenic callus from leaf base segments of oat and barley. We observed, that development of embryogenic structures occured mainly at the intersections of the segments, thus in areas where the tissue had been wounded. The culture response varied dependent on the medium and the genotype. In oat the induction of embryogenic structures was generally easier than in barley. High amounts of embryogenic callus could be obtained reproducibly on medium supplemented with e.g. 2.5 mg/l 2,4-D. The whole regeneration process, starting from the preparation of explants of *in vitro* grown seedlings, culture on induction, shooting and regeneration media and transfer to soil takes only about 10 weeks. Regeneration of albino plantlets occured only very rarely. The regenerated plants were morphologically normal and most of them fertile. The oat genotypes cv. Fuchs and Jumbo were highly responsive and a mean value of 50.4 regenerants per explant could be achieved. The average regeneration frequency over all oat genotypes was 40 plants per explant and is significantly higher than the values (1.3 to 6.8 regenerated plantlets per explant) reported in the literature (H. Chen et al. 1995). This regeneration system could also be used for barley but the frequencies were much lower, on average only about 3 regenerants per explant could be obtained.

The highly efficient oat regeneration system has been used for optimisation of parameters in the particle bombardment process. Highest number of transient transformation events were observed using 58 µg gold particles per bombardment at pressures between 900 and 2000 psi. Freshly isolated seedlings showed an average of 37.8 blue spots per explant whereas in osmotically pretreated explants an average of 60.1 signals could be achieved.

For stable transformation, a selection pressure of 2 mg/l PPT has proven to be efficient to inhibit further growth and development of non-transformed structures.

At present the biolistic gene transfer is the most successful system for transferring foreign genes to cereals. Oat leaf bases are a very promising target for biolistic transformation due to the very high regeneration frequencies, which can be obtained reproducibly.

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CALLUSOGENESIS AND MORPHOGENESIS OF SPRING BARLEY. M.V. Ivanov, Belogorka, Institutskaya 1 Street, C3HUUCX, Leningrad Region 188231, Russia.

The somaclonal modifications arising from unorganized tissue are a major strong source for selection. However, the low regenerative potential is the limiting factor in the use of *in vitro* cell culture for breeding spring barley.

This work is devoted to determining the factors that increase the frequency of regeneration and to analyzse the new forms.

In the years of investigation 54858 embryos of spring barley were explanted. Considerable variability in formation of calluses by sorts and hybrids was observed. Spring barley cultivars such as *Beta Ketseras* var *erectum* and *Northern* var *pallidam* has a low ability to form callus - 20.2% and 22.1%, respectively. Hybrids from interbreeding of sorts having similar levels of callus formation exhibiting dominance and overdominance.

Table 1. Heredity in callusogenesis and regeneration of spring barley.

Type of heredity	Callusogenesis (% of combination)	Regeneration (% of combination
hp>1	21.4	17.9
hp=1	7.1	0.0
0.5>hp>1	14.3	7.1
0 >hp>0.5	14.3	21.4
hp=0	0.0	0.0
0 >hp	42.9	53,6

It is notable that overdominance was observed only with hybridization of sorts close one to one on the type of sign. However, in all cases the ability to create a callus was higher for the cultivar mutants. Callus was derived from different cells: mesophyll, conductive tissue, epidermis etc. Accordingly, in the differentiated tissues the induced morphogenesis arose, not from the whole callus, but from separate parts (mainly coming into contact with nutrient environment) and not all cells of the callus were affected. It is evident that the induced morphogenesis is mainly dependent on the levels of phytohormones and sugars in the nutrient culture. The higher the concentration of sugars the lower the ability to regenerate. The high morphogenetic potential of cultivars *Ida, Orphey, Melody, Roland* with high degree of probability defines the ability for morphogenesis in their hybrids. Crossing of cultivars with a low level of formation of morphogenetic structures gives in following generation of the non-embryogenetic water callus. Crossing of the sorts with a relatively high degree of regeneration (45-54%) produced 39.4 -47.6% embryogenetic reduced calluses. The main type of heredity to regeneration - depression (Table 1) from our point of view is associated with the prevalence of cultivars with a low potential of regeneration. The results of our investigations showed that the causes of different levels of regeneration are:

- breaches in physiology - biochemical bonds between the only cell and whole organism;
- acting of the environment;
- the influences of metabolites as in the cell and the environment;
- the selective sifting out a range of cells;
- breaches in chromosomes.

Those causes largely determine the composition of populations of the regenerants (Table 2).

Table 2. Composition of populations of regenerants of spring barley (%)

Origin of the explant	Fertile	Albino	Sterile
Cultivars	40.2	30.6	29.2
Hybrids	54.4	26.2	19.4

In practically all of the populations we observed albinos and plants with altered architecture. The number of anomalous plants varied from 21.5 to 100%. The quantity of anomalous plants in the population of regenerants was closely dependent on their parental forms. The level of fertility for the regenerants created from the hybrid combination was higher (14-23%) then for the ones created from the cultivars.

Thus, for the creation of fertile plants it is necessary to take into account the biological and mainly morphobiochemical characteristics of the initial cultivars. Also, it is necessary to have in mind that the explants from hybrid combinations have a higher level of regeneration if one of the parents has a high regenerative potential.

Segregation of the *bar* and *gus* genes in transgenic barley.

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Introduction

It is now possible in many economically important cereal species to introduce foreign genes into the genome and monitor expression of the transgene in the subsequent generation. Transformation of cereals is mainly obtained after bombardment of embryogenic tissues with high velocity metal particles carrying the transgenes followed by selection of morphogenic transgenic callus and subsequent regeneration of plants (McElroy and Brettell, 1994). The selectable marker genes giving resistance towards specific antibiotics or herbicides are co-transformed into the embryogenic tissue along with the genes of commercial or scientific interest. Once the transgene has been inserted into the genome transfer of the gene to the following generations is the crucial point. Transgenic plants produced by particle bombardment often contain multiple copies of the transgenes, sometimes located in more than one locus. Loci with repeated transgene sequences are considered to some extent to be unstable and susceptible to suppression of gene expression. If the introduced transgene is of commercial interest it is very important to evaluate the expression pattern and stability of the transgenes in several generations, to ensure that the transgenes are properly inherited and expressed. Such analyses of a large number of transgenic lines produced by particle bombardment have only been reported in maize (Spencer et al., 1992; Register et al., 1994)
This paper presents data on the segregation and expression of the selectable *bar* and non-selectable *gus* genes in plants representing 11 transgenic barley lines.

Material and Methods

Construction of the plasmid pDM803.
The 5'promoter region of the rice actin 1 gene (*Act1*) (McElroy et al., 1990) from the *Act1-bar-nos* gene in plasmid pDM302 (Cao et al., 1992) was removed as a 1.4 kbp *Hin*dIII - *Hin*dIII fragment and the remaining plasmid self-ligated to produce the *bar-nos* containing plasmid pDM3021. In order to delete the polylinker region between the *bar* and *nos* sequences, pDM3021 was digested with *Bam*HI and *Sst*I, blunt-ended by Klenow treatment, and self-ligated to produce the *bar-nos* containing plasmid pDM3022. The promoter of the maize ubiquitin 1 gene (*Ubi1*) (Christensen et al., 1992) was removed from the *Ubi-gus-rbcS* containing plasmid pRG73 (Kyozuka et al., 1993) as a 2.0 kbp *Pst*I-*Pst*I fragment and cloned into the *Pst*I site of pDM3022 to produce the *Ubi-bar-nos* containing plasmid pDM3023. The *Act1-gus-rbcS* gene from

pDMC207 (McElroy et al., 1995) was isolated as a 3.5 kbp *Pst*I-*Apa*I fragment, blunt-ended by Klenow treatment, and cloned into the *Hin*dIII site of pDM3023, which had been blunt-ended by Klenow treatment, to produce the *rbcS-gus-Act1*:*Ubi-bar-nos* containing plasmid pDM803.

Generation and analysis of transgenic plants.
Immature embryos of the cultivar Golden Promise were bombarded with 1 µm DNA coated gold particles 1 day after isolation. The following day the embryos were transferred to selective solidified medium containing 5 mg/L Bialaphos as described by Wan and Lemaux (1994). Every 2 weeks growing callus was transferred to fresh medium and regeneration was initiated 8 to 12 weeks after bombardment. Regenerated green plants were potted and transferred into the greenhouse and allowed to set seeds after self pollination.
Detection of the introduced transgenes by PCR was performed as described by Brinch-Pedersen et al. (1996).
Expression of the *bar* gene was verified by germinating embryos on medium containing 50 mg/L Chlorophenol Red and 1 mg/L Bialaphos (Kramer et al., 1993). GUS activity was determined by incubating half kernels from germinating seeds in X-gluc. (Jefferson, 1988).

Results and Discussion.

Several reports have now described transformation of barley by particle bombardment using either bombardment of immature embryos with the *bar* (Wan and Lemaux, 1994) or hygromycin resistance genes (Hagio et al., 1995) and subsequent selection on Bialaphos or hygromycin respectively. Additionally, Ritala et al. (1994) have obtained transgenic barley after bombardment of immature embryos, but without selection on herbicide or antibiotic containing medium. Freshly isolated microspores have also been used as target for bombardment with subsequent selection on phosphinothricin containing medium (Jähne et al., 1994).
In these reports the transformation frequencies vary from experiment to experiment. A similar variation was found in our experiments. The number of Bialaphos resistant callus lines which could be selected ranged from 40 to 0.1 per 100 bombarded immature embryos in different experiments. From 45% of these cell lines 1 or more green plants could be regenerated. Green plants from 90% of the lines were transformed with the *bar* gene while 80% contained both the *bar* and *gus* genes after bombardment with pDM803. Similar results have been obtained in rice, wheat and maize (Christou et al., 1992; Becker et al., 1994; Nehra et al., 1994; Koziel et al., 1993)
Among the transgenic barley plants produced, 11 independent transgenic lines were randomly selected for analysis of segregation and expression of the *bar* and *gus* genes. A total of 30 to 100 seeds was germinated per line. The analysis showed that 9 of the 11 lines showed segregation of the *bar* gene according to mendelian laws. Additionally the segregation analysis of these 9 lines indicated that 4 lines had 1 locus and 5 lines had 2 loci containing an expressed *bar* gene. R0 and R1 plants representing 9 of the 11 lines showed *gus* gene expression. In these lines the *gus* gene was shown to co-segregate with the *bar* gene in all lines exhibiting mendelian segregation. However, PCR analysis of the *bar* and *gus* genes in the progeny plants indicated that 4 of the 9

lines contained 1 locus with a non-expressed *gus* gene adjacent to an actively expressed *bar* gene. The silent state of the *gus* gene may be due to rearrangements in the *gus* expression cassette in accordance with the situation in maize where the non-selectable *gus* gene expression cassette was more often rearranged than the selectable *bar* gene expression cassette (Register et al., 1994).

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Production of fertile transgenic barley plants expressing barley yellow mosaic virus or barley mild mosaic virus capsid protein gene.

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Introduction Barley yellow mosaic virus (BaYMV) and barley mild mosaic virus (BaMMV) are economically important viruses in East Asia and Europe, which cause yellow mosaic disease in barley. Nucleotide sequences have been determined for the BaYMV and BaMMV capsid protein (CP) genes, which encode CPs of 297 and 251 amino acids, respectively (2,3). In order to produce BaYMV- or BaMMV-resistant transgenic barley, we introduced the CP genes of BaYMV or BaMMV by direct DNA transfer into barley protoplasts.

Materials and Methods DNA fragments which code for the BaYMV or BaMMV CP genes were isolated by polymerase chain reaction (PCR), using synthetic oligonucleotides which correspond to both termini of the CP genes and cDNAs of BaYMV or BaMMV RNA1 as templates. The fragments were cloned to an *E. coli* expression vector pKK223-3 (Pharmacia), and the expression of CP genes in *E. coli* was confirmed by western blot analysis. Ten different plant expression vectors were constructed with the CP genes and rice actin promoter (4) or barley ADP-ribosylation factor (ARF) promoter (H. Kuroda, in preparation), described in Figure 1 and Table 1. Barley transformation was performed as described (1).

Results and Discussion In order to obtain transformed calli, we performed co-transformation with neomysin phosphotranseferase II (nptII) expression vectors, or transformation with nptII expression cassette fused vectors (tandem vectors), and the developing colonies were selected twice with G418 (20mg/l and 30mg/l). We performed a total of 38 DNA transfer experiments using 2.9×10^8 protoplasts (cv. Igri), and consequently obtained 447 plantlets regenerated from 113 independent G418-resistant ($G418^r$) callus lines. Among them, 258 plants have been transferred to soil, 52 plants are growing in the growth chamber, and seven plants have shown fertility, so far. The numbers of transformed plants with various vectors in this study are summarized in Table 1.

We analyzed 49 of $G418^r$ calli obtained in various transformation experiments and detected NptII protein in 47 of the calli by ELISA. By analyzing DNAs extracted from 14 shoots regenerated from NptII protein expressing calli by

genomic PCR, we detected the BaYMV or BaMMV CP gene in three of seven shoots obtained by co-transformation and in all of seven shoots transformed by tandem vectors. Further analysis of four fertile transgenic plants showed that various copies of BaYMV or BaMMV CP genes were integrated into the barley genome (Fig. 1B and C). We also detected the mRNA of the BaYMV and BaMMV CP genes in the roots of transgenic barley (Fig. 1D).

T_1 progenies of two transgenic plants transformed with pSBG229 and pSBG239 (T229-1 and T239-1 in Fig. 1) were germinated in vitro. BaMMV CP genes were detected in both progenies by genomic PCR, thus demonstrating the transmission of the CP genes to the T_1 generation. Mechanical inoculation experiments are planned to examine the BaYMV and BaMMV resistance in the T1 progenies of transgenic barley.

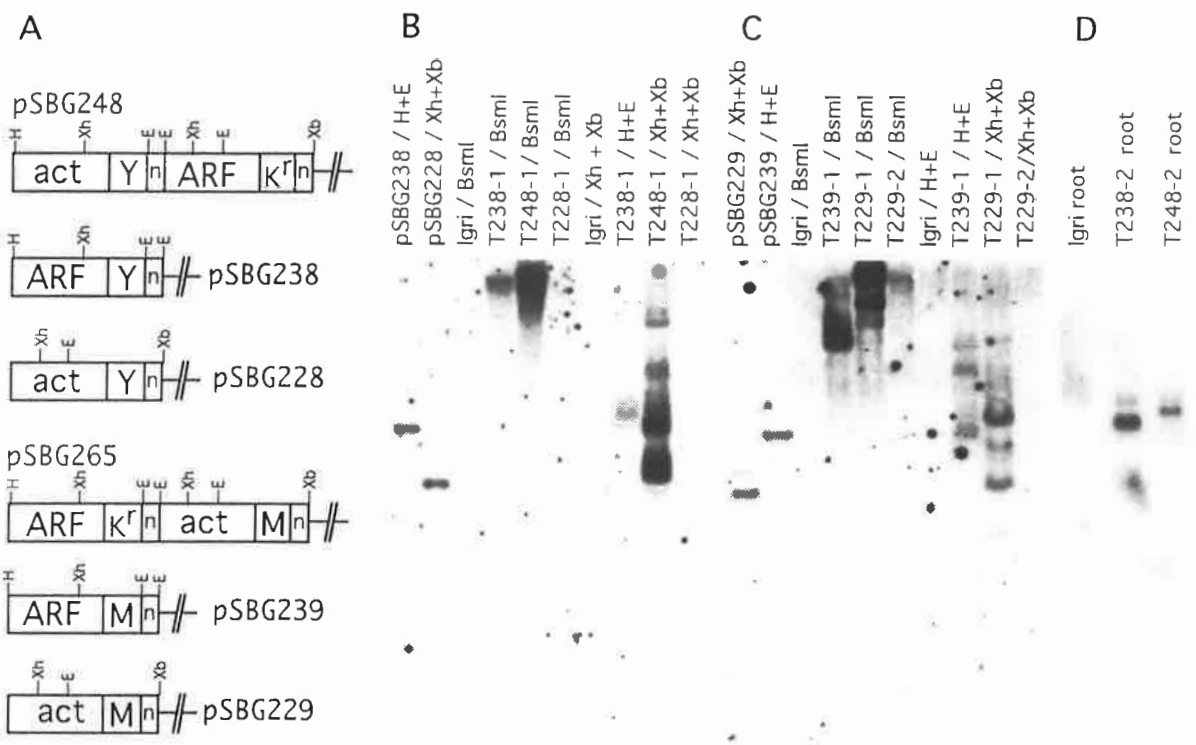

Figure 1 Schematic drawing of transformation vectors (A) and Southern (B,C) and Northern (D) hybridization analysis of transgenic plants. (A) Symbols: ARF, barley ARF promoter; act, rice actin promoter; Y, BaYMV CP gene; M, BaMMV CP gene; K^r, nptII gene; n, terminator of nopaline synthase gene; H, HindIII; E, EcoRI; Xh, XhoI; Xb, XbaI. Every vector has no BsmI site. pSBG260 and pSBG261 are the tandem vectors which have ARF promoter + nptII and ARF + the BaMMV CP gene cassette, which differs in orientation. pSBG250 is also the tandem vector which has act promoter + nptII and act + the BaYMV CP gene cassette. (B,C,D) T228-1; T238-1,2; T229-1,2; T239-1 and T248-1,2 indicate plants transformed with pSBG228, 238, 229, 239 and 248, respectively. Filters were hybridized with digoxigenin-labeled (Boehringer, Inc.) BaYMV (B,D), or BaMMV(C) CP gene probes.

Table 1. Summary of transformation experiments

Vectors	Pro - CP[a]	G418[r] calli (Reg.[b])		PLT[c] (G.Ch.[d])[e]		fertile plants[e]
pSBG244	ARF - BaYMV	27400	(12)	98	(14)	
pSBG248	act - BaYMV	1348	(9)	43	(7)	1
pSBG250	act - BaYMV	1039	(1)	13	(1)	
pSBG228 + M[f]	act - BaYMV	765	(4)	27	(4)	
pSBG238 + M	ARF - BaYMV	1920	(15)	43	(8)	4
pSBG260	ARF - BaMMV	582	(22)	67	(1)	
pSBG261	ARF - BaMMV	487	(3)	24	(1)	
pSBG265	act - BaMMV	1109	(8)	18	(1)	
pSBG229 + M	act - BaMMV	623	(15)	29	(7)	1
pSBG239 + M	ARF - BaMMV	3470	(24)	85	(8)	1

[a]Designates "Promoter and CP gene". [b]Numbers of G418[r] calli which regenerated green shoots. [c]Plantlets. [d]Plantlets transferred to soil and grown in the chamber. [e]These numbers indicates present data. At the end of this study, we expect to have more than 400 fertile transgenic plants. [f]The marker plasmid, pSBG112 (ARF promoter + nptII gene) or pAct1DNeo (act promoter + nptII gene, described in ref. 1.) was co-transformed.

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TITLE: Protoplast transformation of barley suspension cells with a salt tolerance-related gene, HAL1; transformation and characterisation of non-transformed lines.

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ABSTRACT.

Barley (*Hordeum vulgare* L.) suspension cells adapted or not adapted to growth in sodium chloride medium were analysed for ion content and ion uptake capacity. Cells grown in different media have a different capacity for accumulation of sodium and potassium. A gene for salt tolerance, HAL1, was introduced to suspension protoplasts via PEG-mediated DNA uptake. Transformed cells are undergoing molecular analysis before reintroduction into liquid medium and characterization for salt tolerance.

INTRODUCTION.

Soil salinization is an increasing problem for agriculture worldwide. Most crop plants are salt-sensitive and it is therefore desirable to introduce genes for salt tolerance into them. The use of cell suspensions in the study of the physiology of salt tolerance eliminates tissue and whole plant interactions, and allows examination of responses resulting from the introduction of salt-tolerance genes. In this study such a gene, HAL1, isolated from yeast (Gaxiola *et al.* 1992), was introduced into barley suspension cells. Non-transformed lines have been characterised with respect to growth in medium containing sodium chloride. The relationship between sodium and potassium uptake in these cells is being examined using radioactive tracers. Increased cellular potassium may correspond with reduced sodium uptake, limiting NaCl-related metabolic perturbations.

METHODS.

Barley (cv Dissa) suspensions were isolated previously (Funatsuki *et al.* 1992). Cells were maintained on either L1 medium (Lazzeri 1995) but without amino acids, or L7 medium (modified L1 medium; ammonium nitrate concentration, 3 mM, potassium nitrate concentration, 15 mM) with amino acids. 2,4-D (2,4-dichlorophenoxyacetic acid) was added at a concentration of 2 mg l^{-1} for routine maintenance of cells.

Growth of non-transformed cells in sodium chloride.

Cells were acclimated to growth on salt medium over a period of six months. NaCl concentration in the medium was elevated in 50 mM increments and growth rate recorded after each increment. Once a stable growth rate had been achieved the salt concentration was further increased until cells were no longer able to maintain fresh weight increase and therefore deemed unable to acclimate.

Ion content of adapted and non-adapted cells.

Sodium and potassium concentration of cell sap was analysed in a flame photometer. Cells were harvested and placed in Vectaspin 3 tubes (Whatman). Tissue was washed three times with an isoosmotic buffer solution and then frozen in liquid N_2. Sap was extracted from thawed cells by centrifugation at 5000 x g for 5 min.

Radioactive ion uptake experiments.

Uptake of ^{86}Rb by cells adapted to growth in L7 medium, either without NaCl or with 150mM NaCl, was measured. Adapted cells were brought down to a zero NaCl medum prior to the experiment by decreasing salt concentration in 50 mM steps over a period of 3 days. Both cell types were then placed in a potassium free, sodium free

nutrient medium for a further 3 days. On day 7 medium was removed from all cells and tissue was washed with a washing solution of 10 mM MES pH 5.7, 10 mM $CaSO_4$, with mannitol added to provide the same osmolarity as the final nutrient medium. Cells were incubated for 2 h in wash solution prior to time zero. At time zero 20 mM K and ^{86}Rb at a final concentration of 0.0074 MBq ml^{-1} was added to each sample. Sodium chloride was added at a final concentration of 150 mM according to treatment conditions. Samples were removed at intervals and washed three times with isoosmotic washing solution before drying at 60°C for 12 h and ashing at 480°C for 12 h. Ashed tissue was extracted with 100 µl 1M HCl and extracts mixed with 5 ml scintillation fluid (Ultima Gold) for counting.

Protoplast transformation.

The procedure was modified from Lazzeri, 1995. For optimal cell division following transformation 2,4-D concentration was increased to 4 mg l^{-1} in the growth medium. Protoplasts were cotransformed with pAHC25, containing the *bar* gene under a maize ubiquitin promoter for tissue selection with phosphinothricin (PPT), and pRS655, with the HAL1 gene under a CaMV 35S promoter. DNA was added at a concentration of 50 µg per tube. Agarose segments were placed in normal suspension medium containing 1, 2 or 3 mg l^{-1} PPT. Calli which came through selection were picked and maintained on plates containing 1 mg l^{-1} PPT. These cells were tested for the presence of *bar* and HAL1 genes by PCR.

PCR conditions; PCR reactions were prepared using TAQ polymerase (Boehringer) and primers for *bar* or HAL1. Cycling conditions for *bar* were 94°C for 1 min, 55°C for 30 sec, 72°C for 1 min for 35 cycles. Cycling conditions for HAL1 were 95°C for 1 min, 65°C for 30 sec, 72°C for 1 min, 35 cycles.

RESULTS AND DISCUSSION.

Barley cells in L1 medium were able to adapt to sodium chloride concentrations of up to 200 mM. Cells in L7 medium were not able to sustain growth at concentrations greater than 150mM (fig 1). The cells in L1 medium accumulated higher concentrations of potassium and lower concentrations of sodium (fig 2) than cells in L7 medium. The ability of L1 grown cells to limit sodium uptake and substitute by potassium accumulation may have been a contributory factor to their apparently greater salt tolerance.

Salt adaptation affected ^{86}Rb uptake in L7 grown barley cells (fig 3). Adapted cells took up rubidium at a lower rate than non-adapted cells. This work will be continued by comparing L1 and L7 grown cells and by looking at ^{22}Na uptake in cells from both growth media. Down-regulation of sodium uptake may protect the cell from accumulation of this potentially toxic ion. Osmotic balancing could then be effected by production of compatible solutes or accumulation of potassium.

A number of transformed lines were retrieved from selection. All concentrations of PPT produced transgenic lines though the higher concentrations gave most rapid results. Even at concentrations of 3 mg l^{-1} PPT the control cells were not completely killed but growth was inhibited.

All lines were positive for the *bar* gene and growth rates in liquid medium were comparable with nontransformed cells. Initial results suggest that about 70% of lines were successfully cotransformed with HAL1 and this figure is being verified. RNA is currently being extracted for northern analysis and depending on the results of this cell lines will be reintroduced into suspension for growth and uptake studies. Southern analysis of gene integration is also underway.

Fig 1. Growth of barley cells at different concentrations of NaCl a) L1 medium b) L7 medium.

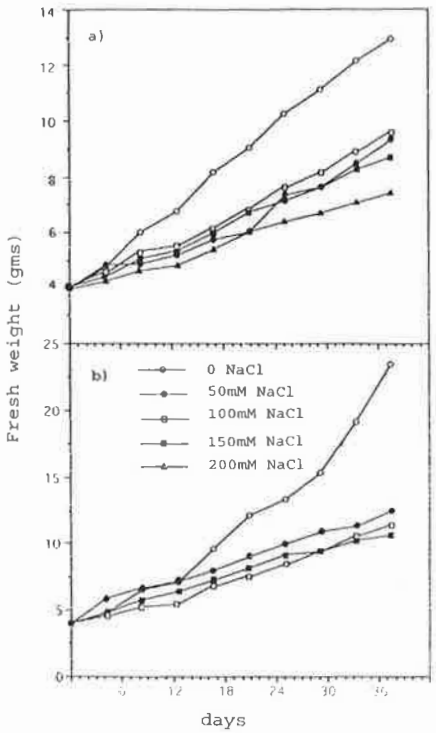


Fig 2. Ion content of cell sap. L1 or L7 medium. 0, 50, 150 mM NaCl in growth medium as indicated (L1, L1/50, L1/100, L1/150 etc.)

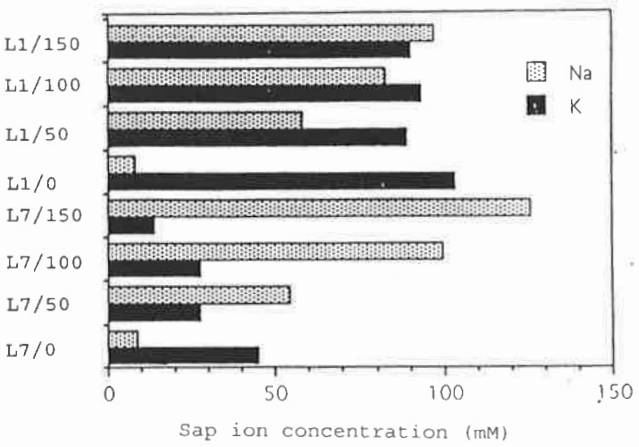
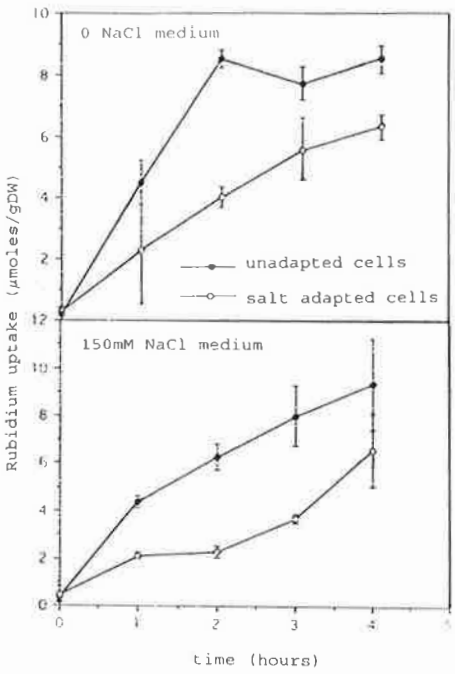


Fig 3. ^{86}Rb uptake into salt adapted and non-adapted cells.



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Genetic modification of malting barley. L. MANNONEN, A. RITALA, A.M. NUUTILA, K. ASPEGREN*, R. PUUPPONEN-PIMIÄ, K. HEMMANN, T.H. TEERI* and V. KAUPPINEN, VTT Biotechnology and Food Research, POB 1505, FIN-02044 VTT, Espoo, Finland. *Institute of Biotechnology, POB 45, FIN-00014 University of Helsinki, Finland.

Introduction. Gene transfer has become a useful tool in plant breeding. It is used to supplement conventional breeding in improving the economically important qualities of industrial plant raw material e.g. nutritional quality, ease of processing or for production of specific compounds. Barley is one of the most important crop plants in the world and has also received considerable attention in tissue culture and genetic transformation research. Successful reports on introduction of foreign genes into barley genome and regeneration of fertile transgenic barley plants thereof has been published (1-5). However, no useful new quality traits has hitherto been reported to be introduced into barley genome. Our interest lies in improving the malting barley by modifying its enzyme spectrum, altering the starch composition and increasing its fungal resistance and research is conducted in each field. As the first task we undertook to complement the enzyme spectrum of malting barley with a heat stable endo-glucanase activity of fungal origin. Barley grain contains about 10% of β-glucan. The heat stable endo-glucanase is expected to improve the mashing process by degrading soluble glucans at elevated temperatures leading to reduced viscosity of the wort and thus to reduced sieving and filtering costs.

Materials and methods. In order to verify the proper action of the heterologous endo-glucanase (EGI) of *Trichoderma reesei*, the gene was first introduced to barley cell cultured cells by particle bombardment. The enzyme produced by the cells was purified, characterized and used in laboratory scale mashing. The gene was also linked to a germination specific promoter of barley α-amylase and transferred to barley plants.

Plant material. Barley cell culture was initiated from immature embryos of a malting barley variety *Pokko* on a modified B5-medium and cultured thereafter as a suspension culture on the same medium (6,7). Immature embryos of the variety *Kymppi* were used as targets for gene transfer for production of transgenic plants. The embryos or isolated scutella were cultivated on solid L2-medium (8) which was modified by replacing the amino acids with 1 ppm casein hydrolysate and reducing the 2,4-D concentration to 1 ppm.

DNA material. For production of EGI in cell culture the cDNA of *egl1* gene was linked to a constitutive promoter of CaMV 35S in plasmid pKAH21 (7,9). For selection of transgenic clones the cells were co-transferred with the *nptII* gene under the same regulation in plasmid pHTT303 (7,9). For production of transgenic plants the *egl1* was linked with barley high pI α-amylase promoter with the first intron in plasmid pKAH80. The promoter element was obtained from Dr.John Rogers in Washington University, St. Louis, MO). It was co-transferred with a selectable marker gene of *bar* linked with the maize ubiquitin promoter with the first exon and intron in plasmid pAHC25 (10).

Transgenic cell culture. The genes were transferred by particle bombardment to barley cells

as described in (6). The cell culture cells were cultivated under 100 ppm geneticin selection on solid medium. After 8 passages of cloning the cells were suspended and the suspension culture was cultivated by weekly subculturing on 75 ppm geneticin selection. Due to the signal sequence in the *egl1* gene the heterologous EGI was secreted in the growth medium where it was collected and purified for characterization (7).

Transgenic plants. The genes were bombarded into immature embryos essentially as described earlier (1) but using the scutellar side or isolated scutella as target. After bombardment the embryos were cultivated under 5-10 ppm bialaphos selection. The growing callus was divided and subcultured on the same medium in dark for 3 passages before transferring to light on regeneration medium MS3018 (1) supplemented with 2-5 ppm bialaphos. The green shoots were transferred to rooting medium MS3019 (1) supplemented with 1 ppm bialaphos. The rooted plants were transferred to soil.

Assay of transgenic plants. The plantlets in regeneration medium were screened for both the *bar* and the *egl1* gene with PCR. The result of the PCR positive plants were confirmed with Southern hybridization using the appropriate gene fragments as probes. EGI activity was assayed from the mature seeds with either tissue printing or enzyme activity measurements. After 3 days of germination the shoot was cut from the seed, the starchy endosperm removed and the aleurone layer printed on nitrocellulose filter. The EGI was detected using polyclonal antibody against EGI (7) and visualized with alkaline phosphatase conjugated to the secondary antibody. EGI was further extracted from the aleurone cells and the starchy endosperm. EGI was separated from the barley endogenous glucanase activity by isoelectric focusing. The IEF gel was incubated with β-glucan substrate in agarose as described in (7) and the activity was visualized by staining the undigested glucan with 1% Congo Red.

Results and discussion. Production of transgenic barley plants is a very slow process although several groups have shown it to be possible. In order to be able to evaluate the potential of new heterologous activities, plant cell cultures offer a good alternative. In our example the heterologous EGI produced by barley cell culture cells appeared to have retained its original properties almost unchanged. The temperature optimum of the enzyme was slightly reduced from 65 °C to 60 °C and the pH optimum from 5.5-6.0 to 5.0-5.5. A slight reduction in molecular weight appeared to be at least partly due to modifications in glycosylation. The enzyme activity profile and specific activity appeared, however, to be unchanged. In order to evaluate the potential of the transgenic malt in mashing process, a laboratory scale mashing experiment was conducted using either the original fungal EGI or the EGI made by the barley cell culture cells added to the mash. Both enzymes had a beneficial effect reducing the soluble β-glucan content in the mash and improving the filtrability of the wort and the extract yield. Furthermore both enzymes acted similarly.

Transgenic plants were produced by transferring a selectable marker together with the *egl1* gene. The growing callus and plantlets thereof were first selected in respect to the co-transferred selectable marker which conferred resistance to the herbicide bialaphos. Of 1660 bombarded embryos 145 green plants were obtained. The β-glucanase coding gene was demonstrated with PCR or Southern blot hybridization from leaves of the plants growing in the green house. One plant of these appeared to contain the *egl1* gene. When germinating

immature embryos of some of the seeds the *egl1* gene was shown to be inherited in the second generation. Several new series of *egl1* transformations are presently in progress.

Due to the germination specific regulation mature seeds of the transgenic plant need to be germinated in order to evaluate the expression of the *egl1* gene. The studies on the detection of heterologous β-glucanase activity in transgenic barley seeds during germination is in progress.

Conclusions. Altough all the major cereals have come within the range of genetic engineering the technique is far from routine. Few useful breeding cultivars are susceptile to genetic engineering and few quality traits are reported to be transferred to cereals such as barley or wheat. Furthermore, little is known on the regulation of genes, on the successful expression of gene products and on the inheritance of the genes in succeeding progeny. We report here on successful transformation of a cultivar currently used as malting barley with a quality trait for heat stable endo-glucanase. We continue our work to make it more efficient as a tool in future barley breeding.

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Barley Transformation *via* DNA uptake into scutellum protoplasts.

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Introduction. Recent advances in molecular biology and the use of novel genetic engineering technologies may offer the opportunity to speed up breeding programmes for the improvement of barley for malting, animal feed and other uses.

Recently, transgenic barley plants have been recovered using particle bombardment (Hagio *et al.* 1995, Wan & Lemaux 1994) and protoplast mediated transformation (Funatsuki *et al.* 1995).

Protoplast methods do not call for highly specialised equipment, they allow the production of large numbers of independent transformants and the selection of the transformants is effective at the stage of microcalli. However, totipotent protoplasts are usualy obtained from cell suspensions, which are difficult to establish, accumulate genetic aberrations over time and rapidly lose their regeneration capability (Lazzeri & Shewry 1993). To avoid these constraints, we have recently developed a new approach which involves the isolation of protoplasts directly from immature scutella. These protoplasts were able to regenerate fertile plants (Nobre *et al.* 1996).

This work examines parameters affecting transient GUS expression from scutellum protoplasts for the development of a procedure for barley transformation via PEG-mediated direct DNA uptake.

Materials and Methods. Scutellar tissues from 1.4-1.6 mm immature embryos of barley cvs. Clipper, Dissa, Derkado and Prisma were pre-cultured and protoplasts were isolated, after vacuum infiltration of scutellar tissues with cell wall digesting enzymes, as described previously (Nobre *et al.* 1996). Protoplasts were kept at 4°C during 3-4 h, before transformation. The effect of the osmolarity of the pre-culture medium on transient gene expression was tested. Two pre-culture media were compared: L8D2C5 (Nobre *et al.* 1996) and L8D2C5h, a similar basal medium, but modified to contain 26 $g.l^{-1}$ maltose, 10 $g.l^{-1}$ glucose and 10 $g.l^{-1}$ fructose.

Two plasmids were used for co-transformations: pAct-1D*gus*, which contains the *uidA* gene under the control of the actin-1D promoter, and pCa*lneo*, containing the *neo* fragment of the *npt*II gene under the control of the CaMV35S promoter and the *Adh1* intron from maize.

Transformation was performed according to a procedure used for barley cell suspensions (Lazzeri *et al.* 1991). PEG-treated protoplasts were kept in washing solution, at 4°C, for 1 h post-transformation. Protoplasts were collected by centrifugation and were embedded in Na-alginate (1.0×10^6 cells/ml), at 4°C, for 24 h.

Embedded protoplasts were co-cultivated with the feeder cell line HTA 42A (Tritordeum suspension line) in L8D1 protoplast medium (Nobre *et al.* 1996) and incubated in the dark for 14 d. After this period, feeders were removed and medium was replaced by a standard suspension medium containing 15 $mg.l^{-1}$ G418 (medium was removed every 7 days), until calli reached 2-3 mm diameter (4-6 weeks); macroscopic calli were then subcultured to somatic embryogenesis induction medium (Nobre *et al.* 1996).

Transient GUS activity was assayed histochemically on cells 5 d after protoplast isolation and was determined as the nº of blue cells/nº protoplasts plated per field.

Results and Discussion. Protoplast survival was reduced by 30-40 %, following PEG-treatment. However, the survival increased when protoplasts were kept at 4°C for 6-8 h, before the PEG treatment. Also, this period of cold treatment greatly reduced protoplast aggregation after transformation.

The effects of the osmolariry of the pre-culture medium on transient GUS expression were evaluated (Table 1).

Table 1 - Effect of the pre-culture medium osmolarity on transient gene expression. Values are the mean of at least two replicates ± SE.

Cultivar	% cells expressing GUS	
	L8D2C5 (~ 300 mOsm)	L8D2C5h (~ 400 mOsm)
Dissa	0.5 ± 0.1	2.9 ± 1.2
Clipper	NT	5.6
Derkado	NT	1.9 ± 0.2

NT - not tested.

Increased osmolarity of the pre-cultured medium resulted in 5-fold increase on transient *gus* gene expression in cv. Dissa. Beneficial effects of the osmoticum conditioning of the tissues prior to transformation were reported previously in stable transformation of maize (Vain *et al.* 1993).

The influence of the PEG treatment on plating efficiency and on the number of resistant calli regenerated after 4 subcultures on selection medium, was evaluated in cvs. Clipper and Prisma pre-cultured in L8D2C5h (Tab. 2).

Table 2 - Effect of PEG treatment on plating efficiency (%) and on the number of resistant calli following selection on G418 (15 $mg.l^{-1}$). Values are the mean of two replicates ± SE.

Cultivar	Plating efficiency (%)			Nº calli from selection/10^6 protoplasts
	Control	Mock Transformation	Transformation	
Clipper	7.7 ± 2.5	4.6	13.0 ± 3.7	22.5 ± 2.5
Prisma	0.9	1.2	4.4	0.0

Protoplast-derived cells started to divide 4-5 d post-transformation. Increased plating efficiency was obtained from transformed protoplasts in both cultivars. No microcallus development was observed in control treatments following selection with G418. Transformed protoplasts from cv. Prisma gave a lower plating effciency as compared with cv. Clipper and no macroscopic callus was regenerated post-selection. Attempts to induce somatic embryogenesis and plant regeneration in protoplast-derived callus are in progress.

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The influence of the medium composition and the medium pH change during the cultivation on embryogenic callus formation and plant regenerations from leaf tissue of barley. T.P.PASTERNAK and T.V.YOUSUKHNO, Institute of Cell Biology and Genetic Engineering, National Academy of Sciences of Ukraine, Zabolotnogo str.,148, 252022, Kiev DSP-22, Ukraine.

Introduction.

Despite the fact that all plant cells contain identical genetic information, callus obtained from somatic cells varies in its competence to express totipotence under identical culture conditions. This is especially true for cereal tissue culture. As compared to other cereal species, barley plants regeneration faces problems of low efficiency and genotype dependent response [1]. For example, only immature explants (embryos, inflorescences, etc.) serve as suitable starting material with high regeneration capacity for in vitro cultivation[2]. All other explants (leaves, mature embryos, etc.) have a reduced capacity for plant regeneration. Therefore, the aim of our investigation was to study the influence of nonphytohormonal factors on embryogenic callus formation from the leaf tissue of barley in vitro. We now present the reliable protocol for regeneration of barley from leaf tissue.

Materials and Methods.

Donor plants. Seeds of cultivars Dera and Defra were kindly provided by Prof. R.R.Mendel, Botanical Institute, TU Braunschweig, Germany. Seeds of cultivar Edem were obtained from Ukranian Breeding and Genetic Institute, Odessa. Deshunked seeds of spring barley cultivars Dera, Defra and Edem were surface sterilized by treating them for 20 minutes in 20% solution of commercial bleach, and then rinsed 4 times in sterile, distilled water. After sterilization seeds were treated in two different ways. First group was put into the tubes with medium (15 ml in each tube). Seeds of the second group were soaked at 40 C in sterile distilled water for 24h. Embryos were excised from surrounding tissue and placed scutella down onto the Petri dishes with the medium without growth regulators (4 plants per 20 ml of the medium in each 10 cm diameter Petri dishes). Plants were outgrown and maintained under controlled conditions at 23-25 ^{0}C and 16 h photoperiod with light intensity 3000 lx.

Culture media. More than 45 original plant medium compositions were tested for their capacity to promote plant growth, callus induction and plant regeneration. Contents of some of these media are listed below. All callus induction media were supplemented with 2 mg/l 2.4-D. For plant growth only phytohormone free media were used. Medium N1 for callus induction consisted of MS salts, B5 vitamins, 20 g/l Sucrose, and 2 mg/l 2.4-D, initial pH 5.8. Medium N2 for callus induction consisted of 10 mM NH_4NO_3, 0.3 mM $CaCl_2$, 3.75 mM KH_2PO_4, 17.4 microM $ZnSO_4$ x$7H_2O$, 0.5 microM $CoCl_2$x$6H_2O$, and 1.25 microM Cu SO_4x$5H_2O$ (all other salts were as in MS medium), plus B5 vitamins, 20g/l Sucrose, and 2 mg/l 2.4-D, initial pH 5.8. The initial pH of the media was adjusted to appropriate value (5.7-6.1) by 0.1N KOH, then agar was added and the media were sterilized by autoclaving for 15 min at 125^{0}C. To monitor the pH value of the media

during cultivation standard pH indicator Chlorophenol Red (Fluka) was added before autoclaving.

Explant preparation and culture conditions. Explants were prepared from 15 to 45 day old plants. Leaves were trimmed down to approximately 2 cm, removed from the seedling axis, basal point and axillary buds, cut into 2 mm perpendicular sections using a scalpel and transfered to induction medium. Explants were cultured in 9 cm diameter Petri dishes on 20 ml aliquots of induction medium on light at 23-25^{0}C. Embryogenic calli formed on induction medium were transfered to appropriate regeneration medium. Obtained plantlets were transferred to growth regulators free medium.

Results and Discussion.

Callus formation/embryogenic callus formation. The frequency of embryogenic callus formation (compact globular callus) had the feedback correlation with the frequency and size of nonembryogenic (soft, watery) callus formation in all our experiments. The nutrient medium content for donor plants growth and presence/absence of the grains together with callus formation medium composition strongly influenced embryogenic callus formation. For example, increasing the $CuSO_4x5H_2O$ content in the medium for plants to 0.25 mg/l led to complete inhibition of callus formation from leaves, while increasing Zn content to 15 mg/l $ZnSO_4$ $x7H_2O$ led to increase in both callus formation frequency and feedback pronounced gradient. For example, for the plants grown on ordinary MS medium only the fourth segments from seedlings axis formed callus. However, callus formation occurred even from the sixth and the seventh segments from the axis of the plants grown on the medium supplemented with 15 mg/l $ZnSO_4$ x $7H_2O$. For the explants of all three cultivars investigated we obtained identical callus induction frequencies. But the embriogenic callus formation frequency from explants of Dera and Defra cvs.(up to 80% from the first segments) was much higher than that from explants of Edem cv. (5 %). The medium pH stabilization by Casein hydrolyzate led to complete inhibition of callus formation from all explants.

Our results also suggest, that there is close correlation between medium acidification and embryogenic callus formation frequency. For example, leaf explants of cv. Edem, which possess low frequency of embryogenic callus formation on the MS callus-induction medium, caused very rapid and strong medium acidification (from 5.7 to 4.5 during 3 days in culture). Leaf explants from cultivars Defra and Dera, on the other hand, decrease pH from 5.7 to 5.0 only after 7 days of cultivation.

Embryos proliferation and plant regeneration. We have tested more than 40 media based on MS salts supplemented with different phytohormone combinations (e.g. 2.4-D, kin, BAP, ABA, GA3). Only media N1, containing high kinetin concentration (2-2.5 mg/l) in combination with high ABA concentration (1-1.5 mg/l), led to further proliferation of somatic embryos, and to plant regeneration after decrease in ABA content. Somatic embryos proliferation frequency on this medium achieved 50-70% and frequency of plant regeneration from proliferated embryos achieved 40-50%. In contrast with medium N1, on the induction medium N2, without phytohormones, we observed further proliferation of

primary embryogenic callus and even plant regeneration without transfer of the explant to the new medium.
A limited number of regenerated plants were further investigated in natural environment, planted into soil, where they developed into normal fertile plants.

To explain the synergetic effect of kinetin and ABA we suggest that both compounds lead to the inhibition of root formation therefore increasing the cytoplasmic pH due to the H^+-pump function [3]. These mechanisms lead to the strong medium acidification during cultivation. This hypothesis is completely confirmed by our pH-measurement data. The pH of the MS medium supplemented with ABA (1mg/l) and kinetin (2mg/l) after 7 days of cultivation of embryogenic callus of Dera dropped to 4.3. It must be noted that synergetic effects of cytokinins with ABA was previously described .

Our results show that real pH of the medium dramatically changes during the cultivation of barley *in vitro*. Monitoring of the medium pH during the process of cultivation suggests that all structures, e.g. explants, formed calli, donor plants and regenerated plants, cause strong acidification of the medium. Moreover, this acidification strongly modulate explants growth and development.

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Expression and inheritance of transgenes in genetically engineered oat. W.P. PAWLOWSKI, K.A. TORBERT, H.W. RINES* and D.A. SOMERS, Department of Agronomy and Plant Genetics, University of Minnesota and *Plant Science Research Unit, USDA-ARS, St. Paul, MN 55108, U.S.A.

Introduction. Microprojectile bombardment has been successfully used to genetically transform a number of crop species (Christou, 1992). In some species, transgenic plants produced by this method are commercialized. For practical application of transgenic plants to crop improvement, stable integration and inheritance of introduced transgenes are essential. Behavior of transgenes in genetically engineered plants must be also understood and predictable.

Somers et al. reported production of transgenic oat (*Avena sativa* L.) in 1992. The method is still being improved to increase transformation efficiency (Torbert et al., 1995). We are conducting analyses of integration, expression and inheritance of transgenes in genetically engineered oat. We expect that results of these analyses will provide us with clues on how to influence the processes of transgene integration and expression in hexaploid oat. It will be a guide for future improvement of the oat transformation technology to enhance transgene stability and assure consistent, high levels of transgene expression in genetically engineered plants. We have studied the patterns of integration of two marker transgenes in oat and followed their expression and inheritance through several generations of transgenic plants.

Material and Methods

Transgenic plants. Transgenic oat tissue cultures were produced by microprojectile bombardment with the plasmid pBARGUS (Fromm et al., 1990). This plasmid carries the *Escherichia coli uidA* gene (Jefferson, 1987) encoding β-glucuronidase (GUS) and the *Streptomyces hygroscopicus bar* gene (Thompson et al., 1987) encoding phosphinothricin (PPT) acetyl transferase (PAT), which provides resistance to the herbicide PPT. The *uidA* gene is under the control of the maize *AdhI* promoter, which confers GUS expression in seeds; the *bar* gene is regulated by the CaMV 35S promoter. Following DNA delivery, transformed tissue cultures were selected on a medium containing the herbicide and transgenic plants were regenerated.

Transgene genotypes. Presence of transgenes in plant DNA was detected by Southern blot analyses (Southern, 1975).

Transgene phenotypes. Transgenic plants were self-pollinated for up to four generations. Harvested seeds were histochemically stained for GUS activity (Jefferson, 1987). Herbicide resistance was tested by painting leaves of transgenic plants with a solution of glufosinate, a herbicide containing PPT (Thompson et al., 1987).

Results

Transgene integration. Transgenic tissue cultures and regenerated transgenic plant lines representing 27 independent transformation events were studied. Analysis of transgene integration patterns revealed extensive rearrangements of transgenic DNA sequences integrated into the oat genome. There were, on average, four copies of the *uidA* gene and three copies of the *bar* gene per haploid genome. Both transgenes, *uidA* and *bar*, always cosegregated at the DNA level.

Expression of GUS. Twenty-three transgenic lines exhibited GUS phenotype expression. Presence of the *uidA* gene was detected in all tested GUS-expressing plants. Cosegregation of the *uidA* transgene with the GUS phenotype was found in 40 % of lines. Individuals that had the *uidA* transgene but showed no GUS expression were detected in 60 % of analyzed lines. The restriction patterns of transgenic DNA in the *uidA*$^+$ but GUS$^-$ individuals were identical to the restriction patterns of the GUS-expressing plants. This excludes a possibility of presence of additional non-functional transgene loci segregating independently from the expressed locus and suggests that silencing of transgene expression was responsible for lack of the transgenic phenotype. Transgene silencing was very common in two transgenic plant lines, in which most of the *uidA*$^+$ individuals did not express the GUS phenotype. It was less frequent in other lines.

Plants from four lines showed no GUS expression. Three of the lines were examined using Southern blot analyses. Two lines showed transgene fragments hybridizing to the *uidA*-specific probe but no fragments corresponding in size to the unit-length *uidA* gene. Therefore, all of the detected *uidA*-hybridizing DNA fragments most likely represented rearranged and non-functional *uidA* copies. One line showed no *uidA*-hybridizing fragments and only some rearranged, probably non-functional, *bar* sequences.

Mendelian inheritance of GUS phenotype. Inheritance of the GUS phenotype was studied in up to four generations of self-pollinated progeny of the primary transgenic plants. Eleven lines segregated in a Mendelian 3:1 ratio for the GUS phenotype. All of them exhibited segregation consistent with Mendelian one-locus segregation in all generations tested. In 9 of the 11 lines, GUS$^+$ homozygotes were identified as plants giving rise to uniformly GUS$^+$ progeny in a following generation. The ratio of GUS$^+$ homozygotes to heterozygotes was most frequently consistent with the 1:2 ratio expected from the Mendelian 1:2:1 segregation of genotypes. All tested GUS$^+$ homozygous progenies continued to produce only uniformly GUS$^+$ offspring in subsequent generations.

Distorted GUS inheritance. Twelve transgenic plant lines showed segregation that did not fit Mendelian inheritance ratios in all or some of the tested generations. In all of these lines the number of GUS$^+$ individuals in progenies segregating for the GUS phenotype was smaller than expected based on the Mendelian segregation ratios. Seventy percent of the abnormally segregating lines produced GUS$^+$ homozygotes. Individual progenies showing segregation distortions were sporadically found also in normally segregating lines. Silencing of the *uidA* gene was the predominant cause of distorted segregation ratios. Transgene silencing was found in all but one abnormally segregating lines and, to a lesser extent, in several lines with apparently normal segregation ratios. Segregation abnormalities caused by transgene silencing were often reversible in the offspring and most abnormally-segregating progenies showed normal, as well as abnormal, inheritance in a following generation. GUS$^-$ individuals giving rise to GUS$^+$ progenies were also frequently observed. Two lines that exhibited distorted inheritance ratios showed no or very limited transgene silencing suggesting that reduced transgene transmission to progeny might have been responsible for abnormal segregation.

Coexpression of two marker genes. The *bar* gene cosegregated with the presence of the PAT phenotype in 30 % of analyzed lines. Individuals which had the *bar* transgene but showed no transgene phenotype were found in 70 % of lines. Most of *bar* silencing cases were discovered in lines in which *uidA* silencing also was found. However, there were five lines with plants showing *bar* silencing but always normal *uidA* expression and one line in

which *uidA* but not *bar* silencing was encountered. Overall, silencing of *uidA* and/or *bar* transgenes was detected in Southern blot analyses in three-fourths of transgenic lines affecting nearly 70 % of all *uidA*/*bar*$^+$ plants in these lines. Either one or both transgenes could be silenced. Silencing of only one of the two transgenes resulted in lack of coexpression of the transgene phenotype and was found in two-thirds of all analyzed lines. Most often the *bar* gene was silenced while the *uidA* gene was normally expressed; PAT$^+$ plants showing no GUS expression were three-fold less frequent.

Discussion. Transgenic oat lines exhibited stable expression of the analyzed transgene phenotypes and Mendelian transgene inheritance consistent with the presence of one transgenic locus. They also produced fixable homozygotes. Stable transgene expression and Mendelian inheritance were consistent across several generations showing that the oat transformation technology can be practically applied for crop improvement. Segregation in the first generation of transgenics was a good predictor of segregation ratios in subsequent generations allowing selection of promising transgenic lines without extensive progeny testing.

About one-half of the analyzed lines exhibited distorted GUS inheritance ratios and 70 % of lines showed lack of coexpression of the two transgene phenotypes even though the corresponding genes were always present together in the plant genome. Transgene silencing appeared to be a primary cause of the irregularities in transgene expression and inheritance. Reduced transgene transmission potentially also contributed to the distorted segregation ratios, although to a much lesser degree. Overall, close to 80 % of all transgenic oat lines showed different types of transgene expression and inheritance abnormalities. Transgene silencing was previously described in several plant species, including tobacco, petunia and Arabidopsis (Finnegan and McElroy, 1994; Flavell, 1994; Matzke and Matzke, 1995). The phenomenon is most frequently associated with the presence of additional copies of genes or transgenes. Matzke and Matzke (1995) proposed that a general gene silencing phenomenon might be responsible for inactivating expression of multiple copies of genes in polyploids. Oat as a hexaploid could, therefore, have a very strong transgene silencing mechanism. However, we have seen no obvious relation between occurrence of transgene silencing in oat and the number of transgene copies in a transgenic line although several transgene copies were often integrated into the oat genome. High frequency of transgene silencing in oat indicates a need for a system stabilizing transgene expression to be used in future transformation endeavors.

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Efficient transformation in barley variety 'Lenins' by particle bombardment.

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Introduction. Genetic transformation in monocot plants can be achieved mostly depending on efficient regeneration procedures of their protoplasts and suspension culture cells. However, we have not yet developed an efficient regeneration procedure of barley protoplasts and cultured cells. We recently found one barley variety 'Lenins' of which calli derived from immature embryos showed high regeneration ability. In this report, using particle bombardment and immature embryos of Lenins, we describe efficient transformation conditions in barley.

Materials and Methods.

Plant Materials : Immature embryos of Lenins were excised out from surface sterilized young caryopses. Three types of immature embryos (A type, 0.5~0.8mm in length; B type, 1~1.3mm and about 0.3mm segments of the B type immature embryo) were precultured scutellum-side up on callus induction medium (a modified MS medium (Jähne *et al.* 1991) supplemented with 30g/l maltose and 2mg/l 2,4-D and solidified 2g/l Gelrite). Thirty immature embryos (target tissues) were placed in a Petri dish in two ways; in a circle around the center of Petri dish (C arrangement), or a square in the center of Petri dish (S arrangement). To evaluate the effect of preculture period, target tissues were incubated at 25℃ in 16 hours light for one to seven days prior to the bombardment .

Plasmid : Plasmids that consist of a reporter gene, *GUS*, or a selective gene, *bar* that expresses resistance to herbicide bialaphos under the control of promoters of rice *Act1* or maize *ubiquitin* gene were used.

Particle bombardment : Plasmid DNA was absorbed to gold particles (1.6μm) as described by Klein *et al.* (1988). Equal amounts (μg) of DNA from the two types of plasmids with either *bar* or *GUS* gene under the same promoter were mixed for co-transformation. The target tissues were positioned 4.5cm (the C arrangement) and 14cm (the S arrangement) below the microprojectile stopping plate and were bombarded twice, using a PDS 1000He (Bio-Rad) with 1100-psi rupture discs.

Callus culture and selection of transformants : Two days after the bombardment, the tissues were transferred to the selection medium (callus induction medium supplemented with 5mg/l bialaphos) and incubated for four weeks. Bialaphos-resistant calli were

selected for through two selection cycles and were transferred to the shoot regeneration medium (callus induction medium supplemented with 2mg/l IAA and 0.05mg/l zeatin instead of 2,4-D). Regenerated shoots were transferred into Plant Boxes containing half strength hormone-free modified MS medium with 5mg/l bialaphos.

GUS assay : Transient *GUS* expression was assayed 24 hours after the bombardments. The tissues were treated with GUS assay buffer at 38°C in the dark for 12 hours (Jefferson *et al.* 1987).

PCR southern analysis : PCR products were amplified using primers designed inside of the *bar* gene and hybridized with DIG-labeled *bar* gene.

Results and Discussion. Higher transient *GUS* expression was detected in the target tissues precultured for two to four days prior to bombardment than those precultured for shorter or longer days. A few days preculture appears to be necessary for target tissue to obtain more viability and recovering ability from wounds by the bombardment. Prolonged preculture period may make the calli hard for the penetration of the particles. Comparing two arrangements of target tissues, higher transient *GUS* expression was observed in the C arrangement than in the S arrangement, suggesting that target tissues placed in a circle received particles more efficiently and uniformly than the other. In respect with promoter activity, rice *Act1* promoter activated *GUS* gene more profoundly than the *ubiquitin* promoter.

108 plants resistant to bialaphos were obtained in a total (Table 1) and these were fertile except one plant. In all fifteen randomly selected resistant plants, *bar* gene was detected by PCR-southern analysis. The average efficiency of transformation based on the number of embryos was 10.4%. Efficiency of co-transformation of *bar* and *GUS* genes was 27.8%.

Bialaphos-resistant plants were obtained in higher rate when the target tissues were precultured for four to five days (Table 1). The preculture appears to be necessary to increases the efficiency of transformation as observed in the transient expression of *GUS*. However, it is not clear why longer preculture time (four or five days) are required, comparing with preculture time in *GUS* expression (two to four days). Comparing the bombardments trials that resulted in the top six high transformation (Table 2), *Act1* promoter and embryo size appears to be related in high transformation. *Act1* promoter may contribute higher bialaphos-tolerance to the calli and resulted in high transformation. The A type embryos that appear to have high cell activity and consequent recovering ability from bombardment damage might reflect to the high

transformation.

The present results indicate that it is possible to obtain barley transformants relatively easily when used immature embryo culture system of variety Lenins.

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Table 1. Effect of preculture periods prior to bombardment on the shoot regeneration and production of bialaphos-resistant plants

Preculture period (days)	No. of embryos (A)	No. of resistant calli with shoots		No. of plantlets resistant to bialaphos	No. of mature plants (B)	B/Ax100
		green	albino			
1	175	51	1	32	24	13.7
2	200	52	0	19	13	6.5
3	200	36	1	24	17	8.5
4	136	39	1	24	17	12.5
5	126	29	2	28	23	18.3
6	119	31	1	16	8	6.7
7	85	21	0	11	6	7.1
Total	1041	259	6	154	108	10.4

Table 2. Numbers of regenerated shoot, plantlet resistant to bialaphos and mature plants in the top six high transformation traials

PP[1] (days)	ES[2]	AT[3]	PS[4]	No. of embryo (A)	No. of resistant calli with shoots		No. of plantlets resistant to bialaphos	No. of mature plants (B)	B/Ax100
					green	albino			
1	A	S	A	25	14	1	6	6	24.0
1	A	C	A	25	9	0	9	7	28.0
1	A	C	U	25	16	0	12	7	28.0
3	A	S	A	25	13	0	13	8	32.0
5	B	S	A	18	3	0	7	6	33.3
5	C	C	A	25	7	0	9	9	36.0

1) PP : Preculture period.
2) ES : Explant size, A and B indicate 0.5~0.8mm and 1~1.3mm immature embryos, respectively. C indicate about 0.3mm segments of 1.0~1.3mm immature embryos.
3) AT : Arrangement of target, C and S indicate the C and the S arrangement, respectively.
4) PS : Promoter sequence, A and U indicate promoter of rice Act1 gene and maize ubiquitin gene, respectively.

Mature Embryos: An Alternative Tissue Culture Explant For Efficient Transformation of Oat. K.A. TORBERT, H.W. RINES*, AND D.A. SOMERS, Department of Agronomy and Plant Genetics, University of Minnesota and *Plant Science Research Unit, USDA-ARS, 411 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN 55108, USA.

Introduction. The oat (*Avena sativa* L.) transformation system established by Somers et al. (1992) at the University of Minnesota utilizes friable, embryogenic callus tissue initiated from immature embryos of the GAF/PARK genotype (Rines and Luke, 1985; Bregitzer et al., 1989) as a source of totipotent target cells. Friable, embryogenic callus has been used to establish transgenic tissue culture lines for development of an alternative selection system (Torbert et al., 1995) and for analysis of transgene expression patterns and transgenic disease resistance. A disadvantage of immature embryo-derived tissue cultures is that production of callus sufficient for microprojectile bombardment requires at least 12 weeks, and tissue cultures are generally used up to one year. Genetic aberrations due to somaclonal variation increase with tissue culture age. We suspect that reduced fertility of regenerated transgenic plants is due to the age effect in friable, embryogenic callus initiated from immature embryos. We investigated methods to reduce the duration of the tissue culture period required to produce transgenic plants. Also, we sought to find a more convenient explant for tissue culture establishment. Cummings et al. (1976) stated that mature embryos could provide a useful alternative for tissue culture establishment. Mature embryos were tested for tissue culture and transformation response.

Materials and Methods. Mature seed of GP-1, which is a selection from the GAF/PARK genotype, and two MN breeding lines, Starter-1 and MN89127, were sterilized for 30 seconds in 95% ethanol, 5 minutes in 2.5% hypochlorite, and rinsed 3X in sterile double deionized water for 5 minutes/rinse. Sterilized seeds were placed in 50 ml sterile double deionized water and left on a shaker (145 rpm) overnight. On the next day, mature embryos were excised and placed scutellum side down on MS2D medium containing MS salts (Murashige and Skoog, 1962), 150 mg l^{-1} asparagine, 0.5 mg l^{-1} thiamine-HCl, 20 g l^{-1} sucrose, 2.0 mg l^{-1} 2,4-D, pH 5.8, solidified with 0.2% Gelrite . The embryos were cultured on the same plate for 8 weeks. Shoots were excised as they appeared during the first 2 to 4 weeks.

Embryogenic appearing callus that developed after 8 weeks was used for DNA delivery via microprojectile bombardment. The construct used in this study was pNGI (Klein et al., 1989), which contains the *npt II* plant selectable marker and the ß-glucuronidase (*uid A*) reporter gene. Gold particles for DNA delivery were coated with this plasmid using procedures in the Biolistic® PDS-1000/He Particle Delivery System manual (BioRad Laboratories, Hercules, CA). Callus-derived tissue was plated onto solid MS2D medium containing 0.2 M sorbitol and 0.2 M mannitol as a 0.4 M osmoticum pretreatment (Vain et al., 1993) for 4 h prior to microprojectile bombardment. After this treatment, callus was bombarded with the PDS-1000/He Particle Delivery System (DuPont). Tissue remained on the osmoticum medium overnight and was transferred to MS2D media for 7 d at 20 °C in the dark. Selection for paromomycin resistant tissue cultures was conducted according to

Torbert et al. (1995). Plant regeneration, NPT II ELISA assay, and ß-glucuronidase (GUS) assays are described in Torbert et al. (1995).

Results and Discussion.

Tissue culture response of mature embryos

The frequency of GP-1 mature embryos that initiated embryogenic callus was more than 50% after ~8 weeks. Starter-1 and MN89127 mature embryos exhibited 33% and 20% callus initiation frequency, respectively, but the callus initiated was primarily non-embryogenic. Extensive experimentation concerning tissue culture response of immature oat embryos has been reported (Cummings et al., 1976; Rines and McCoy, 1981; Bregitzer et al., 1989). Bregitzer et al. (1995) discussed genotype by media interactions in callus initiation from immature embryos. These results suggest that different media may result in increased callus initiation from mature embryos of other genotypes. Accordingly, we plan to try mature embryos of various agronomically elite genotypes on different media combinations in an attempt to increase their tissue culture response.

Selection, regeneration, and fertility of transgenic tissue cultures

Twenty-one microprojectile bombardments of mature embryo-derived callus from GP-1 yielded 68 paromomycin resistant tissue cultures. This converts to 3.2 transgenic tissue cultures per bombardment, which is similar to our previous results (Torbert et al., 1995). Seventy-one percent of the transgenic callus lines exhibited GUS expression. Plant regeneration frequency was 64%, a 28% improvement over results reported for immature embryo-derived callus. The fertility of regenerated plants was greater than 90%, a vast improvement over our previous results (Somers et al., 1992; Torbert et al., 1995). Overall, the mature embryo system increased output of fertile transgenic plants more than 100% over the immature embryo system.

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Transformation of spring barley using the enhanced regeneration system and microprojectile bombardment. B.J. WEIR[1,2], K.J. LAI[1], K. CASWELL[1], N. LEUNG[1], B.G. ROSSNAGEL[2], M. BÅGA[1], K.K. KARTHA[1] and R.N. CHIBBAR[1]. [1] Plant Biotechnology Institute, NRC, 110 Gymnasium Place, Saskatoon, SK, S7N 0W9, Canada, [2] Crop Development Centre, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, S7N 5A8, Canada. NRCC No. 40016.

Introduction. The transformation of barley through recombinant technologies can be beneficial in basic studies related to gene expression and regulation as well as enabling crop improvement. Stable transformation of barley has been reported using electroporation of protoplasts (Salmenkallio-Marttila et al., 1995); and particle bombardment of explants such as microspores (Jähne et al., 1994), microspore derived embryos (Wan and Lemaux, 1994) and immature zygotic embryos (Ritala et al., 1994; Wan and Lemaux, 1994). However, these transformants have been limited to a few malting barley cultivars not grown in western Canada.

Our group has applied the enhanced regeneration system (ERS) for the transformation of barley. The ERS is based on regeneration of plants from isolated scutella derived from immature zygotic embryos (Nehra et al., 1994). Isolated barley scutella were bombarded with gold particles coated with three different plasmid constructs. Putative transformed plants were obtained following culture on an optimized regeneration and selection media for barley. Molecular and histochemical assays were used to confirm the expression and integration of the inserted DNA into the barley genome.

Materials and Methods. Plants of three advanced breeding barley lines, SB92559, a hulless feed barley (BR3); TR328, a hulled feed barley (BR4); and TR232, a malting barley (BH1), were grown in growth chambers (22/17°C, day/night, with 17hr light). Immature seeds were dehulled, surface sterilized, and scutella (1.2 to 2.2 mm) were isolated from the immature embryos and plated on MS (Murashige and Skoog, 1962) medium supplemented with 1.1 mg/l 2,4-D and 0.22 mg/l BA. The medium was solidified with agar (0.8%) and the pH adjusted to 5.8 prior to autoclaving. Cultures were incubated in the dark at 25°C for 3 days prior to bombardment.

Thirty scutella per plate were arranged in a circle approximately 2 cm in diameter and bombarded with DNA-coated gold particles (1.0 μm). The plasmids pBARGUS (Fromm et al., 1990), pRC62 and pGK107 (both developed at the Plant Biotechnology Institute) were used in this study. The gene construct pBARGUS contains the scorable marker *gus* and the selectable *bar* gene which confers resistance to L-PPT (the active ingredient in glufosinate ammonium based herbicides). The plasmids pRC62 and pGK107 contain the *gus:nptII* fusion gene driven by different promoters, and the *nptII* gene confers resistance to aminoglycosidic antibiotics such as Geneticin.

Scutella bombarded with pBARGUS were allowed to form somatic embryos which were cultured for two weeks on 3 mg/l L-PPT and then for two weeks on 1 mg/l L-PPT before putative transgenics were rescued and grown to mature plants. Scutella bombarded with pRC62 or pGK107 were transferred to induction medium containing 10 mg/l Geneticin for 3 weeks, and the surviving somatic embryos were transferred to regeneration medium containing 15 mg/l Geneticin for another 3 weeks. Putative transformants were subsequently rescued and grown to mature plants.

Histochemical and fluorometric assays for ß-glucuronidase (GUS) activity, phosphinothricin acetyltransferase (PAT) assays, plant DNA isolation and Southern blot hybridization were done according to standard protocols.

Paste Assay: Young leaves (4-5 leaf stage) of putative pBARGUS-transformants and control plants were treated with an aqueous solution containing L-PPT. A 2-3 cm segment of the leaf was brushed on both sides with a solution containing either 4 or 40 mg/l L-PPT. A second application of L-PPT was applied 1/2 hour after the first application. Leaves were assessed for damage one week after herbicide application.

Germination assay: Immature zygotic embryos (1.5 to 3.0 mm) of R_0 and R_1 plants and control barley plants were isolated and germinated on selection media. Embryos from pBARGUS-transformants were germinated on media containing 0.5 mg/l L-PPT, whereas pRC62- and pGK107-transformants were germinated on media containing 10 mg/l Geneticin.

Results and Discussion. A summary of bombardment experiments is given in Table 1.

Table 1. Summary of bombardment experiments.

Barley Genotype	Plasmid Construct	Number of Scutella Bombarded	Number of Calli on Highest Selection Level	Selection Agent in Media	Post-selection Survival in Soil
BH1	pBARGUS	2870	101	L-PPT	16 (H16,H18)
BH1	pRC62	2382	9	Geneticin	1
BH1	pGK107	150	5	Geneticin	1
BR3	pBARGUS	2800	45	L-PPT	6
BR3	pRC62	1890	11	Geneticin	2
BR3	pGK107	660	20	Geneticin	2 (H15)
BR4	pBARGUS	570	29	L-PPT	4 (H17)
BR4	pRC62	1320	NR	Geneticin	1 (H4)

NR = not recorded

One BR3 plant (H15) transformed with pGK107 showed histochemical expression of GUS in one leaf and in three immature seeds each from separate tillers. In addition, a fluorometric assay revealed GUS activity in the mature leaves of H15 and PCR analysis revealed the presence of the *gus* gene in the genomic DNA of H15. Southern blot analysis to further confirm the integration of the pGK107 construct into the barley genome is underway. In another putative transformant, Southern blot analysis indicated the integration of the *gus* gene into a BR4 plant (H4) transformed with pRC62.

The leaves of two BH1 plants (H16, H18) and one BR4 plant (H17) transformed with pBARGUS showed no damage when treated with a 4 mg/l solution of L-PPT, but did show damage at the higher level (40 mg/l). Non-transformed control plants showed damage at both levels of L-PPT. The results suggest that the *bar* gene is weakly expressed in these transformants.

To date, fifteen of the putative transformants have grown to maturity and produced seed. Immature embryos from these plants were germinated on selection media. A total of 48 out of 600 embryos have survived this second round of selection. Further molecular and histochemical assays are being conducted to confirm the integration of foreign DNA in the remaining R_0 transformants and the R_1 generation.

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Optimization of the enhanced regeneration system for selected feed and malting barley lines. B.J. WEIR[1,2], K.J. LAI[1], K. CASWELL[1], B.G. ROSSNAGEL[2], K.K. KARTHA[1] and R.N. CHIBBAR[1]. [1] Plant Biotechnology Institute, NRC, 110 Gymnasium Place, Saskatoon, SK, S7N 0W9, Canada, [2] Crop Development Centre, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, S7N 5A8, Canada. NRCC No. 40017.

Introduction. Successful transformation of plants requires a very efficient method of *in vitro* regeneration. Stable transformation of barley has been reported using explants such as protoplasts (Salmenkallio-Marttila et al., 1995), microspores (Jähne et al., 1994; Wan and Lemaux, 1994), and immature zygotic embryos (Ritala et al., 1994; Wan and Lemaux, 1994). However, these transformation events have been limited to a few malting barley cultivars not grown in western Canada.

Recently, the enhanced regeneration system (ERS), which is based on culturing isolated scutella derived from immature zygotic embryos of wheat and barley, was developed at the Plant Biotechnology Institute. This system is reported to work with a wide variety of genotypes but at different efficiencies. In this report we describe the optimization of the ERS for selected feed and malting barley genotypes. We have studied the effects of storage conditions of harvested barley spikes prior to scutella isolation, scutella size and culture media composition on the induction of embryogenic callus and subsequent somatic embryo development.

Materials and Methods. Ten advanced barley breeding lines were grown in growth chambers (22/17°C day/night, 17 hr light). Barley spikes were harvested and stored in plastic bags at 4°C. Immature seeds were removed from the spike, dehulled, and surface sterilized in 4% Javex solution and then rinsed with sterile water. Isolated scutella were placed with the abaxial surface on MS (Murashige and Skoog, 1962) media containing varying levels of the auxin, dichlorophenoxyacetic acid (2,4-D).

Spikes from two lines, TR232 (BH1) and SB92559 (BR3), were harvested and stored at 4° C for either 3 to 6 days or 7 to 10 days. Scutella were measured during isolation and then cultured on one of three MS based media containing varying levels of 2,4-D and a cytokinin, 6-benzylaminopurine (BA). The media examined contained 5×10^{-6}M 2,4-D + 5×10^{-7}M BA (BAR1), 2.5×10^{-6}M 2,4-D + 1×10^{-6}M BA (BAR2), and 3.75×10^{-6}M 2,4-D + 7.5×10^{-7}M BA (BAR3).

Results and Discussion. The ten advanced barley breeding lines varied in their response to 2,4-D concentrations in the regeneration media. Generally, the malting barley lines had higher regeneration frequencies than the feed barley lines. The two lines BH1 and BR3 had the highest regeneration frequencies among the malting and feed lines, respectively, and were used for further experimentation. Genotypes BR3 and BH1 differed in their response to media composition and pre-isolation storage (Tables 1 and 2). The highest % scutella generating embryogenic

Table 1. Effect of media composition, pre-isolation storage of spikes, and scutella size on embryogenic callus formation and shoot regeneration from scutella isolated from immature barley embryos of genotype BR3.

Medium	Pre-isolation Storage (days)	Scutella size examined (mm)	Number of Scutella	% Scutella generating Callus	% Callus generating Shoots	Ave. no. shoots per callus	Scutella size generating shoots
BAR1	3 - 6	1.1 - 2.6	109	79.7	18.4	3.5	1.4 - 1.9
BAR1	7 - 10	1.3 - 3.2	135	14.8	0	-	-
BAR2	3 - 6	0.5 - 2.5	240	55	7.5	2.1	1.3 - 1.5
BAR2	7 - 10	1.1 - 2.7	160	40.6	15.4	2.7	1.2 - 2.2
BAR3	3 - 6	0.8 - 2.9	187	75.4	23.4	3.5	1.2 - 2.1
BAR3	7 - 10	0.5 - 2.6	280	0	0	-	-

Table 2. Effect of media composition, pre-isolation storage of spikes, and scutella size on embryogenic callus formation and shoot regeneration from scutella isolated from immature barley embryos of genotype BH1.

Medium	Pre-isolation Storage (days)	Scutella size examined (mm)	Number of Scutella	% Scutella generating Callus	% Callus generating Shoots	Ave. no. shoots per callus	Scutella size generating shoots
BAR1	3 - 6	0.5 - 2.2	249	42.9	48.6	5.0	1.2 - 1.8
BAR1	7 - 10	1.3 - 2.8	100	25	20	7.2	1.6 - 2.2
BAR2	3 - 6	0.6 - 2.4	265	32.5	22.1	3.9	0.6 - 1.6
BAR2	7 - 10	1.2 - 2.4	180	18.9	26.4	2.0	1.5 - 2.3
BAR3	3 - 6	0.7 - 2.8	242	8.3	50	3.6	1.4 - 2.0
BAR3	7 - 10	1.3 - 2.7	180	14.4	34.6	2.3	1.5 -2.3

callus occurred on BAR3 media for line BR3 and on BAR1 media for BH1. The results of this study suggest that even after 6 days of storage there can be a dramatic drop in the % of regenerating scutella depending on the media and barley line. On BAR3 media, for example, the % of BR3 scutella generating embryogenic callus dropped from 75% (3 to 6 day storage) to 0% (7 to 10 day storage). Generally, a pre-isolation storage of 3 to 6 days resulted in a higher % of scutella generating embryogenic callus for both BR3 and BH1.

The range in size of the scutella generating shoots varied depending upon the regeneration medium, the number of days of pre-isolation storage, and the barley line. However, by selecting BR3 scutella in the range of 1.5 to 2.0 mm the % scutella generating callus, % callus generating shoots, and the average number of shoots per regenerating callus on BAR3 medium increases to 86.9 %, 27.4 % and 4.8 respectively. Similarly, these figures increase to 46.6 %, 60 %, and 5.1 for BH1 plated on BAR1 medium if a size range of 1.4 to 1.8 mm is selected.

Cereal transformation protocols commonly indicate an optimal physiological state of immature embryos with regard to days post-anthesis (DPA). In barley anthesis takes place in the boot, which makes estimation of DPA difficult. Thus, we have used size as an indication of the physiological state for optimal regeneration from isolated barley scutella.

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Section X: Breeding and Quantitative Genetics

SCREENING FOR MALTING QUALITY IN EARLY GENERATIONS

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INTRODUCTION

The number of quality characteristics to which a plant breeder has to respond increases continuously as research provides new information. Regarding malting quality, barley varieties must possess many specific quality characteristics required by the malting and brewing industry. Many of these are also beneficial in feed barley production. Due to the high number of quality parameters barley breeders face a problem in choosing from the very large numbers of new lines they produce. Good malting quality is however, one of the most important aims in many breeding programmes.

The first premise for an efficient malting barley breeding program is that the breeder is well aware of the quality parameters which best predict the good malting barleys. Other premises are that malting potential of new crosses can be evaluated rapidly, reliably and cheaply and as early as possible from small but numerous samples.

At Boreal considerable effort has been devoted to developing malting analyses. The Ministry of Agriculture and Forestry and the Finnish malting industry have, since the mid-1980's, financed several projects, carried out by malting barley researchers and barley breeders, aimed at developing and applying methods suitable for analyzing malting quality from small, early-generation seed samples. In this presentation the possibilities, advantages and problems of using these methods in analyzing breeding materials are evaluated and discussed.

MATERIALS AND METHODS

Malting quality was analysed for part of the breeding material obtained from ear rows (F_3 and F_6, barley milling energy and acid extract viscosity) and after selection from part of the subsequent small and large non-replicated multiplication plots (4 m^2 and 10 m^2, protein content, acid extract viscosity and small-scale micro-malting (20 g) giving extract percentage and wort viscosity results) before the best lines entered replicated yield trials at different locations. All these analyses were made for part of the ear row material 1995. The possibilities for prescreening to predict malting potential, both in preceeding generations and within a generation were evaluated.

RESULTS

Promising results for predicting malting quality were obtained from using protein content, barley milling energy, acid extract viscosity and small-scale micro-malting (20 g). The genetic control of milling energy appeared high and is a repeatable character.

We found however, that it should be used very carefully as a basis for discarding breeding material. Good malting barleys did not necessarily have low milling energy and barleys with low milling energy were not necessarily of good malting quality. Screening material based on acid extract viscosity seemed to be much more reliable, enabling high ß-glucan lines to be discarded. All the breeding lines with a high extract percentage in small-scale micro-malting also showed low acid extract viscosity.

Environmental factors greatly influence malting quality, indicating the difficulty in selecting barley for malting during a breeding program. As a result of selection based on prescreening methods, made by the breeder in a particular generation, the genetic variation in malting quality in subsequent generations narrows. Therefore, more precise and reliable analysis methods, such as small-scale micro-malting, are required to determine the differences in malting quality between genotypes. Milling energy and especially acid extract viscosity are however, good tools for judging malting quality when they are used as selection criteria for discarding lines within a generation. As regards protein content, GE-interaction is an important consideration. Consequently, because protein content is highly correlated with many characteristics it is essential for a breeder to determine the optimum environment - the level of protein content - where he wants the protein related characteristics, e.g. extract percentage and enzyme activity, to be determined.

More detailed results will be presented and discussed in the poster.

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The locus ea-k influence the expressivity of the gene lys.

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Introduction. The locus **ea-k** not only determined the maturity time, but also has wide pleiotropic effect on almost all growth characters of barley: growth rate of seedlings, roots, grain, etc. (Aksenovich A. et al., 1989). In the origin of pleiotropic effects of series of multiple alleles of the locus **ea-k** lays differential responses to the gibberellic acid action (Aksenovich A., 1996).

To the other side, the allele **lys** has different expressivity in different genotypes (Shumny V et al., 1992). Namely, **lys** does not penetrates in genotypes of European group of barley varieties (Bonus, Viner, etc.) with the normal (+) allele of the locus **ea-k**; **lys** satisfactory express in genotypes of early varieties from continental climate (Mary, Nepolegaushii, Hiproly) with the allele **ea-k** (ibid.).

Join two these facts, we asked: Does the locus **ea-k** influence the expressivity of the allele **lys**?

Methods. To clear up this question we selected 3 lines of barley, using following procedures: 1. hybridization of the line Hiproly CI 3947 (donor of the allele **lys**) with donors of 3 alleles of the locus **ea-k**: v. Viner (**+**, normal allele), v. Nepolegaushii (the allele **ea-k**), the line k-27058 (the allele **ea-k 6**). 2. 5 backcrosses on the recurrent parent v. Nepolegaushii to make the uniform genetic background for these 3 lines. 3. 12 generation of the line selection. In F_2 populations after hybridization and each backcrosses we selected 3 homozygotes: (**+/+**, **lys/lys**), (**ea-k/ea-k**, **lys/lys**) and (**ea-k 6/ea-k 6**, **lys/lys**). The **lys/lys** homozygotity have been identified using the lysine/protein grain content dates from Beckman 121m analyzer and polyacrilamide gel electrophoresis of grain proteins (Shumny V. et al., 1992). The homozygotity of alleles **+**, **ea-k**, **ea-k 6** have been identified by field observation of morphogenesis dates and by response of the aleurone to the exogenous gibberellic acid (GA_3, 10^{-6}M), measured by method (Chrispeels et al., 1967). The selection of lines continued 14 years.

Results. Mentioned above 3 lines have uniform genetic background, all homozygous by allele **lys** and each homozygous by alleles **+**, **ea-k**, **ea-k 6**, respectively.

Table. Parameters of **lys/lys** lines with different alleles of the locus **ea-k**.

Line, variety	response of aleurone to GA_3, units of induced a-amylase	lysine content, mg/grain	grain filling time, days	time from seedling to maturity, days
N 249 (**+**)	1755 ± 60	380 ± 42	35	81
N 659 (**ea-k**)	3935 ± 184	540 ± 51	29	70
N 421 (**ea-K 6**)	4621 ± 167	680 ± 56	24	62
v. Viner	1689 ± 60	320 ± 50	36	82
v. Nepolegaushii	2625 ± 132	380 ± 64	32	75

The "normal" allele (+) of the locus **ea-k** prevents the penetration of the allele **lys** in the character "high grain lysine". the content of lysine in grains of the line 249 (**+/+**, **lys/lys**) significantly does not differs from the recurrent parent. It does not mean, that the allele **lys** does not act in grains of the line 249 - the electrophoretic patterns of grain proteins of this line showed increased amounts of proteins CI-1,2; Z and b-amylase, characteristic to **lys** action (Shumny V et al., 1992). But this increase is not enough for sufficient increase of lysine in the grain of the one, comparatively with the recurrent parent. In view of this it is clear wellknown fact, why the segregation in F_2 hybrids between Hiproly CI 3947 with many European barley varieties was not monogenic (1 high lysine: 3 low lysine), but digenic (1:16). To express **lys** one need get rid of normal allele (+) of the **ea-k** locus, which is usually in European varieties of barley.

The allele **ea-k** in the line 659 allow the penetration of the **lys** allele with middle expressivity, the content of lysine in a grain increases to 142 %, if compare with the parent variety or the line 249.

And the allele **ea-k 6** gives the maximum expressivity of the **lys** in the grain of the line 421 (**ea-k 6/ea-k 6**): 179 % of lysine, if compare with the recurrent parent. What is the mechanism of this phenomenon? The allele **lys** affects on the level of synthesis of lysine-rich proteins PSI, CI 1, 2; Z, b-amylase in the grain, but not on the time of its synthesis, the same at 14-30 days of filling both in normal and mutant barley (Rasmussen U. et al., 1988). In the Table has shown the time of grain filling, different for **+**, **ea-k** and **ea-k 6** homozygotes: 35, 29 and 24 days, respectively. Possible, the intensive grain growth of 659 (**ea-k**) and 421 (**ea-k 6**) lines jointed in time with synthesis of lysine-rich pointed above glutelins, while in the line 249(+) the maximum of growth joints with the synthesis of low lysine hordeins.

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Research on the stability of the "hulledness" characteristic of the naked oats genotype.

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Introduction. Attempts at the cultivation of naked oats in Bulgaria have been made as early as the first half of the century (Pers. com.). Because of the lack of officially recorded data about its potential and cultivation, it is assumed that most probably the references are about incidental import of limited quantities of seed from Western Europe.

Research work on naked oats began in Bulgaria in 1985. The aims of the research are motivated by the actual and prospective demands of livestock breeding and wholesome human nutrition. These research aims are, most generally, focused on the following basic trends:

- research and enriching of the collection and creation of new naked oats genetic plasm. The accent is on the stability of the economically important for the country diseases - leaf rust, powdery mildew etc. The duration of the vegetation period is optimized to avoid damages by Frit fly. Because of the undisputable advantages of the winter over the spring varieties, cold-resistant (survival capacity at -10^0C) are sought, which can successfully winter in the southern regions of the country. Naturally, last but not least in importance is the grain quality and the related to it percentage of husked grains;
- research of the basic agricultural requirements, including fertilizer feeding and weed control, aimed at achieving the full realization of the genetic productivity potential of the crops, so that profits would be comparable to those from other small-grain corn crops;
- application and ways of utilization of naked oats as feed and food and establishing it in the market.

The presence of husked grains in the naked oats varieties creates a number of inconveniences related mainly to the various technological practices and purposes of utilization. This calls for seeking genetic selection and other methods for the substantial reduction of husked grains. It has been established that the "huskedness" characteristic (hulledness) is controlled by a single partly dominant gene N-1 together with a small number of modifying genes with varying dominant or epistatic effect (1,4,7,8).

The aim of the present research was to assess the average level and stability of the "huskedness" characteristic in naked oats varieties.

Materials and Methods. The percentage of husked grains in 15 naked oats genotypes from the National Genetic Bank was recorded yearly in natural conditions for the period 1991-1995.

The data has been processed using the Lidanski & Vassileva (5) - proposed approach, where the indexes have been differentiated statistically and classificationally. The stability by mean level has been expressed by the Finlay & Wilkinson (3) regression coefficient (b) and differentiated in 3 classes - high, medium and low. The index variance of stability - S^2d has been used (derived on the same basis-deviations from regression) for assessment of stability by variability (2). This has been differentiated in 4 quarterly classes: homogeneous (Hom.), weakly heterogeneous (Hlow), heterogeneous (H) and strongly heterogeneous (Hst). In determining the type of stability, however, priority is accorded to stability by mean level.

The average values ($\bar{x}$) and the genotype effects (GE) have been determined using the Mead & Gurnow (6) method. The characteristics under consideration have been ranged.

Results and Discussion. No genotypes have been found among the ones under research that do not produce husked grains. The percentage of husked grains varies largely by

Tabl. 1. **Mean values and stability of the "hulledness" characteristic of the varieties**

№	Varieties	Mean values (1991 - 1995)						Stability								Type of stability
								By mean level				By variability				
		x	R	St ±%	G	GE ±%	G	b	R	Kl.	St	S^2d	R	Kl.	St	
1.	*Mina*	5.42	13	0	II	95.3	I	0.08	4	H	0	3.44	9	Hom	0	Uniform
2.	*Adam*	2.48	10	-54.2	III	-10.6	II	2.73	13	L	34.14	0.69	4	Hom	0.20	Nonuniform
3.	*Rhiannon*	1.50	5	-72.3	III	-45.9	III	1.58	10	H	19.69	0.33	2	Hom	0.10	Nonuniform
4.	*Csnyetei*	1.38	3	-74.5	III	-50.3	III	0.35	5	H	4.39	0.31	1	Hom	0.09	Uniform
5.	*Ceasar*	2.34	9	-56.8	III	-15.7	III	3.06	14	L	38.26	4.75	10	Hom	1.38	Nonuniform
6.	*Rhea*	2.94	12	-45.8	III	6.0	II	2.69	12	L	33.65	17.49	14	H	5.09	Uniform
7.	*Tibor*	2.24	8	-58.7	III	19.3	III	2.11	11	L	26.41	5.25	11	Hom	1.53	Nonuniform
8.	*88106095*	1.06	1	-80.4	III	-61.8	III	1.52	9	L	19.02	1.03	5	Hom	0.30	Nonuniform
9.	*88106110*	1.36	2	-74.9	III	-51.0	III	0.36	6	H	4.55	2.53	7	Hom	0.73	Uniform
10.	*88106111*	1.42	4	-73.8	III	-48.8	III	-0.23	2	H	2.90	0.42	3	Hom	0.12	Uniform
11.	*Terra*	2.22	7	-59.0	III	-20.0	III	-1.29	1	L	16.15	3.00	8	Hom	0.87	Nonuniform
12.	*PGR 1703*	2.88	11	-46.9	III	3.8	II	3.17	15	H	39.64	6.26	12	Hom	1.82	Nonuniform
13.	*K. Hadaca*	5.94	14	9.6	II	114.1	I	0.01	3	H	0.07	32.32	15	Hst	9.39	Nonuniform
14.	*Cinsky Nahy*	1.96	6	-63.8	III	-29.4	III	1.31	8	L	16.43	1.53	6	Hom	0.45	Nonuniform
15.	*C. from Austria*	6.48	15	19.6	I	133.5	I	0.72	7	H	9.00	9.22	13	H low	2.68	Uniform

varieties and years. Through the years usually 1-2 genotypes were above the Mina standard, while above the population mean their number is bigger - from 3 in 1993 to 7 in 1992, that is, the genotypes forming a smaller percentage of husked grains predominate. Because of the large variance of the characteristic no definite tendency or regularity has been observed.

The mean level of the "huskedness" of the varieties for the five-year research period can be seen on Table 1. Three local varieties from the former USSR, Csenyetei and Rhiannon show a low percentage (up to 1.5%). The early Austrian genotype proved to be the most husky. The genotype effects vary within a very wide range and are higher in the positive direction.

The other basic characteristic of the varieties - their ecologic stability, assessed by mean level and by variability, is shown in the same table. Only the two stability-by-mean-level classes figure in the experiment - high and low, in a ratio of 6:9 respectively. According to the ranging of the varieties, on the average for the research period, half of the high stability varieties are those with a high percentage of husked grains and the other half - with a low one. Regrettably, though, this group does not contain the genotype exhibiting the lowest "huskedness" (var. 8). On the basis of the ecological stability by variability, expressed by the variance of the deviation from regression (Sd^2), the genotypes are distributed in the 4 quarterly stability classes in a ratio of 12:1:1:1 - homogeneous, weakly heterogeneous, heterogeneous and strongly heterogeneous respectively.

As can be seen, the genotypes of uniform homogeneous stability predominate (80%).

After the combination of the two types of stability, 6 of the varieties under research are characterized by a uniform, and the rest by a nonuniform reaction. In this procedure only 4 of the varieties exhibit co-incidence with the stability-by-mean-level class. In this case, the combinations with dissimilar stability are not ignored and the stability by mean level has been treated as the one with priority. So, for example, for the variety Kiryou Hadaca (var. 13) stability by mean level is determinant, that is, high.

With the combination of the low average levels of the "huskedness" characteristic, the negative genotype effects and the high stability by the two characteristics, it turned out that of selection value are the variety Csenyetei and the local varieties from the former USSR. In the other extreme we have the varieties Mina, Kiryou Hadaca and cultivar from Austria - high mean level and stability of their "huskedness". With the rest of the genotypes, regardless of the fact that their stability by variability is homogeneous, by the first characteristic - by mean level, stability is low.

To summarize, the approach used to assess the stability of the "huskedness" characteristic gives a more precise and adequate characterization of the varieties for "huskedness".

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Response to *in vitro* anther culture of three Brazilian cultivars of barley, *Hordeum vulgare vulgare*. E.M. ASSMANN and H. WINGE, Departamento de Genética-Universidade Federal do Rio Grande do Sul; Caixa postal 15053; 91501-970 Porto Alegre-RS, Brazil.

Introduction. Due to the great demand of brewing industries almost all Brazilian cultivars of barley are of the malting type. For several reasons, possibly including bad adaptation to the low pH soils, common in Southern Brazil, average yield is low. The total need of Brazilian malting industries is of about 200.000 tons/year; but only about half of that amount was produced in the 1995/96 harvest, what explains Brazil being the world second largest malt importer. The limited variability of our barley germplasm (Schildbach,1990; Luizzi and Castro,1991; Maris,1992), reduces the probability of obtaining new genetic combinations for higher yield. *In vitro* anther culture is an important technique to improve selection efficiency and to reduce the long time necessary to obtain a new cultivar. It is well known that the results of anther culture depend on the species, the genotype and sanitary conditions of donor plants, pretreatment, culture media composition and other culture conditions. The very poor results obtained when anthers of four Brazilian cultivars were cutivated *in vitro* using culture media and conditions recommended for cultivars of Northern hemisphere (Revers, 1993), showed the importance of testing some of those factors. For these reasons the present work aimed to analyze the response of three Brazilian barley cultivars to three pretreatments and three culture media compositions.

Material and methods. Three barley cultivars, from different companies, were tested: A-05 (152 spikes; 7660 anthers), BR-2 (105 spikes; 5813 anthers) and MN-599 (101 spikes; 5182 anthers). Pretreatments of 10, 20 and 30 days at 5°C, were as described by Huang and Sunderland (1982) for whole spikes. Anthers from spikes with pollen at uninucleate to first mitotic division states were plated on one of the induction media(25°C, dark). Three induction media were tested: I-1= modified (sugar and growth regulator concentration) from Olsen (1987), and two modified from Hoekstra et al.(1992): I-2=gelling agent, and I-3=gelling agent and growth regulators. Calli/embryos equal to or larger than 1mm were transferred to the respective 1st.regeneration medium (25°C, dim light).Green plantlets were transferred to the respective 2nd.regeneration medium (without growth regulators) in test tubes, and maintained at 25°C, 16hr.light: 1,8klux, for further development. When several shoots and roots had developed the plants were potted in soil with vermiculite (2:1), maintained in culture room for acclimatization and then transferred to greenhouse. The results of calli/embryos induction were analyzed by three way ANOVAs, main effects and interactions tested by SAS program, using spikes as replicates. For data from regeneration ANOVAs without replication and Tuckey test for main effects or interactions (SANEST program) were used. **Results and discussion.** Table 1 summarizes the main results. Although a smaller sample of spikes were tested for 30 days pretreatment, it was clearly harmful to the tested cultivars, since most anthers degenerated. In some of the tested conditions calli/embryos were obtained for cultivars A-05 and BR-2, but none green plantlet was regenerated. These results are similar to the obtained by Szarejko and Kasha (1991). The 30 days results were not included in the statistical analyses. Induction of calli/embryos: all responsive anthers were counted at the 21st (begining of calli/embryo production) and before elimination at the 150th day of induction. There was an effect of cultivar on the number of responsive anthers at 21 days after induction (A-05> BR-2> MN-599) and 150 days (A-05≡ BR-2 > MN-599). No effect of cultivar on the number of transferred

Table 1. Embryogenic callus formation and plant regeneration from anther culture of 3 Brazilian barley cultivars, in 3 different cold pretreatments and 3 culture media. [1. N'= total number of anthers plated less eliminated ones due to contamination]

CULTIVAR	PRE. TREAT. MENT (days)	MEDIA	SPIKE (N)	INDUCTION				STRUCTURE TRANSFERRED		REGENERATION				
				PLATED ANTHER (N)	RESPONDING ANTHERS AT 21 DAYS (%)	RESPONDING ANTHERS AT 150 DAYS (N^{-1})	RESPONDING ANTHERS AT 150 DAYS (%)	X/100 ANTHERS	(N)	X/ 100 STRUCTURES SHOOTS	PLANTLETS ALBINO	PLANTLETS GREEN	GREEN/ 100 ANTHERS PLANTLETS	GREEN/ 100 ANTHERS PLANTS
A05	10	I-1	22	1,196	26.5	807	53.9	126.0	1,507	2.45	1.46	0.13	0.167	0.084
		I-2	22	1,109	35.2	675	45.8	189.5	2,102	2.95	1.19	0	0	0
		I-3	22	1,247	44.4	581	76.8	189.3	2,360	7.33	0.25	0	0	0
	20	I-1	22	1,169	22.2	491	37.7	62.8	734	4.50	4.09	0.27	0.171	0.171
		I-2	23	1,203	40.5	450	52.0	159.0	1,913	6.27	6.69	0.05	0.083	0
		I-3	23	1,197	51.4	666	68.0	210.9	2,524	6.22	3.92	0.36	0.251	0.167
	30	I-1	4	185	58.9	185	7.6	0	0	0	--	--	--	--
		I-2	4	173	30.6	0	0	144.5	250	0	0.40	0	0	0
		I-3	4	185	49.2	185	73.0	561.6	1,039	0	0.96	0	0	0
BR2	10	I-1	19	1,060	13.6	519	60.7	82.6	875	6.40	3.09	1.14	0.943	0.189
		I-2	17	954	22.9	754	53.9	132.6	1,265	10.12	2.53	0.08	0.105	0.105
		I-3	17	976	33.2	425	67.8	107.7	1,047	8.89	2.58	0.19	0.205	0
	20	I-1	15	813	10.6	338	41.7	47.1	383	2.35	8.09	0	0	0
		I-2	13	682	35.6	438	63.5	207.0	1,412	13.39	8.43	3.75	7.771	5.279
		I-3	14	739	44.1	333	74.8	209.2	1,546	10.54	5.11	2.01	4.195	2.571
	30	I-1	3	192	19.8	0	0	0	0	--	--	--	--	--
		I-2	5	294	33.7	61	49.2	165.7	487	0	0.62	0	0	0
		I-3	2	103	0	0	0	0	0	--	--	--	--	--
MN599	10	I-1	19	916	6.3	787	32.9	38.4	352	3.98	16.61	0.85	0.328	0
		I-2	16	844	17.8	631	37.9	200.8	1,695	10.44	5.66	0.47	0.948	0.474
		I-3	15	862	36.0	237	71.3	287.2	2,476	5.62	1.05	0.08	0.232	0.116
	20	I-1	15	820	10.4	550	38.7	35.4	290	7.24	24.83	0.35	0.122	0
		I-2	16	820	11.3	695	21.6	133.1	927	8.53	9.71	0.65	0.732	0.244
		I-3	15	765	28.5	491	36.5	213.6	1,634	11.63	4.53	1.47	3.137	2.092
	30	I-1	1	28	0	28	0	0	0	--	--	--	--	--
		I-2	2	60	0	60	0	0	0	--	--	--	--	--
		I-3	2	67	0	67	0	0	0	--	--	--	--	--

calli/embryos was observed, but there was interaction cultivar x pretreatment: 10 days of cold pretreatment (MN-599 > A-05 > BR-2) and 20 days (MN-599 ≡ A-05 ≡ BR-2). So, it was shown that the cultivar MN-599, with a lower number of responsive anthers, produced when pretreated for 10 days, a larger number of calli/embryos able to be transferred to the regeneration media (≥ 1mm of diameter).There was no effect of pretreatment on the number of responsive anthers or on the number of calli/embryos transferred to the regeneration media besides the interaction already discussed. There was a main effect of culture media on the number of responsive anthers at 21days (I-3 > I-2 > I-1) and at 150 days (I-3 > I-2 ≡ I-1) and also on the number of transferred calli/embryos (I-3 ≡ I-2 > I-1). Although the medium I-3 produced more responsive anthers than the other two, it did not difer from I-2 in the production of transferable calli/embryos. In conclusion, a clear effect of genotype on the frequency of responsive anthers and on calli/embryos induction was observed, as a main effect and interaction with the pretreatment.There was also a main effect of culture medium.Bregitzer (1992), analyzing 15 cultivars and 3 culture media, besides the strong genotypic effect on callus formation also observed a significant interaction between genotype x culture medium, not observed in our results. Regeneration of plantlets: the analysis of the number of albino plantlets per calli/embryo (=embryogenic structures) transferred showed main effects of cultivars and of culture media that are clearly due to the interaction cultivar x culture media (I-1: MN-599 > BR-2 ≡ A-05; I-2 and I-3: MN-599 ≡ BR-2 ≡ A-05). The cultivar MN-599 produced more albino plantlets but only when the worst induction medium (I-1) was used.There was also a main effect of pretreatment (20 days > 10 days), indicating that the pretreatment of 10 days should be preferred for these cultivars. The analyses of the number of green plantlets produced per transferred structure and also per 100 plated anthers did not show any main effect or interaction. Powell (1988) by means of diallel analysis concluded that the frequency of microspore derived green and albino plants is under genotypic control, and that the green plant regeneration can be improved by selection.These conclusions were supported by many other studies.Thompson et al.(1991) disclosed differential transmission of alleles in microspore-derived lines of barley crosses in favor of the more responsive parent. The distorted ratios were associated with two chromosomes, strengthening the genotypic control. Nevertheless, our results indicate that the interaction genotype x culture medium, as well as the time of cold pretreatment are also important factors in albino plant production.

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Selection and properties of high protein barley lines. I.BELICKA, Department of Crop Production, Latvia University of Agriculture, 2 Liela Street, Jelgava, LV-3001, Latvia.

Introduction. Barley for nutritional uses have specialized criteria and it depends on nutritional requirements of the animals or fowl to which grain is to be fed [Newman and Newman,1992]. Barley with high protein content is preferred for feeding and this important trait in barley is extremely desirable in world area where protein is deficient.
This article deals with some breeding results in barley improvement.

Materials and Methods. In Latvia University of Agriculture a breeding program for improvement of barley feed quality began in 1986. We have chosen hull-less with brown grain mutant X-4 by Hiproly, received from Lithuanian Institute of Botany as a high protein donor. Indices characterized mutant X-4 by author are: protein content 15,7%, lysine content 722,0 mg 100 g^{-1}, capacity of tillering 5,4 ± 1,7; kernel number per spike 17,4±2,6, 1000 kernel weight - 58,8 g (Shukene, 1985). Seven cross combinations were realized in 1986.
Mutant X-4 is used as female or male parent. The other components of hybrid combinations were hulled barley varieties with good agronomical traits: Imula, Saima, Tirana, Stende 7197, KM-246-3/78, Ca 34739, Sv.77812.
During 1987 - 1990 hybrid F_1, F_2, F_3, F_4 population were evaluated. The populations were divided into hulled and hull-less barley forms. Both types of elite plants were selected in F_4 and F_5. During 1991-1995 the progenies of them were evaluated.
Trials were established in the Experimental Field of the Faculty of Agriculture. The soil at the site is sod-calcareous heavy loam, humus content 1,8-1,9 %, soil pH - 6,8 - 7,1. In control nursery the size of plots were 7,5 m^2 in 2 - 3 replicates. During experimental period meteorological conditions differed year by year. Prolonged period of drought and heat in 1993 (April - June), 1994 (in July), but excess of precipitation in 1995 (May - June) influenced plant growth and barley yield level.
The protein content (N x 6,25) of grains were determined by the Kjeldal method.

Results and Discussion. Barley grains in F_1 were hulled. In such agronomical traits of F_1 hybrid as kernel weight and number per spike, length of ears, height of plants were noted midparent value or heterosis effect. In all cases F_1 hybrid ears were lax compared to its parent. The protein content in grains of F_1 generations were at the level of midparent value (13,6 - 15,2 % in 5 cross combinations) or at the level with lowest protein content of parents (11,6-13,6 % in 2 cross combinations). The protein content of mutant X-4 was 16,7%. In F_2 generations the wide range of segregation was noted. There were barley with hulled and hull - less grains, both in different colours from dark brown to light colour. In the F_2 generations, the hull-less grains, especially those with dark colour, had the highest protein content, but the protein content of hulled grains was lower by 1,5-5,2 %.
Our attention focussed to hulled and hull-less barley with light colour of grains. The progenies of 97 hulled and 90 hull-less plants were evaluated to protein

content. The protein content level of hulled lines was 8,1-17,5 %, but that of hulled lines was 12,5-23,0 %. The lines divided into 3 groups accordingly to protein content and results are represented in Table 1.

Table 1. Protein content of hulled and hull-less barley lines (1991)

Barley forms	Number of lines	Protein content					
		< 13,0 %		13,1 - 15,0 %		> 15,1 %	
		number	%	number	%	number	%
Hulled	97	60	61,8	22	22,7	15	15,5
Hull - less	90	26	28,9	37	41,1	27	30,0
Total	187	86	46,0	59	31,6	42	22,4

The hulled barley had 60 lines (61,8 %) with lower protein content and only 15 lines (15,5 %) with protein content > 15,1 %.
The majority of hull-less barley progenies were included in the above groups with higher protein content: > 15 % and 13,1 - 15,0 %; accordingly 27 lines (30,0 %) and 37 lines (41,1 %). The same data, that hull-less barley showed to have a higher content of protein when compared to covered barley noted by other authors too. (A.Hodjkov, 1985, A.Heen et al, 1991).
During 1994 - 1995 in control nursery programs selected with highest protein content hulled and hull-less lines were estimated (Table 2).

Table 2, The characteristic of high protein barley lines (average 1994 - 1995)

Lines	Grain yield			Protein content %	Protein yield kg ha^{-1}	1000 kernel weight g
	t ha^{-1}	± to standard	%			
1	2	3	4	5	6	7
* Hulled lines (protein content 13,1 - 15 %)						
Abava - standard	5,0	-	100,0	10,4	520	52,2
L-234(Ca34739 x X-4)	4,4	-0,6	88,0	13,8	607	44,4
L-237(X-4 x Stende 7197)	4,3	-0,7	86,0	13,3	572	45,8
L-276(X-4 x Stende7197)	3,4	-1,6	68,0	14,8	503	53,6
L-264(Imula x X-4)	5,2	+0,2	104,0	13,7	712	56,1
L-285(Imula x X-4)	4,5	-0,5	90,0	13,6	612	50,3
L-272(Tirana x X-4)	5,5	+0,5	110,0	13,6	748	53,1
* Dates of 1994						
Hulled lines (protein content > 15,1 %)						
Abava - standard	4,6	-	100,0	10,6	488	49,5
L-233(Imula x X-4)	2,4	-2,2	52,2	16,5	396	50,4
L-261(Imula x X-4)	2,7	-1,9	58,7	15,8	427	51,3
L-235(Ca34732 x X-4)	2,8	-1,8	60,8	15,5	434	51,5
L-257(X-4 x Sv.77812)	2,7	-1,9	58,7	15,2	410	47,4
L-290(X-4 x Sv.77812)	2,8	-1,8	60,8	15,2	425	60,3
L-279(X-4 X Stende7197)	3,0	-1,6	65,2	16,1	483	52,7

1	2	3	4	5	6	7
Hull-less lines (protein content > 15,1 %)						
Abava - standard	4,4	-	100,0	10,5	462	49,8
X-4 - hull-less mutant	2,3	-2,1	52,2	17,2	395	52,0
L-238(X-4 x Stende7197)	2,3	-2,1	52,2	16,2	372	52,7
L-239(Ca34739 x X-4)	2,3	-2,1	52,2	16,0	368	53,4
L-240(Ca3479 x X-4)	2,4	-2,0	54,5	15,4	369	53,9
L-241(Ca3479 x X-4)	1,8	-2,6	40,9	17,3	311	55,7
L-297(Tirana x X-4)	2,7	-1,7	61,4	18,1	489	52,5
L-299(Imula x X-4)	4,1	-0,3	93,2	17,9	734	53,6
L-302(X-4 x Stende7197)	2,6	-1,8	59,1	17,8	463	53,8

In our trials the barley lines in the group with protein content level 13,1 - 15 % were showed higher grain yield: 86 - 110,0 % to standard variety 'Abava' (5,0 t ha^{-1}), and protein yield 119,0 - 143,8 % to standard variety 'Abava' (520 kg ha^{-1}). By point of grain yield these lines are more perspectives.
The grain and protein yield level of barley lines in group with higher protein content (> 15,1 %) were lower: 52,2 - 86,9 % and 81,1 - 98,3 % compared to standard variety 'Abava' (grain yield 4,6 t ha^{-1}, protein yield 488 kg ha^{-1}).
Hull-less barley lines have the highest protein content: 15,4 - 18,1 %, but their grain yield level was the lowest compared to barley lines with lower protein content. As promising lines are L-297 (Tirana x X-4), L-299 (Imula x X-4) are L-302 (X-4 x Stende 7197).
The investigations of agronomical and nutritional traits should be continued.

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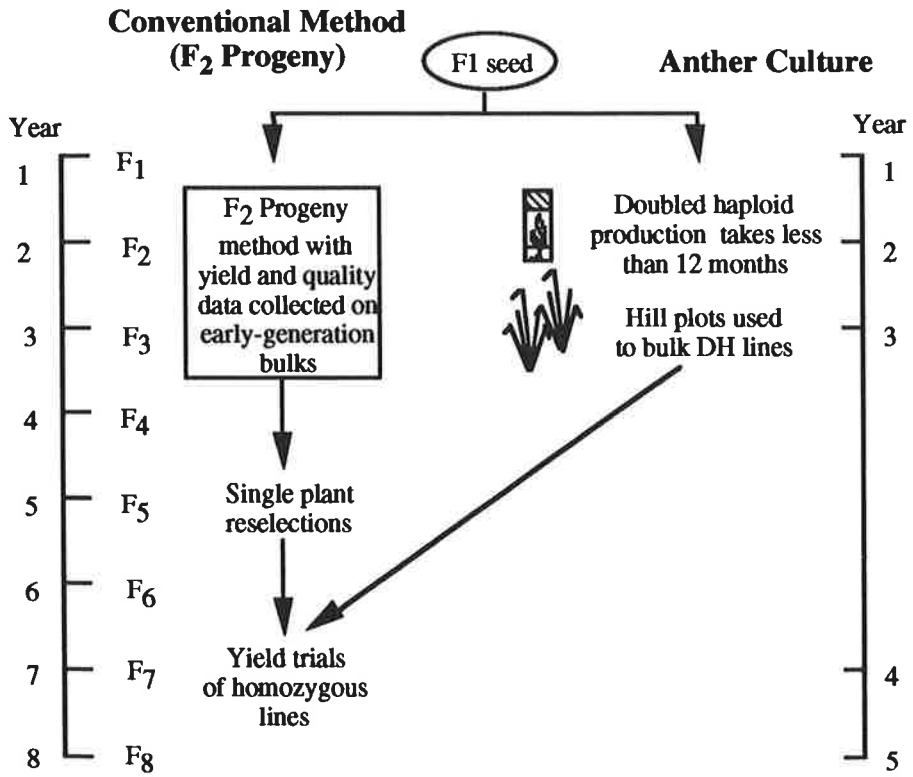
Production and utilisation of barley doubled haploids in the Western Australian Barley Improvement Program. S. BROUGHTON[1] and R. F. GILMOUR[2], [1]Crop Industries, Agriculture Western Australia, Baron-Hay Court, South Perth WA 6151, Australia, [2]ForBio Systems Pty Ltd, PO Box 1556, Coorparoo DC Qld 4151, Australia.

Introduction. Anther culture derived doubled haploids (DHs) are being used to accelerate the development and release of barley cultivars with improved malting characteristics in Western Australia. This technology was introduced in 1993 with the establishment of an anther culture laboratory to service the State's major barley breeding program. This paper reports on the protocol used to generate DH lines, genotype response of Western Australian crosses and field performance of DH lines.

DHs in the Breeding Program The Western Australian Barley Improvement Program utilises an F_2 progeny method in the conventional breeding program. The generation and bulking of DH lines takes approximately two years and the lines enter breeders' trials at a stage equivalent to reselected homozygous material, saving three years (Figure 1). Eight to ten crosses are selected each year (using cross evaluation strategies) with the aim of generating approximately 200 DHs for each population. To date, the program has generated 22 populations with a mean population size of 220.

Figure 1: Integration of doubled haploids into the conventional barley breeding program.



Materials and Methods.

Production of Doubled Haploids Donor plants (F_1) are grown in growth rooms with day/night temperatures of 15/12°C. Spikes are harvested when the microspores are at the uninucleate stage. Single excised spikes are sealed in 9 cm petri-dishes then cold pretreated for 3-4 weeks at 4°C. Following pretreatment, anthers are plated onto an induction medium and incubated in the dark for six weeks at 25°C. Developing embryoids and calli are then transferred to regeneration medium under low light. FHG medium (Hunter, 1988) is used for the induction and regeneration media with maltose added at 60 g/L and 30 g/L,

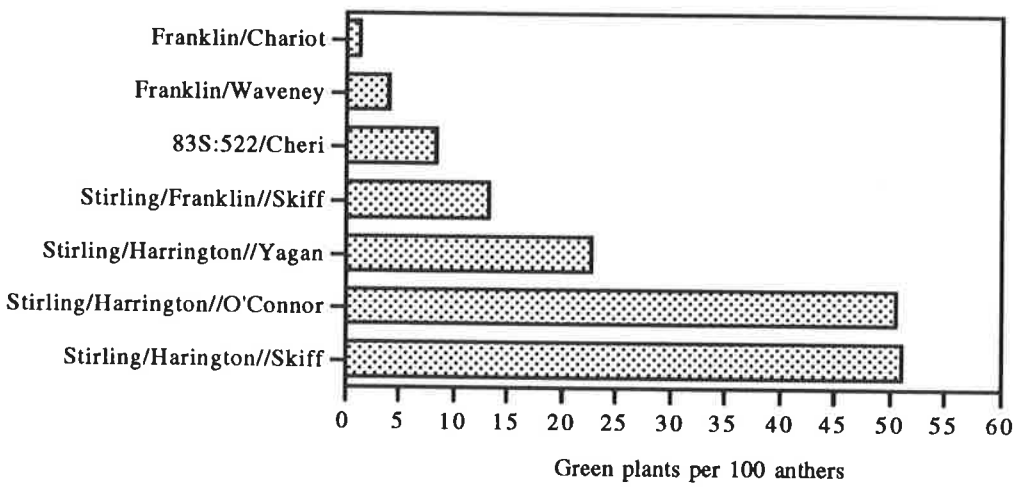
respectively. Indole-3-acetic acid (IAA) and 6-benzylaminopurine (BAP) are added to the induction (1 mg/L) and regeneration media (0.4 mg/L) and both media are solidified with agarose. Green shoots are transferred to vials containing hormone-free Murashige and Skoog basal medium with sucrose (20 g/L) and solidified with agar. Regenerant plants are then transplanted to soil, grown to maturity, and seed harvested from lines which have undergone spontaneous chromosome doubling.

Field Trials - 1995 DH populations from three crosses were generated in 1993, bulked in 1994 and entered field trials in 1995. Lines from each cross and locally grown cultivars were sown in single replicate trials at two sites in 5 row plots. Sites were chosen to reflect the expected adaptation of each cross. The plots were trimmed from 5 m to 3 m near flowering. Agronomic and physical grain characteristics were measured. Yields were spatially adjusted for within site trends using the methods of Cullis and Gleeson (1991) and the program TwoD, within S-PLUS (1993).

Results and Discussion.

Anther culture response of crosses and parental cultivars Cultivars and crosses (all two-row spring types) varied considerably in their responsiveness to anther culture. Green plant regeneration over 22 crosses ranged from 1.3 to 51.2 green plants per 100 anthers (Figure 2). Data averaged over all crosses indicate that 36% of anthers responded producing 16 green and 43 albino plants per 100 anthers. Testing parent cultivars revealed that Stirling (Western Australian cultivar) is reasonably responsive (26.7 greens/100 anthers) whilst Franklin (Tasmanian cultivar) responds poorly (1.8 greens/100 anthers).

Figure 2: Green plant regeneration from a range of crosses in the WA Barley Improvement Program.



Fate of regenerant plants The program has relied on spontaneous chromosome doubling to produce fertile DH plants. Ploidy is determined by phenotype observation and haploids and tetraploids are discarded. Data averaged from nearly five thousand regenerants from twelve crosses in 1993-4 indicated that 65% of surviving regenerants were fertile DHs (range 54-71%), with the remainder either haploid or sterile (26%), or tetraploid (9%). When regenerant losses *in vitro* and in pots are included (10-15%), the expected recovery of fertile DHs is approximately 55%.

Field Trials Crosses were well adapted to the Western Australian growing season in 1995 with grain yields similar to that achieved by the local parent, Stirling, at all test sites (Table 1). An average of 23% of lines in each cross had a similar or greater yield than Stirling.

Table 1: Mean grain yield and range for three doubled haploid populations grown in 1995.

Genotype	No. lines		Yield (kg ha^{-1}) Newdegate <325 mm [a]	Wongan Hills 325-450 mm [a]	Gairdner River >450 mm [a]
Control;					
Stirling	–	*mean*	2030	2070	1940
DH lines;					
Stg/Harr//Skiff [b]	310	*mean*	1320	–	1640
		range	*590 - 2080*		*320 - 3670*
Stg/Harr//O'Connor [b]	423	*mean*	2090	1800	–
		range	*1000 - 2980*	*470 - 3030*	
Stg/Harr//Yagan [b]	127	*mean*	1810	1620	–
		range	*880 - 2840*	*440 - 2370*	

[a] long term annual average rainfall
[b] Stg/Harr = Stirling/Harrington

The populations were culled principally on the basis of their yield, development, straw strength, neck break, lodging and shattering relative to locally grown cultivars. Remaining lines (309) were then screened for grain plumpness, brightness and hectolitre weight and further selections were made on the basis of these characters. Selections (132) were retained for barley beta-amylase measurements prior to inclusion to 1996 trials where they will be tested at three to five sites in two replicate trials. Concomitant with these trials, malting quality evaluation will be conducted on the remaining 1995 samples. A further eight DH populations, bulked in 1995 will enter field testing for the first time in 1996.

Further improvements in the production of DHs are envisaged with the use of isolated microspore culture, together with the introduction of the Bulbosum method for genotypes which respond poorly to anther and microspore culture. Marker-assisted selection has also commenced in 1996 and selected DH populations are being screened for the Yd2 and mlo gene which provide resistance to barley yellow dwarf virus and powdery mildew, respectively. The introduction of marker-assisted selection in the F_2 stage for selection of donor plants is expected to further increase the overall breeding efficiency.

Conclusion. Doubled haploids are beginning to have a major impact on the Western Australian Barley Improvement Program. The most advanced DH lines will enter widescale regional testing in 1998. In combination with cross evaluation for malting quality potential, the proportion of high yielding, superior quality material is expected to increase.

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INFLUENCE OF PLOIDY LEVELS OF HORDEUM MURINUM ON COMPATIBILITY WITH HORDEUM VULGARE V.E. Chernov, North-West Biotechnology Centre, Podbelskogo 9, S.-Petersburg, Pushkin, 189620, Russia.

Introduction. The Hordeum of Murina Nevski series possesses several valuable traits, among them resistance to drought and salt stress (Nevski, 1941; Bothmer, Jacobsen, Baden et al., 1991). In spite of H. murinum and H. vulgare systematical closeness and their belonging to the same section of genus Hordeum, introgression of the H. murinum genome into the genome of H. vulgare has not been obtained. The aim of our investigation was to estimate the crossability between H. murinum specimens of different ploidy and geographical origin and reveal the main barriers to interspecific compatibility.

Materials and Methods. H. murinum specimens of different ploidy level (5 specimens - 2n=2x=14; 4 specimens - 2n=4x=28; 4 specimens - 2n=6x=42) and H. vulgare cv. Roland and cv. Betzes were used in crosses.

Reciprocal crosses were conducted between H. murinum and H. vulgare specimens. The extent of seed set and embryo differentiation was measured on the 14th day after pollination. 14 day embryos were planted onto a culture (N6 medium with kinetin (1 mg/1)) for germination (Chu,Wang, Sun et al., 1975). Anomalies of germination and seedling lethality were measured in the hybrid combinations.

Luminescent microscopy was used to study the growth of pollen tubes in the combinations exhibiting absence of seed set. Chromosome numbers in somatic and sporogenic tissue were investigated in squashed aceto-orcein preparation.

Results and Discussion. Reciprocal differences occurred in crosses between H murinum (2x) and H. vulgare: seed set was higher in all combinations including H. vulgare as pollinator (in H. murinum (2x) x H. vulgare crosses - 11-20%, in reciprocal crosses - 0-2.4%) A low frequency of seed set can not be prescribed only to incompatibility at the progamic stage. Analysis of pollen tube growth in the crosses exhibiting the absence of seed set revealed that in the majority of cases the pollen tubes reached the ovary. Thus, the lack of seed set in this combination is connected with some disturbance of fertilization or maybe with disturbances in a very early stage of embryo- and endospermo-genesis preventing a continuation of further development.

Using H. vulgare as the female in combination with H. murinum (4x) exhibited a trend to increasing seed set frequencies (in H. vulgare x H. murinum crosses - 41-80% if compared to reciprocal combinations - 5-40%). After 14 days the seeds have a differentiated embryo and watery endosperm.

Statistically significant differences have been observed in crosses involving hexaploid specimens of H murinum ssp. leporinum and H. murinum ssp. hrasdanicum with cultivated barley in all the studied combinations. Using H. vulgare as a maternal form resulted in 65-74% seed set. In reciprocal combinations the frequency of seed set comprised 0-65%. Regardless of the direction of the cross, the embryos in hybrid seed were well differentiated, and the endosperm was

differentiated, but a bit shrunken.

Thus, the higher the ploidy level of H. murinum, the lower the degree of incompatibility in embryo and endosperm development in crosses between H. vulgare and specimens of Murina series.

When planted onto N6 medium, most of the 14-day-old hybrid embryos in reciprocal crosses with H. murinum (4x, 6x) germinated, but many seedlings exhibited morphogenetic anomalies (lack of roots, curved coleoptiles). All normal and abnormal seedlings obtained from the embryos that emerged from crosses of H. vulgare with different tetraploid H. murinum specimens died before planting into soil on the stage of 1-2 leaf stage. Some of the seedlings died on the early ontogenic stages in the crosses including hexaploid cytotype of H. murinum. Some of the plants were planted into sterile soil at the 3rd leaf stage. The majority of the plants died after producing 2-5 shoots. Only 2 plants developed to the stage of spike formation, but they exhibited instability in somatic tissues and some chromosome elimination was observed. Cells with 23-27 chromosomes were found in one plant and cells with 25-28 chromosomes were found in the other. Meiosis was observed in the second plant. Chromosome number in PMCs varied from 23 to 28 and the plant itself was weak with necrotic spots on the leaves.

Thus, the success of crossing of H. murinum with H. vulgare depends on the ploidy level of H. murinum. However, cytogenetic instability and high lethality of the seedlings suggest genomic and physiological incompatibility of these taxons.

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Genetic analysis of a two-row x six-row cross of barley using doubled-haploid lines. T.M. CHOO and K.M. HO, Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada (AAFC), Ottawa, Ontario K1A 0C6, Canada; P.Y. JUI, Eastern Region, Research Branch, AAFC, Ottawa, Ontario K1A 0C6, Canada; and R.A. MARTIN, Research Centre, AAFC, Charlottetown, Prince Edward Island C1A 7M8, Canada.

Introduction. In an extensive investigation, Harlan et al. (1940) found that two-row/six-row crosses were 'strikingly inferior'. Not only did six-row selections from 149 two-row/six-row crosses yield lower than selections from 209 six-row/six-row crosses, but two-row selections from the two-row/six-row crosses also yielded lower than selections from 21 two-row/two-row crosses. In contrast, Lambert and Liang (1952) were able to identify lines from a two-row/six-row cross that were outstanding in yield, test weight, and stiffness of straw. Improved varieties have also been developed from two-row/six-row crosses in Canada and elsewhere. Therefore, a study was initiated to determine the yield potential of a two-row/six-row cross of barley. The effects of six marker loci: *V* (two-row), *Re2* (purple lemmas), *s* (short rachilla hairs), *R* (rough awns), *Est1* (esterase 1), and *Est5* (esterase 5) on six agronomic traits were also investigated.

Materials and Methods. One hundred and ninety doubled-haploid (DH) lines were derived from a Leger/CI9831 cross of barley by the bulbosum method. Leger is well adapted to Eastern Canada, it possesses the *vv*, *re2re2*, *ss*, *rr*, *Est1Ca*, and *Est5Te* genes. On the other hand, CI9831 is an introduction, possessing the *VV*, *Re2Re2*, *SS*, *RR*, *Est1Pr*, and *Est5Ri* genes. The DH lines and the two parents were tested for grain yield, test weight, seed weight, plant height, lodging, and heading/maturity date in four-row plots in a randomized complete block design with four replications at two locations (Charlottetown and Ottawa) in Eastern Canada in 1993. The two parents were each entered five times in each replication. A transgressive line was defined as one with a mean value of an agronomic trait outside the parental range. A superior line was a transgressive line with yield or test weight higher than Leger, seed weight greater than CI9831, plant height shorter than Leger, lodging resistance better than Leger, or heading/maturity date earlier than CI9831.

Results and Discussion. The two parents differed only in two of the six agronomic traits, i.e. yield and seed weight (Table 1). Leger outyielded CI9831 by 60% at Charlottetown and 61% at Ottawa. CI9831 seed outweighed Leger seed by 29% at Charlottetown and 28% at Ottawa. The mean yield and seed weight of the two parents was 25% and 5% higher than that of DH lines, respectively. On average, the two parents were 4% taller than the DH lines at Charlottetown and as tall as the

DH lines at Ottawa. These results suggested that additive x additive epistasis was present for yield, seed weight, and plant height. There were many transgressive lines, but the majority of them were inferior. None of the DH lines outyielded Leger, but some two-row lines yielded significantly higher than the two-row parent CI9831. In fact, one two-row line outyielded CI9831 by 31%. This suggested that it is possible to transfer desirable genes from six-row varieties to two-row. However, further studies are needed to determine if superior six-row lines can be developed from two-row/six-row crosses. Under the growing conditions in Eastern Canada, six-row lines outyielded two-row by at least 20% (Table 2). Six-row lines, however, were associated with low test weight, low seed weight, and severe lodging. The *R* , *s*, and *Est5* loci also had a significant effect on some of these traits, but the *Est1* locus had no effect on any of the six traits. The effect of the *Re2* locus was probably due to its close linkage with the *V* locus.

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Table 1. Agronomic traits of the 190 doubled-haploid (DH) lines derived from a Leger/CI9831 cross of barley.

Trait	Mean			No. of transgressive DH lines	
	Leger	CI9831	DH	inferior	superior
Charlottetown					
Yield (t ha^{-1})	4.8 a	3.0 b	(3.2)[z]	45	0
Test weight (kg hl^{-1})	65.2 a	64.9 a	(62.0)	137	5
Seed weight (mg)	39.6 a	47.6 b	42.0)	90	73
Height (cm)	109.5 a	114.7 a	(107.7)	13	56
Lodging (1-9)[Y]	4.5 a	4.9 a	4.8	58	77
Heading (days)	60.2 a	60.2 a	60.8	92	46
Ottawa					
Yield (t ha^{-1})	3.6 a	2.2 b	(2.3)	22	0
Test weight (kg hl^{-1})	62.8 a	62.5 a	(59.7)	97	4
Seed weight (mg)	34.6 a	44.2 b	40.1	31	52
Height (cm)	95.1 a	89.1 a	93.0	0	0
Lodging (1-9)	0.5 a	0.6 a	0.9	12	0
Maturity (days)	93.6 a	93.1 a	92.9	0	15

a, b Means followed by different letters were different at the 0.05 level.

z Means in parentheses were different from their respective mean values of parents at the 0.05 level.

Y 1 = no lodging, 9 = severe lodging.

Table 2. Agronomic traits of ten classes of the 190 barley doubled-haploid lines grown at Charlottetown (C) and Ottawa (O) in 1993.

Comparison	Yield ($t\ ha^{-1}$)		Test weight ($kg\ hl^{-1}$)		Seed weight (mg)		Height (cm)		Lodging (1-9)[z]		Heading/Maturity (days)[y]	
	C	O	C	O	C	O	C	O	C	O	C	O
Spike type												
Two-row (94)[x]	2.7a	2.1a	62.6a	60.0a	51.1a	47.0a	112.0a	92.4a	3.1a	0.9a	61.2a	92.9a
Six-row (96)	3.5b	2.6b	61.3b	60.0a	34.4b	33.4b	103.6b	93.5a	6.2b	0.9a	61.0a	92.9a
Lemma color												
Purple (97)	2.8a	2.2a	62.7a	59.9a	49.2a	45.7a	111.2a	92.4a	3.6a	0.9a	61.2a	93.0a
Yellow (93)	3.5b	2.6b	61.5b	59.8a	36.0b	34.4b	104.6b	93.6a	5.8b	0.8a	60.9a	92.9a
Rachilla hairs												
Long (96)	3.2a	2.4a	62.3a	60.3a	42.2a	39.6a	108.1a	92.7a	4.7a	0.9a	61.1a	93.8a
Short (94)	3.0b	2.2b	61.6b	59.1b	43.2a	40.8b	107.4a	93.3a	4.6a	0.8a	61.1a	94.0a
Awn Type												
Rough (99)	3.3a	2.5a	62.3a	60.3a	40.8a	38.8a	106.9a	92.4a	5.0a	0.9a	60.8a	93.9a
Smooth (91)	3.0b	2.2b	61.6b	59.1b	44.6b	41.7b	108.6b	93.5a	4.3b	0.9a	61.4b	93.9a
Esterase 5												
TeTe (83)	3.1a	2.3a	61.7a	58.7a	43.0a	40.5a	107.6a	92.1a	4.8a	0.9a	60.7a	93.8a
RiRi (50)	3.3b	2.5b	62.6b	61.3b	40.6b	38.2b	107.4a	94.2b	4.8a	0.9a	60.8a	93.9a

a,b Means in each comparison followed by different letters were different at the 0.05 level.

z 1 = no lodging, 9 = severe lodging.

y Heading data for Charlottetown, maturity date for Ottawa.

x The number of doubled-haploid lines for each marker class is given in parentheses. The allelic identity at the *Est1* and *Est5* loci was not determined for the other 57 lines.

Variation in Rates of Embryo Development and Plant Generation in *in vitro* culture of Korean Barley Cultivars pollinated with *Hordeum bulbosum*

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Introduction

The breeding method by haploid production has a few merits and use of *Hordeum bulbosum* method(interspecific hybridization of *H.vulgare* with *H.bulbosum*) is reported by many researchers(5,11). So, we need reliable *bulbosum* methods such as haploid production, embryo and plant generation from *in vitro* cultured haploidy embryos, and authentication of haploids. This study objective is to establish an effective *bulbosum* method for production of genetically stable normal barley hybrids in Korea.

Materials and methods

The seeds of *H.bulbosum*(GBC(2x) and Spenish diploid(4x)) have been gotten from Canada, sown, multiplicated and used for pollen production. The eleven *H.vulgare* plants bred in Korea were planted, controlled in anthesis time and crossed with *H.bolbosum* pollens. After vernalization in natural open field, the plants were under conditions of 20 ^{o}C, 12hr light, and 15 ^{o}C, 12hr dark. Pollens were applied carefully to the barley stigmas after 3-4 days of emasculation, and 75 ppm of gibberellic acid was applied 2 days after pollination. Dissection of embryos(15-21 days after pollination) from sterilized fruits was done and cultured on B5 medium with light intensity of 2,500 lux(24hr) and temperature of 20 ^{o}C. Chromosome number and morphology were observed in roo-tip squash using acetocarmine.

Results and discussion

In *H.vulgare*, *H.bulbosum* and their haploids, the chromosome number of parents and their hybrids were 14 and 7, respectively. The most of doubled haploidy plants by colchicine had similar morphology and growth pattern to *H.vulgare*, but a few of doubled haploidy plants with abnormal karyotypes were observed. In the latter plants, three pairs of *H.bulbosum* which were eliminated, seemed to be added to whole chromosome(2n=14) of *H.vulgare*, but more detailed observation would be needed for confirming the assumption. Many researchers(1, 2, 3, 7) reported that the most chromosomes from interspecific crosses between *H.vulgare* and *H.bulbosum* were from the *H.vulgare* of disomic, but according to Jensen(6) and Pickering(9), a part of chromosomes of *H.bulbosum* were added to *H.vulgare*.

Esterase zymograms in mature grains of *H.bulbosum, H.vulgare* and its doubled haploids by isoelectric focusing(pH 3-10) showed more clear bands than peroxidase did. The bands of

colchicine-induced diploids of Dongbori #1 were almost same as those of *H.vulgare*, and those results suggested that the hybrids had no chromosomes from *H.bulbosum*.

Averaged values of culm, spike and awn lengths in the original and doubled haploids(Suwon #234, Suwon #235 and Chalssalbori) are not significantly different(Fig. 1). The results showed the similar trend to wheat(4).

The rates of embryo development of major barley cultivars pollinated with *H.bulbosum* varied among cultivars with averaged value of 26%(Table 1). The embryo develpoment rates were greatest in naked barley(33%) followed by two- rowed barley(25%), and covered barley(20%). Those variations of embryo development rates among cultivars were reported by others(8, 9). Pickering(10) reported that spring type had worse rates of embryo development than winter type did. However, the naked barley(facultative type) was greatest in embryo development in this study. In general, the development rate pollinated with GBC was better than that with Spenish diploid.

The rates of plant generation from *in vitro* culture of embryos in major barley cultivars pollinated with *H.bulbosum* were 18-55% with averaged value of 39%. The generation rates pollinated with GBC(41%) were higher than those with Spenish diploid(39%). The rates of embryo development and plant generation from embryos were very various among different cultivars. The rates of plant generation in Paldalbori and Doosan 12 were greater than in other cultivars. Also, there was no correlation between the rates of embryo development and plant generation.

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Table 1. The rates of embryo development and plant generation from *in vitro* culture of embryos in major barley cultivars pollinated with *H.bulbosum*.

Barley group	Cultivar	*Hordeum bulbosum*	No. of florets*	Developed embryo		No. of embryos*	Generated plants	
				No.	Rate(%)		No.	Rate(%)
Covered	Kangbori	GBC	1147	296	26	201	75	37
		Sp.D.	925	195	21	147	67	46
	Dongbori 1	GBC	753	119	16	100	40	40
		Sp.D.	827	147	18	110	39	36
	Oweolbori	GBC	717	120	17	100	47	47
		Sp.D.	986	240	24	168	48	29
	Paldalbori	GBC	1272	195	15	179	99	55
		Sp.D.	1039	159	15	136	65	48
	Saeolbori	GBC	1181	202	17	152	43	28
		Sp.D.	789	217	28	136	43	32
	Total		9636	1890	20	1429	566	40
Naked	Baekdong	GBC	1097	517	47	414	168	41
		Sp.D.	514	156	30	124	40	32
	Youngsan-bori	GBC	893	283	32	198	64	32
		Sp.D.	304	96	32	63	27	43
	Neulssal-bori	GBC	1147	318	28	233	106	46
		Sp.D.	1539	301	20	142	43	30
	Mudeung-ssalbori	GBC	1307	493	38	269	116	43
		Sp.D.	595	311	52	170	41	24
	Total		7396	2475	33	1613	605	38
Two-rowed	Doosan 12	GBC	403	113	28	83	40	48
		Sp.D.	363	104	29	71	31	44
	Doosan 22	GBC	146	22	15	17	3	18
		Sp.D.	182	30	17	20	7	35
	Total		1094	269	25	191	81	42
	Total	GBC	10063	2678	27	1946	801	41
		Sp.D.	8063	1956	24	1287	451	35
	Grand total		18126	4634	26	3233	1252	39

* Number of florets pollinated.

The relative efficiency of isolated microspore culture and anther culture for doubled haploid production. P.A. DAVIES and S. MORTON, Field Crops Pathology Unit, SARDI, GPO Box 397, Adelaide SA 5001, Australia.

Introducion Anther culture (AC) production of doubled haploids (DHs) is a relatively new technique to assist and accelerate barley breeding (Kuhlmann and Foroughi-Wehr, 1989; Pickering and Devaux, 1992). A number of technical advances have increased the efficiency of doubled haploid production to a level that is adequate for breeding purposes. These include: cold pretreatment of anthers (Huang and Sunderland, 1982); substitution of agarose for agar (Lyne *et al.,* 1986); optimisation of reduced nitrogen (Olsen, 1987) and the substitution of maltose for sucrose (Hunter, 1987).

Despite these advances, the efficiency of production of fertile, green DH plants through anther culture is very low and relatively costly. In a practical breeding program, the frequency of regeneration is highly genotype dependent and ranges between 0.05-0.50 green plants per anther. Since there are approximately 2,000-4,000 microspores per anther, each with the genetic capacity to produce at least one new doubled haploid plant, there is great potential for increasing the proportion of microspores which develop into doubled haploid plants.

Isolated microspore culture (IMC) is a technique in which the microspores are removed from the anther prior to culture. Research by Hoekstra *et al.* (1992) found that using the highly responsive winter cultivar 'Igri', higher frequencies of DHs could be produced by IMC than with anther culture. Our research aims to investigate the potential of IMC technology for use with spring barley breeding germplasm.

Materials and Methods. Donor plants of the winter cultivar Igri and a breeder's spring F_1 hybrid Amagi Nijo x WI2585 were grown in plant growth cabinets at 17°C/14°C day/night with a 14h photoperiod. Spikes were collected when microspores were at the early to mid-uninucleate stage of development, surface sterilised by spraying with 70% ethanol and subjected to cold pretreatment for 2 weeks at 4°C in the dark. Anthers from one side of each spike were used for AC and anthers from the opposite side were used for IMC so that direct comparsions could be made between the two methods.

For IMC anthers were placed in a microblender chamber (Eberbach Corp. Michigan, USA) containing 20ml of liquid KFWC induction medium. KFWC induction medium is the "medium C" described by Kuhlmann and Foroughi-Wehr (1989) containing 1.0mg.l^{-1} indole-3-acetic acid (IAA) and 1.0mg.l^{-1} 6-benzylaminopurine (BAP) and modified to contain 60g.l^{-1} maltose plus 100

mg.l^{-1} of the antibiotic Cefotaxime, with the omission of Ficoll 400 and barley starch. The microblender chamber was powered by a Waring blender motor and operated for 15-25s at high speed. The debris was separated from the microspores by filtering through a 100µM sieve. Microspores remaining in the chamber were washed out with fresh KFWC medium. Microspores were then pelleted at 100 *xg* for 10 min and resuspended in 580 mM maltose and 5 mM MES, pH 5.9 which was overlaid with 300 mM mannitol and 5 mM MES, pH 5.9. (Mordhorst and Lörz, 1993). Following centrifugation at 85 *xg* for 8 min, microspores were collected from the interface and cultured at densities between 5 x 10^4.ml^{-1} and 1 x 10^5.ml^{-1} in KFWC medium. Microspores were plated in volumes of 2ml in 5cm diameter petri-dishes or 1ml in 3cm petri-dishes. Cells were subcultured after 10-12 days by centrifugation at 100 *xg* for 5min followed by resuspension in KFWC regeneration medium. KFWC regeneration medium has the same salts and vitamins as the induction medium but maltose is reduced to 30g.l^{-1} and IAA and BAP are reduced to 0.4mg.l^{-1}. After a further 10-12 days, microcolonies were counted and subcultured to KFWC induction medium solidified with 0.3% Phytagel™ (Sigma) for 4 weeks and then transferred to solidified KFWC regeneration medium for 4 weeks before counting green regenerant shoots.

For AC anthers were plated directly onto solidified KFWC induction medium for 4 weeks followed by transfer to solidified KFWC regeneration medium for a further 4 weeks before counting green regenerant shoots.

All cultures were incubated at 25°C in the dark until subcultured to solidified regeneration medium, when they were exposed to 50µE.m^{-2}.s^{-1} light with a 16h photoperiod.

Results and Discussion

The frequency of microspores dividing and growing to form microcolonies ranged between 0 - 8.2%. Table 1 indicates a similar mean percentage of microcolony formation for both Igri and the Amagi Nijo x WI2585 hybrid. However the frequency of green plant regeneration was much higher for Igri than Amagi Nijo x WI2585 for both IMC and AC. The most important feature of these data is that the paired comparisons between AC and IMC always result in a greater frequency of green plant regeneration for the IMC treatments. In the case of Igri there was a 90 fold improvement and in the case of Amagi Nijo x WI2585 there was a 12 fold improvement of IMC over AC.

The frequency of regeneration for Igri AC is lower than normally expected from this cultivar probably because the donor plants were grown in conditions with higher than optimal temperatures and a longer than optimal photoperiod for a winter barley.

Table 1. Colony formation and green plant regeneration from anther cultures and isolated microspore cultures of the highly responsive winter cultivar 'Igri' and the spring breeders hybrid Amagi Nijo x WI2585. The results from each genotype are the mean of at least 8 separate cultures from 2 independent experiments.

	IMC		AC
Genotype	Microspores forming colonies (%)	Green regenerants per 100 anthers	Green regenerants per 100 anthers
Igri	3.8	910.0	10.9
Amagi Nijo x WI2585	3.2	74.6	6.3

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Molecular Genetic Analysis of Recurrent Selection in Oat: Genetic Diversity, Allelic Shifts and QTLs. D.L. DE KOEYER, D.D. STUTHMAN and R.L. PHILLIPS, Dept. of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108, USA.

Introduction. Recurrent selection (RS) has been effectively used to improve agronomic traits in self-pollinated crops. Recurrent selection is a cyclic process in which superior genotypes are selected and intermated to form an improved population. In theory, the frequency of favorable alleles in a population is increased using this procedure. Genetic variability is also expected to be maintained during RS to allow for continued progress.

Few studies have examined the long-term genetic changes caused by RS. In corn (*Zea mays* L.), the effects of genetic drift in three RS programs were significant and reduced selection progress (Helms et al., 1989). Changes in allelic frequencies at eight isoenzyme loci were associated with changes due to selection for improved grain yield in corn (Stuber et al., 1980). Rocheford (1994) identified ribosomal DNA intergenic spacer hybridization patterns that were more prevalent in populations that had undergone seven cycles of half-sib RS and eleven cycles of reciprocal RS compared to the original populations. Quantitative trait loci that influence protein and starch concentrations in the Illinois long-term selection experiments were detected (Goldman et al., 1993). Populations that have been selected over several generations may be particularly useful in detecting marker-trait associations.

Recurrent selection to enhance grain yield of oat has been ongoing since 1968 at the University of Minnesota. Yield increases of 7.5% per cycle have been reported (Pomeranke and Stuthman, 1992). Currently, the program is in the seventh cycle of selection. The availability of a RFLP linkage map of hexaploid oat (O'Donoughue et al., 1995) allows for the molecular genetic analysis of this population.

The main objectives of this research are (i) to measure the changes in genetic diversity through seven cycles of RS for grain yield, (ii) to evaluate the changes in allelic frequencies during RS, and (iii) to identify the location of quantitative trait loci governing grain yield and other agronomic traits.

Materials and Methods.

Recurrent Selection: The RS program for enhanced grain yield in oat was described by Stuthman and Stucker (1975). The original parents were selected primarily on the basis of high grain yield. These parents consisted of five named cultivars and seven breeding lines. Each cycle involves: (a) intermating in a partial diallel (63 crosses), (b) 10 F_4 - derived F_6 lines per cross (630 total), (c) evaluation in hill plots at a single location, and (d) selection of 21 lines to be parents for the next cycle.

Pedigree Analysis: The coefficients of parentage (COP) values among the twelve original parents were determined from information provided by Souza and Sorrells (1988). Detailed pedigrees of the RS material are available and these were used to calculate COP values within each cycle.

RFLP Analysis: DNA was isolated from 2-3 wk-old seedlings. The tissue samples were bulks of 25 seedlings per genotype. DNA was digested using EcoRI, EcoRV, or DraI. Southern blotting, DNA hybridizations, autoradiograph exposures were done using the methods described by Sambrook et al. (1989). Twenty-seven cDNA clones were used to screen the 12 original parents for polymorphisms. These probes detected 58 loci, 46 of which mapped to 35 of the current 38 RFLP linkage groups in hexaploid oat (O'Donoughue et al., 1995). Probes that revealed polymorphisms among the twelve original parents were tested on blots with digested DNA from each of the 21 parents for each of the seven cycles. RFLP alleles at each locus were scored for presence or absence on the x-ray films. A total of 83 bands were scored and these data were used to produce a genetic distance matrix using the procedure described by Nei (1972) with the software NTSYS (Rohlf, 1993). Allelic frequencies were also determined for each locus in each cycle of selection. The statistical methods of Nei (1987) were used to detect allelic shifts that were significantly greater than those expected by random drift alone.

QTL Associations: The RS parents were planted in hill plots at Rosemount and St. Paul, MN, in 1994 and 1995. Experimental design was a randomized complete block with four replicates at each location. Grain yield, heading date and plant height were measured. Four linkage groups that contained loci showing significant allelic shifts were studied in greater detail to localize putative QTLs. Thirty-five loci on these linkage groups were screened for polymorphisms and tested for allelic shifts. Regression analysis was performed on allelic frequencies across cycles of selection to determine the average change in allelic frequency per cycle for each locus. Supergene software (Boutin et al., 1995) will be used to generate marker-based pedigrees and graphical genotypes for the four linkage groups. Simple linear regression will be used to identify significant marker-trait associations.

Results and Discussion.

Genetic Diversity: The average COP value among the parents for each cycle has increased substantially over the cycles of selection. Nei's genetic distance, calculated from RFLP data, decreased from cycle 0 to cycle 7. Both of these measurements indicate that there has been a considerable reduction in the genetic diversity within the RS population. The relatively small effective population sizes maintained and the high selection pressure within the population have likely contributed to these results. Fortunately, the loss of genetic diversity has not affected selection response for grain yield, as reported by Pomeranke and Stuthman (1992). These findings are in contrast to RS studies with corn which indicate that genetic drift has reduced selection response (Helms et al., 1989).

Allelic Shifts: Alleles at six loci showed large significant shifts. The loci are: Xcdo346b, Xcdo1473a, Xcdo1436c and Xcdo1168b which map to linkage groups 1, 6, 11, and 28, respectively. Xcdo484c and Xcdo1174d also showed significant changes in allelic frequencies, but are not mapped. Two of the four

mapped loci showed significant associations with agronomic traits measured in a Kanota x Ogle RIL mapping population (Siripoonwiwat et al.,1996). Another locus maps 8.2 cM from a grain yield QTL. Our results indicate that we have identified genomic regions with some of the favorable alleles which were selected during the RS process. Frequencies of alleles at isoenzyme loci and ribosomal DNA intergenic spacer-length composition also changed following RS for grain yield in maize populations (Stuber et al., 1980; Rocheford, 1994).

QTL Associations: Marker-trait associations can be identified in RS or breeding populations when systematic approaches are used, detailed pedigrees are kept, and selection response is large. Changes in allelic frequencies that are greater than that expected by random drift alone gives an indication that the marker is linked to a QTL. Testing other markers on a linkage group will help localize the QTL. The theoretical expectations of this approach are related to the fact that molecular markers that are linked to a QTL will show an inverse relationship between change in frequency per cycle of selection and distance in cM from the QTL. To date, results from the analysis of the four linkage groups agree with this hypothesis. Examples of the marker-based pedigrees, graphical genotypes, and results of the QTL analysis will be presented at the conference.

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C.H.P. Einfeldt[1], S.Ceccarelli[2], A. Gland-Zwerger[1], and H.H. Geiger[1]. Role of heterozygosity and heterogeneity as tools to improve performance of barley under drought stress.
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1. Introduction

Barley is the predominant crop in drought-prone areas of West Asia and North Africa. Time and amount of rainfall have a large variability in these environments. Possible buffer mechanisms against such unpredictable environmental conditions are the increase of intergenotypic diversity (heterogeneity) and intragenotypic diversity (heterozygosity). The objectives of this study were to compare
- doubled haploid lines (DHL) in mixed *vs.* pure stand (influence of heterogeneity)
- F_2 populations *vs.* corresponding DHL mixtures (influence of heterozygosity).

2. Materials and Methods

The parent lines consisted of six DHL derived from drought adapted landraces (gene pool "landraces") and from double crosses between adapted and non-adapted materials (gene pool "experimental lines"). Nine crosses were made between these six parental DHL. The F_2 generation and eight DHL were produced from each cross. The eight DHL of a cross were tested in pure stand and as a mixture. The six parental DHL, all nine F_2 populations, five DHL mixtures and 40 DHL in pure stand were evaluated for various agronomic and morphological traits in four environments (Tel Hadya 1994 and 1995, Breda 1994 and 1995) in North Syria. Due to shortage of seeds four DHL mixtures and the corresponding 32 DHL in pure stand could be tested in the environments Tel Hadya 1995 and Breda 1995 only.

3. Results

The level of stress differed widely among environments. Precipitation, grain yield, biomass yield, and harvest index were highest in the moderate stress environment Tel Hadya 1994. Tel Hadya 1995 and Breda 1994 with about 300 mm precipitation can be regarded as environments with medium stress. Breda 1995 was an environment with severe stress where rainfall was 40 % and grain yield 70 % less than in Tel Hadya 1995.
The F_2 populations outyielded the DHL mixtures and the mean of the DHL in pure stand in all four environments (Fig.1). The superiority of the F_2 populations increased with the stress level. On the other hand, the yield advantage of F_2 populations over the five local checks decreased with increasing stress.
Averaged over all populations and locations, the effect of heterozygosity was highly significant for all traits except number of fertile tillers. In contrast, a significant mixing effect could be observed for days to heading and plant height only. The advantage of the F_2 populations over the DHL mixtures was highest for grain yield (26.6%). For the other agronomic and morphological traits the superiority ranged between 10 and 15 %. However, the number of days to heading and the number of fertile tillers, both regarded as adaptive traits under drought stress, remained almost

unaffected by heterozygosity.

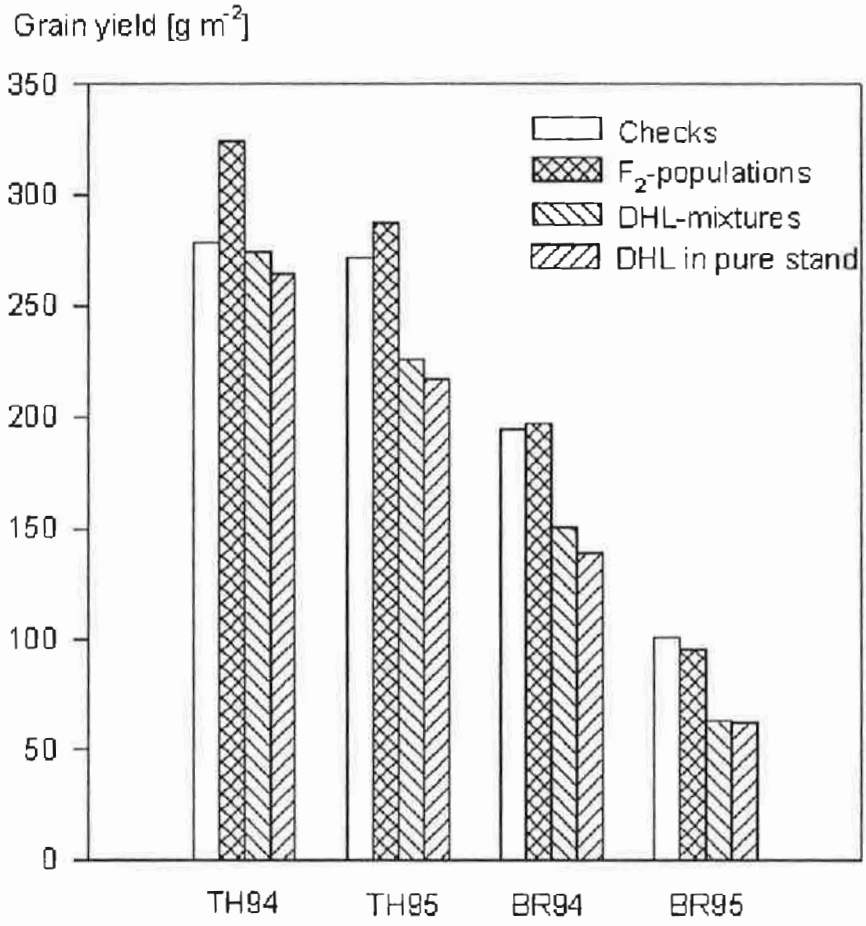

Fig.1 Mean values for grain yield of five checks (two local landraces, three selections from landraces), five F_2 populations, and five corresponding sets of eight doubled haploid lines (DHL) in mixture and in pure stand tested in four environments in North Syria (TH94 = Tel Hadya 1994, TH95 = Tel Hadya 1995, BR94 = Breda 1994, BR95 = Breda 1995).

F_2-population and midparent values were strongly correlated for grain yield ($r = 0.83$, $P = 0.01$) and harvest index ($r = 0.84$, $P = 0.01$) but uncorrelated for biomass. The two types of crosses (EL x EL and LR x EL) reacted differently to increasing drought stress with regard to the effect of heterozygosity. In EL x EL crosses, high levels of F_2 superiority were already observed under medium stress, but in LR x EL crosses only under severe stress.

4. Discussion

Heterozygosity appears to be a much more important factor for grain yield and biomass production under stress conditions than the effect of heterogeneity. The yield increase due to heterozygosity was substantially higher than found by Finlay (1964) in a comparison of F_2 barley hybrids and their midparent values in stress environments of South Australia. The superiority of the F_2 populations reflects a greater vigor during the whole vegetation period (demonstrated by higher scores for early growth vigor and larger gain in biomass). Interestingly, the growth period of the F_2 populations was on average only 1.2 days shorter than of DHL mixtures, whereas Oosterom and Acevedo (1992) found a negative relationship between early growth vigor and growth duration in homozygous barley germplasm in North Syria. Since barley is a predominantly self-pollinated crop, the exploitation of heterosis is difficult. A higher outcrossing rate could be achieved by recurrent selection for open pollination and introgressing genes for cross-fertilization from wild relatives into current breeding populations. The positive relationship between F_2-population and midparent value for grain yield would suggest the use of adapted germplasm as parents. But, still the relationship between midparent performance level and relative amount of heterosis needs to be examined.

5. Acknowledgements

The generous financial support by the Eiselen-Foundation Ulm, Germany, is gratefully acknowledged.

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Morphological, genetical and molecular studies of developmental mutants in barley.

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The structure of adult barley plant is divided, on the basis of morphological observations, into six distinct developmental regions and specific phytomeric units are assigned to each region. Particularly, the rachilla comprised a first metameric region that bears leaves and the sterile glumes. Further along there are two phytomers that produce leaf-like structure: the lemma and the rachilla tip. Several morphological mutants are available in barley, in which metameric regions undergo to process of transitions, organ modifications, addition and reduction of phytomers.

In this work, a new mutation (*leafy lemma*, *lel*) which controls barley awn development has been studied. Morphological studies of the mutant has been performed in order to extablish homologies among normal and mutated plant structures. The inheritance of the mutation was studied through segregation analysis in some different crosses. Chromosome assigment through RFLP mapping of the mutated genes has been done.

Inheritance of another transitional mutation affecting plant height (*curly dwarf*, *cud*) has been confirmed through segregation analysis and the chromosome assignment of the mutation was performed via RFLP analysis.

Use of major genes for increasing oat grain yield and grain quality. L.C. FEDERIZZI; F.IF. CARVALHO,S.C. MILACH; M.PACHECO ; J.F. BARBOSA NETO. Faculdade de Agronomia , C.postal 776. 90001-970 Porto Alegre , Brazil.

Introduction. Oat was a crop introduced in Brazil , by the Spainard in th 19 th century, and it has been limited to the sub-tropical areas in the South. Brazil was a traditional importer of oats from Argentina and Chile . Area grown with oats was less than 10000 hectares in 1980. Cultivars used by farmers were introduced from abroad with low adaptation and poor grain yield and grain quality. High grain yield is a result of the perfect fit of the genotype to target environment. As a consequence genes responsables for the crop adaptation are of major interess in breeding oats for non traditional areas. The main objective of our breeding program was to study and identifying major genes for traits related to adaptation and to manipulate these genes in order to obtain plants with early maturity ,short plant height and compact panicle types.

Material and Methods. The breeding program start in 1974 with the introduction of inbreed lines and F2 and F3 segregating populations from the University of Wisconsin. A continous introduction of germplasm had been made since 1979 trough the Quaker Oat International Nursery "QOIN" . Local selection was made and crosses within the germplasm permited genetic studies for several caracteres of agronomic importance. New varieties and inbreed lines selected with basis in the major genes were tested for agonomic performance in six locations in three different states of Brazil for the last four years.

Results and Discussion. Since 1982 , 17 varieties were released by the program , most of varieties selected from the segreganting populations of the QOIN. New varieties and lines of the program have yielded around 3000 kg/ha (Table 1) and with excellent test weight (table 2) . Major genes for short plant height , earliness ,crown rust resistance and panicle type have been described and incorporated in the program oat lines (CRUZ & FEDERIZZI,1996; BERTAGNOLLI et al.,1993; FEDERIZZI et al.,1996).

Earliness is a main trait in this area ,because oat are grown before soybean (the major cash crop) and it should be ready for harvest before november. Diferent sources of genes for earliness were studied and used in the program (FEDERIZZI et al. ,1996). Varieties released by the program were the early ones in Brazil and they are harvest 20 days before the oldest cultivars making the double cropping with soybean possible (Table 3).

The oldest varieties were very tall (140 cm) with frequent losses in grain yield due to the lodging . Two different genetic systems were identified in the lines of the program and all recent varieties released are of short plant height (less 100 cm) and with stem stiffeness preventing lodging in the farmers fields (Table 4) (BERTAGNOLLI et al. ,1993).

A gene for compact panicle type also was used in order to change the number of flower/panicle and increase the total number of grain/panicle.(BERTAGNOLLI AND FEDERIZZI,1994). It represents a important strategy to increase grain yield ,in an environment were most of tillers did not survive and the yields are given by the grains produced in the main stem of oat plant.In 1996 the first variety with compact panicle of the program will be released to the farmers. Different genes for resistance to crown rust were indentified and varieties with different combinations of genes were recently released by the program (CRUZ & FEDERIZZI, 1996).

Besides the gain in grain yield and grain quality , the use of major genes turns selection more efficient , allowing the extensive test of more crosses in the same space of time. Selection criteria should be changed when adaptative genes are the target in order to couple with new

plant ideotype. Plants with genes for earliness and short plant height should be selected for high growth rate and higher biomassa production in order to compete with standard varieties. New genes that may increase the final crop adaptation should be incorporated in the program in order to mantain the current yield trends.

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TABLE 1. Mean grain yield of UFRGS varieties in six locations of Brazil , in four different years.

VARIETY	1992	1993	1994	1995	Mean	% BC
UFRGS14	3322	2829	2954	2715	2955	112
UFRGS 15	3140	2885	3181	2736	2985	113
UFRGS 16	2922	2962	2968	2781	2908	110
UFRGS 901717	3497	3147	3004	2457	3026	114
UFRGS 884068	2711	3076	2405	2475	2682	101
UFRGS 91905	-	3622	2862	2949	3144	119
BEST CHECK	2055	2744	2954	2838	2648	100

TABLE 3. Mean number of days from emergence to heading of UFRGS varieties in four locations and three years.

VARIETY	1993	1994	1995	MEAN
UFRGS 14	88	86	87	87
UFRGS 15	95	93	95	94
UFRGS 16	96	93	94	94
UFRGS 901717	87	86	84	86
UFRGS 884068	93	92	91	92
UFRGS 91905	95	93	92	93

TABLE 4. Mean plant height of UFRGS varieties in four locations and three years.

VARIETY	1993	1994	1995	MEAN
UFRGS 14	92	89	89	90
UFRGS 15	90	89	89	89
UFRGS 16	98	100	106	101
UFRGS 901717	94	91	100	95
UFRGS 884068	90	83	89	87
UFRGS 91905	96	86	92	91

TABLE 2. Means test weight of UFRGS varieties in four locations and three years.

VARIETY	1993	1994	1995	MEAN
UFRGS 14	49	50	53	50.6
UFRGS 15	49	47	53	49.6
UFRGS 16	52	48	54	51.3
UFRGS 901717	53	54	56	54.3
UFRGS 884068	53	48	52	51.0
UFRGS 91905	50	48	54	50.6

Oat Production and Genetic Breeding at the University of Passo Fundo, 1977/1995.
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Production. During the last nineteen years (1976/1994), the acreage and the production of oats have increased in Brazil. In 1994, 275.835 ha of grain oats were cultivated, 662% more than in 1976. Production of grain was estimated on 257.279 metric tons, 560% more than in 1976 and a yield of 933 kg/ha (Table 1).

Crown rust (*Puccinia coronata* Cda.), stem rust (*Puccinia graminis* f.sp. *avenae)* and barley yellow dwarf virus are the most important diseases limiting the oat production in Brazil. Beyond the genetic resistant or tolerant releasead cultivars, most of the large farms use fungicides, but most of small ones do not.

Aluminum toxicity is also an important limiting factor for oats growth in many acid soils in Brazil.

The most important production regions are in Southern Brazil, states of Rio Grande do Sul (65%), Paraná (24%) and Santa Catarina (6%). In 1976, Brazil imported 37,2% of oats consumed, and in 1989, only 3,2%. About 25,000 metric tons are used for human food while most were utilized for feed, especially for horses. The consumptiom of oat for human food increased in the last years, especialy due the B-glucan.

In South Brazil, oats are the most important winter crop for pasture, planted isolated or associated with ryegrass, rye or legumes. An estimated area of 2,000,000 ha of pasture oats, mostly, black oats (*Avena strigosa Schreb*), are grown in this region. This latter increase is due to an increase in dairyng and beef fattening during the winter.

Oats are also important as conservet forage, silage and hay, especialy in dairy production systems. Many farmers in Rio Grande do Sul sow oats (*Avena sativa* L.) immediately following summer crops, graze them during the winter and harvest grain from the regrowth.

During the last few years increased cultivation of oats for winter soil cover has become important in soil erosion control. Utilization of oats in this system is also important for control of the wheat disease "take all" (*Gaeumannomyces graminis* var. *tritici*), weed control by allelopathic effect and to form a "mulching" for soybean and others summer crops, mainly through the no tillage system. For this purpose the area was estimated in1994 about 1,000,000 ha in South Brazil.

Current investigations are concerned with various breeding programmes which are carried out at the University of Passo Fundo (Passo Fundo, RS), State University of Rio Grande do Sul (Porto Alegre, RS), Cooperativa Regional Triticola Serrana Ltda (Ijuí, RS) and Cooperativa Agrária Mista Entre Rios Ltda (Entre Rios, Guarapuava, PR).

Genetic Breeding. Since 1977 the University of Passo Fundo is carrying out a programme in oat genetic breeding. The main objetives of the program is the development of new cultivars with higher yield potential, better adaptability to the different regions, better grain quality and resistance to the most important diseases, such as crown rust (*Puccinia coronata* Cda.), stem rust (*Puccinia graminis* f.sp. *avenae*) and barley yellow dwarf virus (BYDV). The programme is based on an annual introduction of genetic material from the University of Winconsin and Texas A & M, through the project "Breeding Oat Cultivars Suitable for Developing Countries" and populations proceedings from crosses carried out at the University of Passo Fundo. The gool of this crosses is to produce new genetic combinations through the sexual recombination of adapted germplasm in South of Brazil.

The crosses are being made in net house during almost are year around. In average have been done thirty different parental combinations by year and the seed set abtained is about 50%. Two biotechnology techniques in oats are being developed at the Biotechnology laboratory of the University of Passo Fundo: somaclonal variation and haploid inducing by intergeneric crosses (oat x maize and oat x millet). The callus induction and plant regeneration "in vitro" technique was stabilished with the purpose to obtain genetic variability through tissue culture to contribute to the oat breeding program. Several positive variations in quantitative and qualitative traits were observed in oat somaclones. One selected somaclone is being avaluated in 1996 regional trial. The development of new methodologies to obtain haploid plants can contribute, in a helpful way, to accelerate the breeding process and new cultivar release, because it is possible to get homozigous and uniform plants in only one generation from segregating populations. The method that have been used during the selection of the segregating material is the genealogic.

Table 1: Cultivation, area, production and yield of oats in Brazil over the period 1976-1994

Year	Area (ha)	%	Production (metric tons)	%	Yield (kg/ha)
1976	36.205	100	38.962	100	1.076
1981	90.231	249	98.475	253	1.091
1986	127.855	353	153.663	343	1.045
1991	263.385	727	228.332	585	867
1992	284.027	784	295.283	758	1.040
1993	265. 916	734	260.977	670	981
1994	275.835	762	257.279	660	933

As a result from nineteen years of these activities, seventeen new oat cultivars were released with a significant improvement over the older cultivars Coronado and Suregrain. Six cultivars (UPF 7, UPF 13, UPF 14, UPF 15, UPF 16 and UPF 17) are currently recommended for cultivation in several regions in Brazil, mainly in South Brazil. Eleven cultivars were eliminated due to their susceptibility to crown rust. Pedigree and other phenotypic charateristics of the new oat cultivars developed at the University of Passo Fundo are shown in Table 2. All cultivars provide from the germplasm introduced by the project Oat Cultivars Suitable for Deloping Countries with cooperation with the University of Winconsin and Texas A&M University (USA).

The cultivar UPF 16 have the most seeds disponibility (46%) in 1996 in the State of Rio Grande do Sul. The UPF cultivars represented, in the last year, 81% of the certificated seeds produced.

Table 2: Identification, pedigree, grain yield, test weight, grain weight, number of days to heading and plant height of oat cultivars developed at the Passo Fundo University, in Passo Fundo, 1992/95

Identification	Pedigree	Grain yield (kg/ha)	Test Wt. (kg/hl)	GrainWt. (mg)	Heading (days)	Plant Ht. (cm)
UPF 16	Coronado/X1799-2/Sel.Passo Fundo// X3530-40	2.796	54	29	93	92
UPF 17	Coronado/X1799-2/ Sel. Passo Fundo// X3530-40	2.616	53	34	92	89
UPF 15	QR306=COKER82-83// IL3376/OA338	2.607	53	31	98	109
UPF 14	XI205/X2286-2	2.032	50	25	100	94
UFRGS10(ck)	-	1.925	46	22	95	100
UPF 7	TCFP/X2503-1	1.814	44	24	101	94
UPF 13	QUADCROSS/PC25	1.560	46	24	101	110
UFRGS 7 (ck)	-	1.392	41	19	91	89

Hybrid female sterility in barley. Y. FUKUSHIMA* and T. KONISHI** , *Faculty of Agriculture, Kyushu University, Fukuoka 812, Japan.*

Introduction. Partial sterility was found in hybrids between Ethiopian varieties and Tayeh 1 (Chinese landrace), although chromosome configrations at meiosis of the hybrids were normal, suggesting the hybrid sterility is caused by gene interaction (Konishi and Linde-Laursen 1988). Then, we examined the characteristics and genetic behavior of the hybrid sterility, and further investigated geographical distribution of genotypes for the hybrid sterility with special reference to phylogenetic differentiation.

Materials and Methods. Two Ethiopian varieties, Mota 10 and Adi Abun 5, were used as the parents to examine the sterility in the hybrids with Tayeh 1. Reciprocal crosses were conducted between Mota 10 and Tayeh 1, and F_1 plants were grown, together with their parents. F_2 plants of Tayeh 1 x Mota 10 and their F_3 progenies were examined individually for seed fertility. The seed fertility per plant was expressed as the average of seed fertility of three spikes, which was determined by dividing the number of seeds by that of florets in the central rows of each spike. Further, reciprocal backcrosses were made between Adi Abun 5 and Tayeh 1, and seed set after pollination was tested.

Geographical distribution of genotypes for the hybrid sterility was investigated using F_1 plants of 385 barley varieties and 26 strains of *H. spontaneum* crossed with both of the tester stocks, Tayeh 1 and Mota 10.

Results and Discussion.

Characteristics of the hybrid sterility. Two kinds of F_1 plants derived from reciprocal crosses between Mota 10 and Tayeh 1 were grown, together with their parents. Table 1 shows all the F_1 spikes exhibited partially sterile (62-92%), while the parental ones were highly fertile (89-100%). And, no difference in seed fertility was found between reciprocal F_1 plants, indicating no cytoplasmic effect on the hybrid sterility. Similar results were obtained when the F_1 plants were grown in different years, and from F_1 plants of Adi Abun 5 x Tayeh 1 (Fukushima and Konishi 1994), indicating the hybrid sterility is not significantly influenced by genetic backgrounds and years. It should be noticed here that sterile florets were randomly scattered, not clustered, in each spike of the F_1 plants. Furthermore, pollens in the F_1 plants were well stained with aceto-carmine to be almost completely fertile, so the hybrid sterility might not be caused by pollen abnormality.

Table 1. Comparison of seed fertility per spike between reciprocal F_1 plants of Mota 10 x Tayeh 1.

Cross		Seed fertility (%)		No. of spikes tested
		Mean	Range	
Mota10 x Tayeh 1		78.0	62 - 92	30
Tayeh1 x Mota 10		77.2	64 - 87	28
(Parent)	Mota 10	99.6	96 - 100	20
	Tayeh 1	98.7	89 - 100	20

Genetic analysis of the hybrid sterility. Reciprocal backcrosses were made between Adi Abun 5 and Tayeh 1. As shown in Table 2, when the F_1 plant was backcrossed to the parents, Adi Abun 5 and Tayeh 1, average seed sets were 90.1 and 88.7%. In the reverse crosses, however, the seed sets reduced to 64.7 and 66.6% without completely fertile spikes. The difference between the reciprocal backcrosses indicated that, when the F_1 plant was used as the female parent, seed set was around 25% lower as compared to when the F_1 plant was the male parent, suggesting that the hybrid sterility is induced by sterile female gametes of the F_1 plants.

Table 2. Seed set per spike in reciprocal backcrosses between Adi Abun 5 and Tayeh 1.

Cross	Seed set (%)		No. of spikes crossed
	Mean	Range	
Adi Abun 5 x F_1#	90.1	80 – 100	9
Tayeh 1 x F_1	88.7	73 – 100	8
F_1 x Adi Abun 5	64.7	48 – 79	7
F_1 x Tayeh 1	66.6	52 – 84	6

F_1: Adi Abun 5 x Tayeh 1.

As illustrated in Fig. 1, frequency distribution of seed fertility in F_2 population of Tayeh 1 x Mota 10 showed a wide variation ranging from 42 to 100%. A total of 133 F_2 individuals were roughly classified into three groups based on the seed fertility; semi-sterile (less than 70%), partially sterile (70–90%), and fertile (more than 90%) ones. A segregation ratio of 18:31:84 was observed. Further investigation was carried on examining segregation for seed fertility in F_3 progenies. Semi-sterile and fertile plants segregated in progenies of the semi-sterile F_2 individuals, and semi-sterile, partially sterile and fertile plants were found in progenies of the partially sterile F_2 individuals. Fertile F_2 individuals produced homogeneously fertile F_3 progenies.

From these results, it may be concluded that the hybrid sterility is controlled by two recessive genes (*sfg1* and *sfg2* for sterile female gamete, tentatively written *a* and *b*), and that Mota 10 and Adi Abun 5 carry *AA bb*, while Tayeh 1 contains *aa BB*. Their F_1 genotype is *Aa Bb*, and four types of *A B*, *a B*, *A b* and *a b* gametes appear in both of the

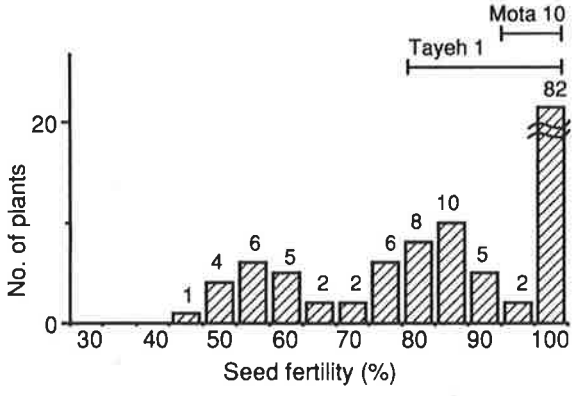

Fig. 1 Frequency distribution of seed fertility in F_2 population of Tayeh 1 x Mota 10.

male and female sides, each with the same (25%) frequency. As the *a b* female gametes become sterile, seed fertility of the F_1 plants decreases to 75% theoretically. Further, segregation (18:31:84) of seed fertility in the F_2 population mentioned above well fit to a ratio of 2:3:7 (χ^2 = 1.47, P= 0.50), assuming that genotypes of semi-sterile, partially sterile and fertile F_2 individuals were *aa Bb* or *Aa bb* (theoretical seed fertility=50%), *Aa Bb* (75%), and *AA BB*, *AA* -- or -- *BB* (100%), respectively.

Geographical distribution of genotypes for the hybrid sterility. A distinct difference in genotype for the hybrid sterility between East Asian and Ethiopian varieties was found in Table 3. Namely, about a half of the East Asian varieties contained the *aa BB* genotype, while one-third of Ethiopian varieties carried the *AA bb* genotype. The remains of both regions and all but one varieties of other regions are of the *AA BB* genotype, same as all the strains of *H. spontaneum* examined.

Table 3. Geographical distribution of genotypes for the hybrid sterility.

Region	Number of varieties and strains examined			
	AA BB	*AA bb*	*aa BB*	Total
Japan, Korea, and China	58	–	54	112
India, Nepal and Pakistan	13	1	–	14
S.W. Asia, Turkey and Europe	82	–	–	82
Ethiopia	120	57	–	177
H. spontaenum	26	–	–	26

These results suggest that the prototype for the hybrid sterility was of the *AA BB* genotype, and that two types of recessive mutation occurred independently at the *A* locus in East Asia and at the *B* locus in Ethiopia during the dispersal of barley and new genotypes of *aa BB* and *AA bb* were established.

Summary. Hybrid sterility is governed by two duplicate genes, *sfg1* and *sfg2*, and the female gametes of *sfg1 sfg2* become sterile, resulting in that seed fertility of F_1 plants between *Sfg1 sfg2* and *sfg1 Sfg2* genotypes decreases to 75% theoretically. Further, the seed fertility is not significantly influenced by cytoplasms and genetic backgrounds, and years grown the F_1 plants. About a half of East Asian varieties possess the *sfg1 Sfg2* genotype, while one-third of Ethiopian varieties carry the *Sfg1 sfg2* genotype. The rest of the varieties in these regions are of the *Sfg1 Sfg2* genotype, same as the varieties in other regions of the world and strains of *H. spontaneum* examined. From these results, geographical differentiation of barley is discussed.

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Variation in the agronomic characters of barley derived from anther culture compared with the original cultivars. A. HANG and K. SATTERFIELD, USDA-ARS and University of Idaho, P.O. Box 307, Aberdeen, Idaho 83210, USA

Introduction. The technique of anther or microspore culture has been improved in recent years with the modification of growth media (Finnie et al. 1989, Ziauddin et al. 1992, Hou et al. 1993), selection of microspores at a particular stage of development, and treatment of the excised spikes at low temperature (Devaux et al. 1993). Large numbers of green plants have been regenerated from many cultivars and F_1 hybrids. Field evaluation of plants derived from F_1 hybrids have been reported (Friedt and Foroughi-Wehr 1983, Rossnagel et al. 1987, Powell et al. 1992). The objective of this study is to investigate the variation in the agronomic characters of barley plants derived from anther culture compared with the original cultivars and select valuable mutants for barley improvement.

Material and Methods. Seed of three lines derived from anther culture of 'Andre', fifteen lines derived from 'Hector', ten lines from 'Morex', twenty-five lines from 'Moravian III', and nine lines from 'Otis' were planted along with selfed seeds derived from the original cultivars; under irrigation at Aberdeen and on dryland at Tetonia, Idaho in four replications at each location, using randomized complete block design, each block consisting of four rows, 2.40 m long and 0.30m apart. The two center rows in each block were harvested. The following characteristics were recorded: heading date, plant height, 100 seed weight, and seed yield. Analysis of variance and Duncan's multiple range test were used for mean comparisons.

Results and Discussion. Under irrigation at Aberdeen, three lines derived from Andre were significantly different from the original cultivar in heading date, plant height, and seed weight. Derived lines were earlier, taller, and seed weight was increased compared to the original cultivar. There was no significant difference in seed yield. Fifteen lines derived from Hector had heading dates ranging from 58.8-62.2 days from planting, with at least five derived lines later than the cultivar and eight lines shorter than Hector. Seed weight was decreased in some lines and no difference was found in seed yield of Hector derived lines. Lines derived from Otis and Moravian III were very similar to the original cultivars, with the exception of one line derived from Moravian III (Table 1). Line number 6 derived form Moravian III was consistently later, shorter, and higher yielding at both locations. Half of the derived lines from Morex were later than the cultivar, but other characters were not significantly different. The patterns of variation in four agronomic characters of all derived lines at Tetonia were similar to those at Aberdeen. Powell et al. (1984) found that anther culture derived lines from 'Sabarlis' showed significant differences in seven characters including days to awn emergence, plant height, neck length, number of fertile tillers, number of grains on the main tiller, grain yield, and thousand grain weight. Overall, the derived plants tended to be late and were taller, with a longer neck and fewer tillers. These results may be somewhat similar to those lines derived from Hector or Morex which were late to mature, or lines derived from Andre which were taller than the cultivar, in our study. However, lines derived from

Moravian III (with the exception of line 6) and Otis were not significantly different in heading date and plant height from their parental cultivars. The patterns of variation in agronomic characters were also different depending on cultivar genotypes. This variation may be used to select desirable characters. If we overcome albinism, anther culture can be an effective method in crop improvement.

Table 1. Means of heading date, plant height, seed weight, and seed yield of Moravian III and lines derived from microspore culture under irrigation at Aberdeen, Idaho.

Cultivar/ derived lines	Heading (No. of days)		Plant height (cm)		100 seed weight (gm)		Seed yield (gm)	
Moravian III	60.8	bc*	87.7	ab	4.8	ab	817.9	bc
1	61.2	bc	91.5	ab	4.5	cd	865.5	abc
2	61.5	bc	88.2	ab	4.6	bcd	831.5	abc
3	61.0	bc	88.9	ab	4.5	cd	801.2	bc
4	61.0	bc	87.7	ab	4.5	cd	782.5	c
5	61.5	bc	89.5	ab	4.5	cd	884.3	abc
6	63.5	a	78.4	c	5.0	a	972.5	a
7	60.8	bc	89.5	ab	4.6	bcd	936.3	ab
8	60.0	cd	90.8	ab	4.7	bc	902.2	abc
9	58.5	d	91.5	ab	4.5	cd	873.1	abc
10	58.5	d	91.5	ab	4.5	cd	885.6	abc
11	60.5	bcd	89.5	ab	4.5	cd	832.0	abc
12	62.5	ab	86.3	b	4.5	cd	863.2	abc
13	60.8	bc	90.5	ab	4.5	cd	780.3	c
14	59.8	cd	88.9	ab	4.8	ab	823.4	c
15	61.2	bc	89.5	ab	4.4	d	910.0	abc
16	60.8	bc	90.5	ab	4.8	ab	895.2	abc
17	60.5	bcd	86.4	b	4.6	bcd	872.3	abc
18	61.0	bc	92.4	a	4.6	bcd	849.2	abc
20	61.2	bc	90.2	ab	4.7	bc	868.9	abc
21	60.5	bcd	90.2	ab	4.6	bcd	864.9	abc
22	61.0	bc	88.2	bc	4.5	cd	816.0	bc
25	62.2	ab	89.8	ab	4.6	bcd	903.4	abc
26	61.8	abc	88.9	ab	4.5	cd	819.4	bc
27	61.8	abc	91.8	a	4.8	ab	855.8	abc
28	61.0	bc	89.9	ab	4.6	bcd	831.9	abc

*Means within a row followed by the same letter are not significantly different at P=0.05 of the Duncan's multiple range test.

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Recurrent Selection for Yield Stability in a Broad-Based Oat Population.

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Introduction. Oat yields are strongly influenced by environmental conditions, with the result that yields often fluctuate greatly from year to year. Improving the yield stability of oats is an important objective of this research. However, the gain from selection for yield and yield stability will be limited by the amount of genetic variability present in the populations under selection. Problems of adaptation tend to inhibit exchange of elite germplasm from one global region to another, and thereby restrict the useful genetic variability available to a breeder in a given area. The research described here addressed these two issues simultaneously by developing a broad-based germplasm pool containing genes from oat lines from geographically diverse regions, and by attempting to improve yield potential and stability in the population via selection. The objectives of this research were, first, to determine if yield and yield stability of oats can be improved via recurrent selection based on testing in widely divergent environments. Secondly we wanted to develop a broad-based gene pool to facilitate germplasm exchange among U.S. and Scandinavian oat breeding programs, and to incorporate wild species (*Avena sativa* L.) germplasm into oat breeding programs.

Materials and Methods. To facilitate germplasm exchange by recombining genes from various sources, a broad-based gene pool was constructed from 20 cultivars and lines. The parents were chosen based on the results of yield trials conducted on a diverse set of genotypes tested simultaneously in Norway and Iowa. The parental material included one cultivar ("Sheldon") and nine experimental oat lines from Iowa. Five of these lines represented introgression materials expected to contain 12.5% *A. sterilis* germplasm from different accessions. Three other midwestern U.S. cultivars, "Don", "Ogle", and "Premier",were used as parents. Two Canadian cultivars, "AC Lotta" (source of the hulless trait) and "Newman", were used and provided genes for photoperiod insensitivity. Five Scandinavian oat cultivars and lines were also used, including "Lena", "Frigg", "Martin", and "Munin". Frey et al. (1988) described a method to complete one cycle of recurrent selection per year in spring oats. This method was modified to produce enough seed from each line to test in five locations each year. Random crosses among selected parents were made in a circular fashion to develop new cycle populations in the fall greenhouse. Five S0 progeny were produced per cross to form a full-sib (FS) family. FS S0 families were increased in the spring greenhouse to produce FS S1 families. At each of the five locations, three replications of the FS S1 families plus checks were grown in randomized complete block designs of hill plots spaced 0.3 m apart with 30 seeds sown per plot. This basic protocol varied slightly among the different cycles in the following manners. Cycle 0 (C0) was developed directly from single crosses among the 20 inbred parents, using a complete diallel without reciprocals or selfs to produce 190 C0 FS families. The five locations used in the evaluation were: Ames, Kanawha, and Nashua in Iowa; Kapp, Norway; and Aberdeen, ID. The C0 families were independently culled for height and heading date. Remaining families were selected based on mean yield over three locations, considering Iowa means as one location. Hulless families were compared separately, and 46 non-hulless lines and four hulless families were selected to be parents of the next cycle. S2 plants of each parent family were crossed five times to make 250 C1 families. The same locations were used to evaluate the C1 population, but all locations suffered a very poor growing season, which caused late maturity to hamper the evaluation experiment. No data were available from Norway in time to make selections in C1.

Instead, the 7 highest yielding families were chosen each from IA and ID, and the remaining parent families were chosen based on rank sums. A total of 38 non-hulless and 2 hulless parent families were chosen and used to develop 192 C2 FS families. Selections among C2 families were based on mean yield over the 5 locations (Aas, Norway replaced Kapp in this and following years), with 30 parent families chosen (including two hulless types) to develop 210 C3 FS families. In 1995, the C3 population was tested along with random samples of families from previous cycles to evaluate the gain from selection in this program. The entries included: 20 original inbred parents, 100 randomly chosen families from each of the C0-C2 populations, and all 210 C3 families. The entries were randomly assigned to four sets, so that each set had all 20 original parents, 25 families from Cycles 0,1, and 2, and 52 or 53 C3 families. Some parent lines were duplicated as entries to make 156 entries per set. The entries within sets were arranged in 12x13 triple lattices at each location, and a sets within replications design was used at each of the five locations. Yields were adjusted for lattice block effects and also adjusted to account for segregation of the hulless trait in some families. Samples of 100 seeds of each entry from two IA locations were scored for % hulless (HLS), and the yield of each family was adjusted as follows: Adj. Yield = Yield/[(HLS*GP) + (1-HLS)], where GP = 0.73, the average 1995 IA groat %. Means over the three IA locations were used to represent one IA environment.

Results and Discussion.No significant difference among cycles were observed for heading date or plant height. Significant differences among cycles were observed for grain yield across and within environments (Table 1.) The rate of gain for yield was greatest in Idaho (1.2%/cycle) and lowest in Norway (no significant linear regression of mean yield on cycle). This compares to a rate of gain of 2.6% per cycle reported by Pomeranke and Stuthman (1992) after five cycles of selection for yield in an adapted population based on single-environment yield evaluations. An exception to the trend of improvement was that the mean yield of C3 was greater than that of C2 only in Iowa. To determine changes in stability over cycles of selection, we estimated genotypic correlations among environments, calculated as $r_g = \sigma^2_G/(\sigma^2_G+\sigma^2_{GE*})$, where σ^2_{GE*} is the genotype by environment variance corrected for heterogeneous genotypic variances within the different environments. (Table 2; Dickerson, 1962).The change in stability over cycles followed the same trend as did grain yield: increasing with each cycle except C3. Direct comparisons of genotypic variances cannot be made among all of the cyclesbecause the parents were inbred lines, while the C3 families were tested as FS S1's and the other cycles were evaluated as FS S2's. However, it appears that genotypic variance for grain yield has been maintained in the population (Table 2), which should allow further progress from selection. We have identified several families in the later cycle populations that exhibit high yield potentials across environments. An example of one of these, IA94366 from C3, is shown in Table 3. The heterogenous nature of these segregating families may provide buffering against environmental influences, and this complicates comparisons with inbred lines. We plan to incorporate such selected lines into our cultivar development programs in an attempt to broaden the genetic base and improve the yield potential and stability of our cultivars. In conclusion, this selection method was successful at improving yield of the population across and within diverse environments, although at a lower rate than would be expected of selection for yield in a single environment. In addition, the later cycles tended to exhibit greater yield stability over environments. However, the most recent cycle of selection did not follow the trend of improvements. This may have been due to poor yield evaluations in C2, rather than due to inherent problems with the methods used. Because genetic variation for yield has been maintained in the population, we can determine if gain from selection can continue in this population by continuing the program for several more cycles.

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Table 1. Cycle means across and within locations for grain yield (g/plot).

Cycle	Overall	Iowa	Idaho	Norway
Parents	53.4±0.6	26.4±0.3	96.3±1.0	38.4±0.5
C0	54.4±0.7	26.6±0.3	98.1±1.0	38.6±0.5
C1	55.4±0.7	26.6±0.3	99.5±1.0	39.9±0.5
C2	56.2±0.7	27.2±0.3	101.6±1.0	39.9±0.5
C3	55.6±0.5	27.3±0.2	100.2±1.0	39.4±0.4
b-value	0.62±0.18	0.24±0.05	1.13±0.35	0.33±0.18
%/year	1.2%	0.9%	1.2%	NS

Table 2. Genotypic variances, genoptye by environmental variances, and genotypic correlations among environments for each cycle of selection.

Cycle	σ^2_G	σ^2_{GE}	r_g
Parents	20.1±1.5	190.5±14.6	0.10
C0	7.1±2.1	19.7±2.1	0.42
C1	10.4±3.0	26.3±2.8	0.51
C2	11.6±3.1	25.6±2.7	0.55
C3	8.9±1.9	26.8±1.9	0.45

Table 3. Grain yields (g/plot) and ranks of a superior C3 FS family (IA94366) and best checks across environments.

	Iowa		Idaho		Norway	
Entry	**yield**	**rank**	**yield**	**rank**	**yield**	**rank**
IA94366	30.2	1	113.7	2	41.1	10
Ogle	29.4	5	96.6	85	34.9	99
H688-4	28.9	8	102.1	37	37.8	49
H61-3-3	28.8	10	95.7	93	38.7	36
Newman	28.1	20	100.0	56	43.9	3
Martin	22.7	156	105.8	22	40.7	14
Lena	25.8	111	105.5	23	43.9	4
LSD(1)	2.5		8.6		4.8	
LSD(2)	2.1		7.0		4.0	

LSD(1) is the least significant difference for comparing IA94366 to checks at α=0.05.
LSD(2) is the least significant difference for comparing checks at α=0.05.

Biotechnology as a possible component of spring barley breeding. S.A. IGNATOVA, V.D. NAVOLOTSKY, **S.Ph. LUKYANYUK**, M.L. MACHNOVSKAYA. Breeding and Genetics Institute, Odessa 270036, Ukraine.

Introduction. The terms of variety creation in self-fertile species are limited by the duration of breeding process technology.

Mathematical models have been used to demonstrate that doubled haploids can result in significant acceleration of plant breeding programs (1). Various authors conclude that each self-pollinated haploid plant can present different combinations of the genes from the parents, but the progeny of each doubled haploid is quite identical, and potentially each haploid plant can become a variety (2,3). In the traditional spring barley breeding it takes 5-6 years to create constant lines, and 8-12 years to develop a variety. In the united research of plant breeders and biotechnologists of our institute we made an attempt to work out a technological model of creating variety material on the basis of homozygous lines from spring barley haploids.

Material and Methods. The method described by K. Kasha (4) and O. Iensen (5) is the base of receiving haploids in the crosses of H. vulgare and H. bulbosum. To determine the efficiency of haploid yield the conditions of haploid formation in Odessa cultivation region have been studied, as well as the term of excision, growth conditions in vitro and the influence of both partners on the yield of complete plants.

Results and Discussion. To decrease the progamic incompatibility it has been proposed to process the flowers of the maternal forms with γ-aminocapronic acid (0.1%) and proline (200 mg/1), and the treated embryos demonstrated increased viability.

During a postgamic period, a spraying of the pollinated plant with a mixture of gibberellic acid (75 mg/1) and boric acid (0.015%) twice after pollination had a positive effect on seed formation. In the experimental hybridization of H. vulgare and H. bulbosum absence or death of the embryos in the spring barley combinations was observed in 30 -54.5%, depending on genotypes and growth conditions. As to our opinion, it resulted from the drought which was often observed in the period of haploid germ formation. In the process of studying of the haploid embryos autonomicity in comparison with the diploid ones, it has been determined that the haploid embryos need physiologically active substances for their development and better differentiation and further germinating ability - such as addition of 0.05-0.1 mg/1 of abscisic acid to the main nutrient media to be cultivated in vitro in addition to kinetin and indolacetic acid. Premature death of haploid germs in unfavourable drought conditions was reduced by cutting the ears on the fifth day, and they were held in artificial climate conditions +18°C in the day and +13°C in the night. The factor which could possibly influence the yield of viable haploid embryos was the clone of H. bulbosum. This made it necessary to select the optimum genotype. The process of haploid doubling by means of diploidization, most often by colchicine presented in different ways was a limiting factor in the number of lines developed from haploids. This process was characterized by a high level of death. The plants that survived set quite a small number of seeds which was not enough for the first field estimation. In this connection it has been proposed to use

hormoneless nutrient medium MS with 2,4D and enriched with proline and glutamine. Plant yield in this case increased. The proposed cultivation methods increased the number of spontaneously doubled haploid regenerants (up to 29.2%) and increased the efficiency of colchicine treatment. After we completed some technical studies with haploid and doubled haploid embryos, we began in 1984 a special program in which F_1 hybrids were selected for yield, resistance to lodging, diseases and drought conditions. After this our efforts were concentrated on creation of doubled haploids from these F_1 combinations and selection of the best of them with complex desired traits. Our research has switched to the technological scheme of breeding with lines from haploids: 5-15 F_1 combinations and F_1 x Hordeum bulbosum (300-1000 DH_1 lines in year 1); studies of DH_2 lines in the field and propagation of the best lines through embryo culture and select 100-300 of the best lines. Preliminary test of 80-300 DH_3 lines for yield and resistance to diseases in years 2 and 3. Competition and ecological testing of 10-15 of the best DH_4-DH_6 lines in years 3 to 6; test for yield, resistance and technological characters in years 5 and 6. The state tests the varieties. As the result of this work several new perspective genotypes were produced. Two from them (Odesskiy 115 and Preriya from 1990-1992) are perspective varieties.

Another 3 potential varieties were referred to the State test in 1994-95. Our results demonstrate the possibility of successfully using biotechnology to reduce the time required to produce new varieties of spring barley.

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Characterization of semi-dwarf barleys. P.E. Jedel, J.H. Helm, and M.J. Cortez, Field Crop Development Centre, Alberta Agriculture, Food and Rural Development, 5030 50 Avenue, Lacombe, AB T4L 1V8, Canada.

Introduction. Semi-dwarf barleys are grown in western Canada because of their increased straw strength and resistance to lodging (Briggs 1990). Reduced lodging and faster recovery lead to significant yield advantages (Briggs 1990, Jedel and Helm 1991). The purpose of this research was to characterize semi-dwarf genotypes and assess the potential and ease of measurement criteria.

Materials and Methods. Twelve cultivars and lines were selected for this study: Ellice (two-rowed, standard height); Leduc and Noble (six-rowed, standard height); Duke, Samson and Winchester (six-rowed, semi-dwarf); Falcon (six-rowed, semi-dwarf, hulless); and I78737, I80047, I80650, I82469 and I83022 (semi-dwarf lines selected from germplasm introductions). Twenty-five plants of each genotype were space planted on May 23, 1991 in a 3.7 m row. Rows were 0.3 m apart. The experimental design was a randomized complete block with four replications. The study was conducted at Lacombe, AB Canada (Black Chernozemic, Penhold loam).

Development (days to anthesis, days to physiological maturity) was measured on a row basis. Grain filling period was calculated as the period from anthesis to maturity. Other field traits were measured on a plant basis and included: plant height (excluding awns); final leaf number of the main culm; length and width of the penultimate leaf; tiller number per plant (at anthesis); plant diameter (at approximately 1 cm above the soil surface) (used to calculate the ratio of tillers to plant diameter). The main culm was identified at the five-leaf stage by tagging. Twenty plants per row were pulled after maturity to take measurements on the main culm and three tillers per plant: culm length (with and without spike); nodes per spike; kernels per spike; internode diameters (top and bottom elongated); internode lengths (top and bottom elongated); and kernel weights. Spike length and spike compactness (nodes per cm) were calculated. Yield and spike numbers were measured on a plant basis. Data were analyzed using PROC GLM and PROC CORR of SAS (SAS Institute Inc., Cary, NC).

Results. Duke was shorter than the standard height cultivars, Ellice, Leduc and Noble, but not significantly so (Table 1). All the other cultivars and lines tested were significantly shorter than the standard height checks, except Samson that was not significantly shorter than Leduc. The introductions were shorter than the semi-dwarf cultivars, Duke, Falcon, Samson, and Winchester.

Duke had an intermediate final leaf number of the main culm (Table 1). The introductions I78737, I82469 and I83022 had low leaf numbers. Low leaf number is not a semi-dwarf trait *per se* as I80047, I80650, Falcon and Samson had leaf numbers similar to the standard checks, Ellice, Leduc and Noble. Duke, Falcon, and Samson had very wide penultimate leaves, while Ellice, I78737 and I83022 have very narrow leaves. Duke, Ellice, Falcon, Leduc, Noble, Samson, Winchester, I80650, and I82469 had very long penultimate leaves, while I78737, I80047 and I83022 had short leaves.

Table 1. Morphological and phenological traits of barley grown at Lacombe, AB.

Genotype	Height	Final leaf	Leaf width	Leaf length	Anthesis	Physio-logical maturity	Yield per plant
	cm	no.	mm	cm	d	d	g
Duke	92.9	8.5	27.0	28.7	59	105	3.8
Ellice	98.7	10.1	17.4	27.7	59	99	1.8
Falcon	77.4	9.9	24.7	29.0	58	100	3.1
Leduc	96.2	9.0	20.4	24.7	54	98	3.2
Noble	99.6	9.3	21.1	29.3	57	101	3.1
Samson	81.4	9.2	25.6	28.4	61	109	3.6
Winchester	75.2	9.7	21.5	24.1	57	102	3.2
I78737	52.4	8.1	18.9	18.0	57	103	1.8
I80047	62.0	9.9	21.5	23.4	58	103	2.3
I80650	51.9	9.5	20.7	24.6	59	102	2.7
I82469	69.0	8.0	20.1	27.2	59	102	1.2
I83022	58.0	8.0	16.1	21.5	56	96	0.8
$LSD_{0.05}$	16.0	1.3	3.0	5.6	1.6	3.0	1.2

Tiller, spike and plant diameters for these spaced plants were very variable from plant to plant within the rows. Ellice have the highest number of tillers and spikes per plant while I78737 had the lowest (data not shown). The two-row I83022 also had a high number of tillers per plant. The ratio of tillers to plant diameter was highly variable. Falcon, Leduc, Noble, Samson, Winchester and I80650 had as high of yield per plant as Duke the highest per plant yielder (Table 1).

Duke, Ellice, Falcon, Samson, I80047, I80650, and I82469 had very late anthesis (Table 1). The same was true for physiological maturity for all but Ellice. I83022 was an early genotype and had a short grain-filling period. Duke, Leduc, Samson, Winchester, I78737, and I80047 had long grain-filling periods.

All of the correlations between traits measured on the main culm and on tillers were highly significant, although for some traits the correlation was higher than for others (Table 2).

The introductions had significantly shorter culms (49 to 64 cm with spike, 44 to 55 cm without) than Duke (90 cm with, 80 cm without), while Falcon, Samson and Winchester had intermediate culm lengths (73 to 76 cm with, 62 to 67 without). Duke and Samson had wide top internode diameters (4.1 to 4.2 mm) while I78737, I82469 and I83022 had very slender top internodes (2.5 to 3.3 mm). Duke, Falcon,

Table 2. Correlation between morphological traits measured on main culms and on three tillers (plant basis). (All correlations were significant at $P<0.001$.)

Trait	Correlation r^2	Trait	Correlation r^2
Culm length (total)	0.94	Internode diameter (top)	0.67
Culm length (without spike)	0.93	Internode diameter (bottom)	0.80
Spike length	0.83	Internode length (top)	0.86
Nodes per spike	0.93	Internode length (bottom)	0.39
Kernels per spike	0.95	Kernel weight	0.75
Compactness	0.53		

Noble and Samson had wide bottom internodes (4.2 to 4.7 mm) while I78737, I82469 and I83022 had slender bottom internodes (2.5 to 3.3 mm). Duke, Leduc and Noble had long top internodes (36 to 40 cm) while the introductions and Ellice had short top internodes (14 to 26 cm). Duke, Falcon and Samson had long spikes (10 to 11 cm) with many kernels per spike (69 to 78 seeds), while the introductions had short spikes (6 to 9 cm) and I82469 and I83022 had few kernels per spike (12 to 19 seeds). Of the semi-dwarf types, Duke, Falcon, Samson and I82469 had many nodes per spike (25 to 29 nodes). While having few kernels per spike, I82469 and I83022 did have very high kernel weights (60 to 61 mg).

Discussion. Some traits were too variable to be of use as selection criteria. Although a wide plant base with few tillers may suggest strong and wide stems, measurement of plant diameter was difficult and variable due to unevenness of tiller distribution and the pressure put on the plant during the measurement. However, there was a close relationship between the main culm and the tillers for all the individual stem traits measured. Therefore, it should be possible to randomly select tillers for desired traits such as internode diameter.

Duke, Falcon and Samson were late cultivars with long and wide leaves, wide stems, long spikes and many kernels per spike. While wide stems and leaves and long heads may be desirable, for a short growing season, the lateness is not desirable. The lower leaf number of some of the semi-dwarfs may also be undesirable when the end-use is for forage. The introductions were much shorter than the adapted semi-dwarf cultivars and had very different morphological traits than them. If these lines contain different semi-dwarf genes than found in the current semi-dwarf cultivars, their use may break undesirable linkages.

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Induction And Genetic Analysis of A Multinode, Branched, Dwarf Mutant In Barley.

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Introduction. Artificial induced mutation is an effective method to create new genetic variations in crops. Many morphologic mutants have been isolated in barley by the method. R. T. Ramage and P. Curtis (1980) obtained 26 morphological mutants such as dwarf, erectoide, elongated outer glume, uniculm etc. from the treatment of an entry of a 2—rowed barley Glacier with 800 rads of fission neutrons. Genetic research showed that all of them were monofactoria recessives. H. E. B. Larsson(1982) described two kinds of branching spike mutants from two loci in 2—rowed barley. We have also induced a new multinode, branched dwarf and mutant by treating the naturally dried seeds of barley elite line 6121 with $^{60}C_0$—gamma ray, and studied its inheritance since 1988.

Materials and Methods. A semi—winter 2—rowed malting barley elite entry 6121 of tall stem and good yield potentiality was used as mother material in the mutation induction. 1000 natural wind dried seeds of 6121 were irradiated with 30 krads of $^{60}C_0$—r ray, at a dosage rate of 100 rads/min in Beijing in Autum of 1988. Then the treated seeds were taken to Zhumadian, Henan province and planted in the field. When maturation, only one spike per plant of the M_1 was harvested and mixed up in May of 1989. 1000 seeds of M_2 randomly taken from the mixed were planted with mother line 6121 as a check in Autum of the same year. M_2 was compared with 6121 for morphologic characters all growing period through to screen mutants. Only the mutants were gathered in for M_3 seed in May of 1990. The research on inheritance of the mutation was caried out in Beijing. First, as female the isolated mutant 93—597 (P_1) was crossed to Bowman (P_2), a 2—rowed malting barley variety from USA in 1993. Then, the F_1 was backcrossed to 93—597 and Bowman respectively to produce F_2, B_1C_1 and B_2C_1 seeds besides self—fertilized in 1994. The P_1, P_2, F_1, and B_2C_1 was planted in a 8—rowed and 4—rowed plots respectively. The data of plant height, ear length, internode number, branching character were collected from more than 30 plants for P_1, P_2, F_1 and B_2C_1, more than 100 plants for B_1C_1 and more than 250 plants for F_2 populations respectively.

Results and Dicussion. The M_1 and M_2 plants were compared to those of the mother line 6121 all the growing period through. There was not any significant variation occured in M_1 population. Almost the 1000 M_2 plants did not differ from those of the mother except for a multinode, branched and dwarf mutant. The induction mutation frequency was 0. 1% aproximately. When planted in Beijing in 1991 and 1992 for puration and seed multiplication the mutant performed stable in all morphologic characters. It was named as multinode, branched, dwarf mutant 93—597 based on the mutation traits. Compared with mother line 6121, the mutant 93—597 became winter with prostrated seedling. Plant height reduced to 73. 8 cm from 96. 7 cm, internodes increased to 17—19 from 5—6. The internode below rachis turned into very short, only 1—2 cm long. The secondary shoots initiated from the lateral buds on the second and the third internodes below the rachis of the main stem and tillers and fully developed with normal spike and seeds. The mutant had 2—4 spikes on a culm generally (see picture 1). The leaves of the mutant were narrower, the auricles larger, the grains per spike (14. 8) fewer and the 1000—kernel weight (32. 4g) lighter than those of the mother 6121 respec tively. The main morpholagic characters of 93—597, Bowman and F_1, F_2, B_1C_1, B_2C_1, B_2C_1 of 93—597 × Bowman were listed in table 1. Plant height, internode number and stem branching of the F_1 and B_1C_1 were identical to those of the male parent Bowman. The three traits segregated at 3:1 and 1:1 ratioes in F2

and B_2C_1 respectively, showing that each may be controlled by a pair of recessive genes. But this was the result of seperate analysis on the three characters. If was there any genetic relation among them? This could only be deduced from the analysis of the segregation and recombinations of the three characters in F_2 and B_1C_1 populations. For convenience, before doing so, let the small letter m stand for multinode gene, b for branching gene, d for dwarf gene. Thus genes for the three correspondent wild characters should be M, B and D resperctively. Because three pairs of the traits and genes were considred in the case, there should be 8 kinds of phenotypes and genotypes in F_2 and B_1C_1 populations, if no complete genetic linkage existed. But only two kinds of phenotypes were observed in F_2 and B_1C_1 (table 2). Further, the ratioes of the wild to the mutation in the two populations fitted to 3:1 and 1:1 of the theoretical proportions for one pair of genes respectively. It could be seen the three mutation characters multinode, branching and dwarf were controled by three pairs of complete linking recessive genes or only by a pair of genes with pleotropic effects. Neverthless, the mutations occured at three different gene loci in once irradiation treatment were very rare from the view—point of mutation. The three mutant characters of 93—597 could be a pleotropic syndrome and determined by the same pair of recessive pleotropic genes. Its gene sign was degnated as mbd tentatively. In our research the male parent Bowman has smooth awns, carring r gene on its chromosome 7. The genetic linkage between mbd and r was studied also. The result showed that there was not any linkage between the two genes. They inherited independently each other (table 3). Therefore, the gene mbd for multinodes, branching and dwarf of the mutant 93—597 must be on a certain chromosome except for the chromosome 7. However H. E. B. Larsson (1982,1983) found an earbranching mutant gene s^1 was allelic and recessive to the short rachilla hair gene s^1 on chromosome 7, and a new gene bir for branching inflorescent rachilla was located on barley chromosome 7, less than 1 cM distal from the locus for lax—a. Dormling, I. et al (1975) reported that barley under short photoperiod of 12 hr. appeared growth disorders such as secondary shoots and spikes in the upper parts of stem at the transition zone of the vegetative and generative system. That the wild gene MBD in 6121 transformed into the mutant gene mbd in 93—597 could change the growth and development reaction of the genotype to environments. 93—597 can be used in studing on development physiology of barley. Mutant gene mbd may be a good marker in barley linkage research. Now the location of mbd on chromosome is in progress.

Table 1. genetic fitting test for the three morphologic mutant characters multinode , branching and dwarf in barley

genera tions	internode		shoot		plant		expected ratioes	X^2	P
	normal (5—6)	many (17—19)	normal	branch	tall	dwarf			
P_1	—	30	—	30	—	30	—		
P_2	30	—	30	—	30	—	—		
B_2C_1	33	36	33	36	33	36	1:1	0.130	0.6—0.7
B_2C_1	30	—	30	—	30	—	—		
F_1	30	—	30	—	30	—	—		
F_2	219	71	219	71	219	71	3:1	0.041	.8—.9

* tall: p_2—93.5cm, F_1—97.8cm, F_2—97.0cm, B_1C_1—84.0cm, B_2C_1—82.6cm; * dwarf: p_1—73.8cm, F_2—65.6cm, B_1C_1—55.8cm.

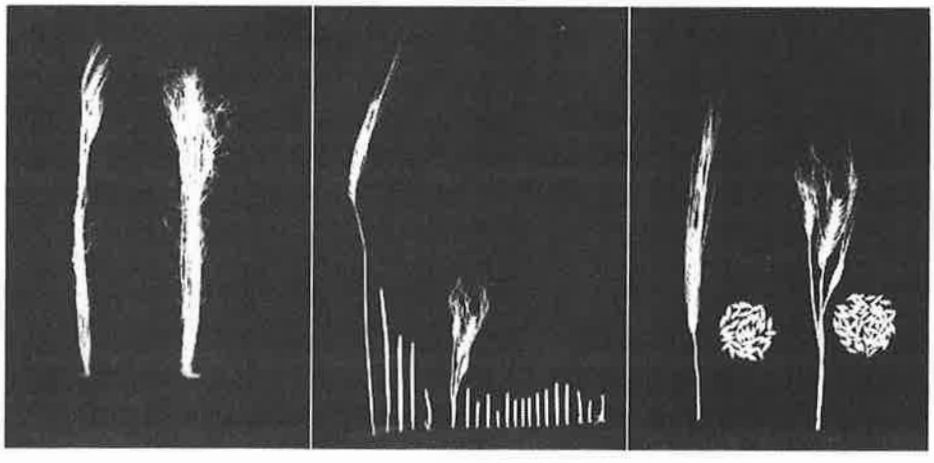
Table 2. comprehensive genetic fitting test for the three mutant characters multinodes, branching and dwarf in barley

generations	genotypes *							
	$M_mB_bD_b$	M_mB_bdd	M_mb_bbD	M_mbbdd	mmB_bD_d	mmB_bdd	$mmbbD_d$	mmbbdd
B_1C_1	33	0	0	0	0	0	0	36
F_2	219	0	0	0	0	0	0	71

* m: multinode gene, b: branching gene, d: dwarf gene; M, B and D: correspondent wild character genes.

Table 3. Genetic linkage test for multinode, branching and dwarf pleotropic syndrome gene mbd and smooth awn gene 'r' in F_2 population

genotypes	MBD-R-	MBD—rr	mbdmbdR—	mbdmbdrr	x^2	P
individuals	162	54	56	18		
expected ratio	9	3	3	1	0.0567	0.85—0.90



Picture 1. plants, spikes, internodes and grains of the multinode, branched and dwarf mutant 93—597

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Characterization of a barley shrunken endosperm mutant, *seg8*.

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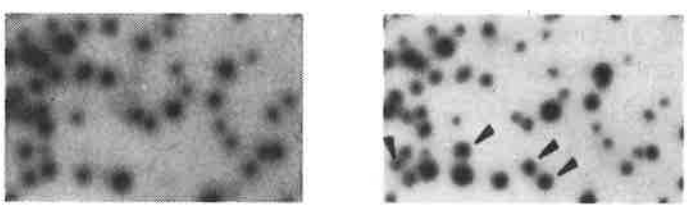
Introduction. *Seg8* is one of eight maternal-effect mutants that have been identified in barley that show a shrunken endosperm phenotype. These mutants do not express xenia, which means the phenotype depends on the genotype of the maternal plant and is not affected by the pollen source. The mutant's inheritance pattern is monofactorial recessive, and embryo development is normal. Every phase of growth except endosperm development, such as seedling emergence, vegetative growth and pollination appear to be normal(1). Various hypotheses about the mechanism of action of the *seg* mutants have included: 1) transport of assimilates into the endosperm may be blocked; 2) a growth factor, normally present in the unfertilized egg cell, may be missing; 3) a growth factor, normally transported into the endosperm during early endosperm development, may be missing in the mutant. The reduced sink size of these mutants provides a model to study the relationships among maternal tissue, endosperm, and embryo in developing cereal seed. The focus of this study is to define the mutant gene *seg8* and it's action in developing barley seed. The study of spontaneous or induced mutants is an alternative to the production of transgenic plants to determine effects of specific gene action. In this aspect, the *seg* series is a natural resource for the study of sink/source relationships, especially in cereal plants. At fertilization, the fusion of two polar nuclei and a sperm cell to form the endosperm mother cell occurs in the maternally controlled environment. All cellular materials originate from the megasporocyte, which is controlled by a genetically programmed sequence of gene action in the reproductive organs of the mother plant. These maternal origin materials are degenerated in time after fertilization and replaced with newly synthesized cellular units by the action of genetic information from the progeny nuclei. However, in the initial stages of progeny growth, cellular metabolism depends on the maternal origin set of enzymes. The role of the cytoplasmic materials in this initial developmental stage is a largely unknown aspect of plant developmental biology. *Seg8*, the mutant in this study, was a spontaneous mutant of barley line 60-Ab-1810-53, which was later released as a component of the cultivar Klages(1).

Experimental. Developing seed samples of *seg8* and it's counterpart Klages were harvested from the age of 4 to 8 days after anthesis and immediately frozen in liquid nitrogen. In this seed sample, three different metameric structures are mixed: maternal origin tissues (pericarp, testa, vascular bundle etc.), embryo and endosperm. Total RNA was extracted from the frozen seed sample with guanidine thiocyanate, and poly(A)$^+$RNA was purified on an oligo d(T) column. Two µg of Klages poly(A)$^+$RNA was used for cDNA synthesis using a RiboClone kit (Promega). Size-selected double strand cDNA was ligated to the

λZapII vector and packaged with the Gigapack in vitro packaging system (Stratagene). A total of 30,000 pfu from the unamplified primary cDNA library were differentially screened. Plaque lifts were initially hybridized with first strand cDNA probes from *seg8* and then with Klages cDNA. Selected plaques were isolated and subsequently converted to pBluescript SK by in vivo excision. Plasmid DNA was sequenced by the dideoxy method using Sequenase (USB). DNA sequence data were analyzed using the GCG program (Madison, WI) and Blast search in NCBI. Genomic DNA was extracted from first and second leaves by a slightly modified CTAB method. Genomic DNA (8 µg/lane) was restricted with BamHI and HindIII and probed with the clone seg8492 which was identified in the differential screening. *Seg8* and Klages were crossed and F1, F2 and F3 progenies were propagated to measure segregation ratios.

Results and Discussion. ***Differential screening and sequence analysis.*** Six candidate clones were identified among cDNA clones expressed exclusively or at an elevated level in Klages developing seed. One of six candidate clones, named seg8492, was identified as a putative aspartate aminotransferase gene (Fig. 1).



A B

Figure 1. The secondary screening result. Hybridization with the first strand cDNA of *seg8* (A) and Klages (B)on the Klages cDNA library. Arrows represent differentially hybridized clones, later identified as a putative aspartate aminotransferase.

The deduced N-terminal amino acid sequence (67 a.a.) of this clone (insert size: 1.5 Kb) has 73%-93% identity with aspartate aminotransferase (E.C. 2.6.1.1 : AAT) of various plant species(2,3,4).

RFLP segregation pattern in F2 plants. Genomic DNA is extracted from both parent plants, *seg8* (seg8-/-) and Klages (seg8+/+) and 16 F_2 plants. The phenotype of all F_2 seeds is wild type (plump: normal shape). In the F_2 plants, the homozygous mutant allele, seg8-/- should exist in the ratio of 1:3, and this genotypic segregation pattern can be detected in F_3 seeds. Genomic DNA was restricted with BamHI and HindIII and Southern blotted. When hybridized with seg 8492, a RFLP resulted which produced bands of 7.5 kb. In two of sixteen F_2 plants, the RFLP pattern matched that of the *seg8* mutant (Fig. 2). The 7.5 Kb band is missing in *seg8*. We have shown a genomic difference between

seg8 and Klages and the identity of the probe. There is not evidence to indicate that AAT is the *seg8* mutant gene. Also, multiple candidate clones including AAT may be down regulated by the direct or indirect action of the mutant gene. Further characterization of these candidate clones will be the next research target to reveal the secret of this mutation.

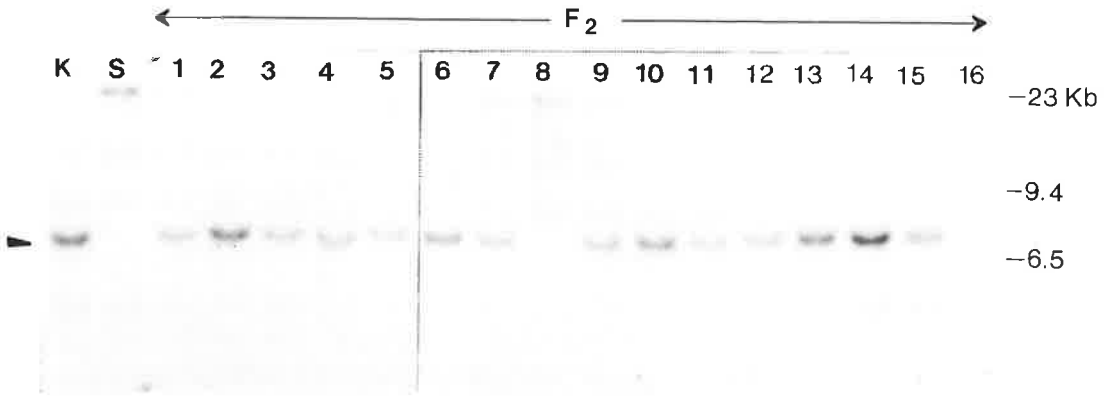


Figure 2. Genomic southern analysis of Klages and *seg8*. Arrow indicates missing 7.5 Kb band in *seg8*. The F_2 plant No.8 and No.16 have the same missing band that was found in *seg8*. K: Klages, S: *seg8*, Number 1-16: F_2 plants. Migration of DNA size standards is indicated on right side.

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Barley Breeding Program at the State Stende Plant Breeding and Agricultural Research Station. S.KALININA, I.PRIEKULE, A.NICGALE,State Stende Plant Breeding and Agricultural Research Station, Dizstende, Talsi Reg.,LV-3298, Latvia

Introduction. Spring barley is the most important cereal crop in Latvia (I.Belicka et al.,1991). In 1995 the acreage of spring barley was 275676 ha, it comprises 43.1% of the total area of cereals. The cultivars 'Abava', 'Imula'and 'Rasa'(bred at Stende) have been included in the recommended list since 1980,1990 and 1996 respectively. In 1995 these cultivars occupied 56,5% of the barley planted area in Latvia.

Materials and Methods. We have the following directions for producing of the new cultivars: the collection testing, breeding work, disease resistance testing on natural and provoked backgrounds, ecological testing and elaboration of agrotechnical recommendations.

Results and Disscussion. We have 638 cultivars and numbers in collection nursery from 30 countries. The best material has been used for hybridization. 40-50% of the Stende Station perspective hybrid lines have been involved in crosses using the backcross method for barley quality improvement. At the same time we have used the classical and bulbosum methods in collaboration with the Latvian Institute of Biology of Academy of Sciences.

The barley breeding scheme (in guidelines, Fig.1): F_1 generation is planted in the field. F_2 is divided into 2 parts.

*First - the material is used for the further breeding;

F_3 - plant selectin, F - stock selection, control nursery, previous, competition and ecological tests.

*Second - the material is used for testing of disease resistence in a separate field. F_3 the inoculation of heads with loose smut (Ustilago nuda). This material of F_4 has been tested for resistance to loose smut and leaf diseases - powderly mildew, rust, leaf spots. The healthy plants have been selected and analuzed morphologically; F_5 - testing of disease resistance; F_6 - testing of disease resistance, yield ability, lodging resistance. The best stocks have been involved in the further breeding process. The breeding material has been tested for disease resistance in the following generations too. The perspective lines have been estimated in other breeding institutes on ecological tests of cultivars. They are obtained in agrotechnical trials, where they receive recommendations for cultivation: sowing season, seeding rate, response to nitrogen fertilizer and pesticides.

The perspective lines and cultivars are being examined for malting quality in the Brewer's Association of Latvia laboratory. Recognized malting cultivars to obtain for lager is 'Abava' and for dark beer 'Rasa'.

Barley breeding group works at seed growing of cultivars 'Abava', 'Imula', 'Rasa', 'Sencis' and 'Ansis', bred at Stende. New cultivars 'Sencis'and 'Ansis'are delivered to

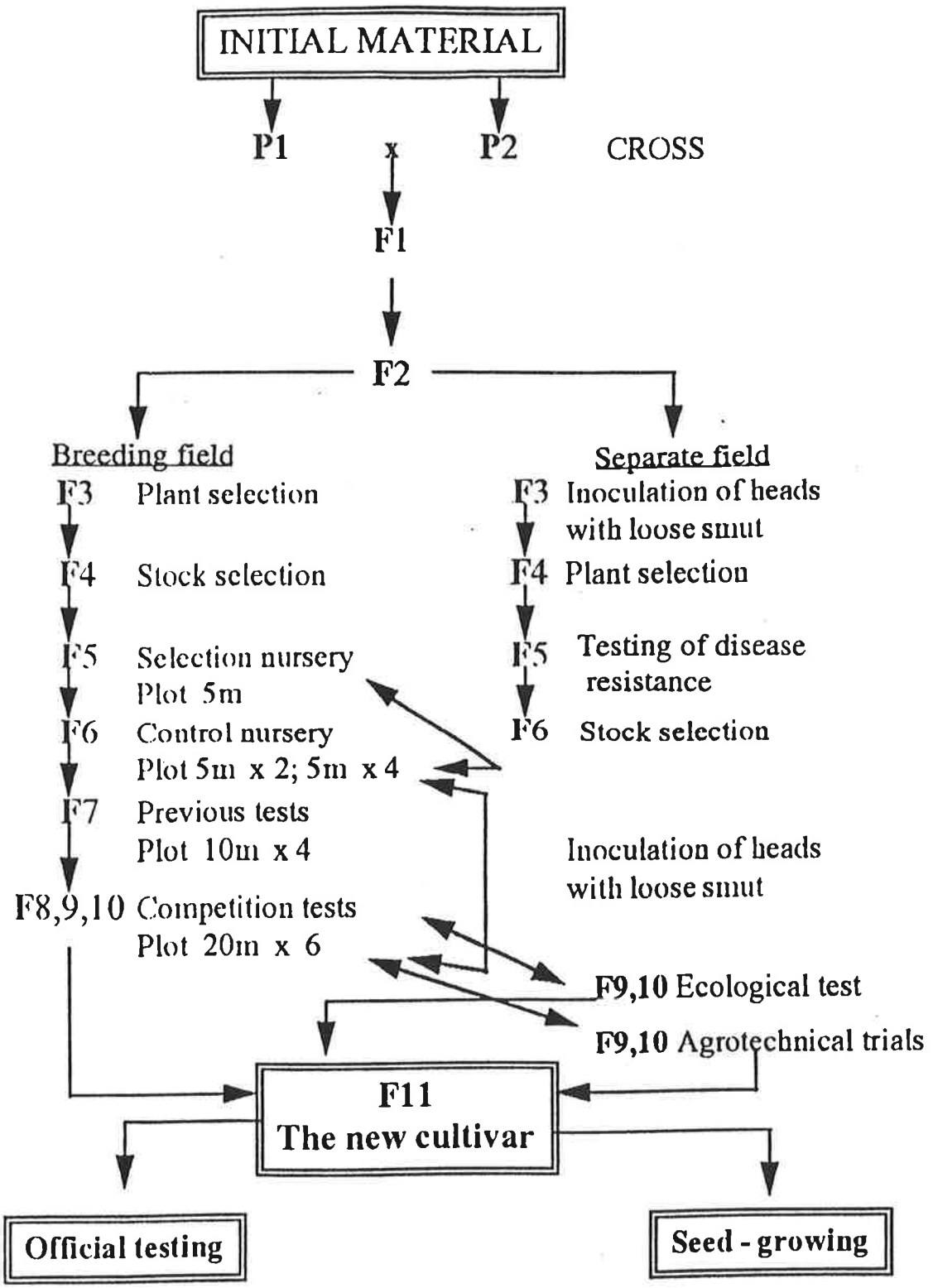

Fig.1. Barley Breeding Group working diagram

official test.

'Sencis'is 2-rowed, combines early maturing and high yields, resistant to loose smut and powderly mildew, good malt quality (S.Kalinina, A.Nicgale, 1995).
'Ansis'is 2-rowed, middle late ripening, high yielding (6.0 - 7.0 t/ha), resistant to loose smut and powderly mildew, with good malt quality (protein content approximately 10,8%, extract content 82,3%, 1000 kernel weight - 49.0 g), good resistance to lodging, straw is 20 cm shorter v s stand. 'Abava'.

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Breeding of very early oat maturing in late fall. M. Katsura, Y. Ueyama and M. Matsuura, Grass Breeding Lab., Kyushu Natl. Agric. Exp. Stn. , Suya 2421, Nishigoshi, Kumamoto 861-11, Japan.

Introduction. Oat is mostly used as forage in Japan. However, compared with other forage crops such as corn, sorghum and Italian ryegrass, oat is a minor crop. The area of forage oat is estimated at about 10,000 hectares. It is mainly distributed in Kyushu, which is located in southwestern part of Japan.

Oat is generally sown in fall and harvested in the next spring, so called fall-sown cultivation. In the case that oat is used as forage, another cultivation system has been adopted in the warm region of Japan. In this cultivation system, so called summer-sown cultivation, oat is sown in late summer and harvested after December within the year. In the warm region of Japan, there is a tendency to sow corn earlier than usual for evading typhoons in late summer and to transplant paddy rice early in spring, that is in March. So, it is necessary to make good use of the field from late summer to early spring after harvesting rice or corn. Oat can be an important choice as well as Italian ryegrass. We have been engaged in the breeding of oat for silage that can be sown in late summer since 1988 and we released a new variety in June 1996.

Breeding strategy. Considering the conditions of summer-sown cultivation, the day-length shifts from long to short-day, and the temperature from high to low. Late summer heat continues till mid September, and this period corresponds to the germination and seedling stage. In addition, the panicle emergence or the growth afterwards is greatly influenced by short-day and low temperature. So, summer-sown cultivation is severe way to oat, which is long-day plant and generally grown under relatively cool climate. However, in order to overcome such unfavorable conditions, we have established the following breeding objectives: 1) stable germination under high temperature, 2) early heading in fall and attaining beyond the milk stage at harvest, 3) high yield. Particularly, we focused on the early heading in fall.

Materials and Methods. For the crossing we chose two varieties, Guelatao and Hayate. Guelatao, released by the National Institute of Agriculture Research in Mexico, has a superior characteristic to be able to attain to heading in early fall. However, it does not have lodging resistance. Japanese variety Hayate has been used in summer-sown cultivation for a long time,

although the heading date in fall is later than Guelatao.

The cross, Guelatao × Hayate, was made in the greenhouse in 1988. The F1, F2 and F3 were advanced in the greenhouse. The F4 plants were grown in late summer to winter, and we conducted the individual selection for the early heading in fall. The F5 lines derived from the F4 were selected for the early heading in fall again. The F6 was selected for good growth in late fall, when it meets low temperature. The F7 was evaluated for yield and the other agronomic characteristics. As a result, we selected Kyushu-1 and Kyushu-2 lines.

Results and Discussion. The characteristics of Kyushu-1 and Kyushu-2 are shown in Table 1. The significant characteristic of Kyushu-1 and Kyushu-2 is that the heading date in summer-sown is earlier than the present leading varieties in Japan, moreover, their dry matter yield is a little bit higher than other varieties (Table 1). In these points, we accomplished our breeding objectives to some extent. These promising lines can reach beyond the milk stage at harvest with high dry-matter percentage and can be preserved as silage directly after cutting. They can contribute to the effective use of the field when other crops cannot be grown.

Table 1. Characteristics of Kyushu lines in summer-sown field test [a)]

Variety, line	Days from sowing [b)] to heading [c)]	Plant height [d)] (cm)	Dry matter yield (ton/ha)	Dry matter percentage (%)
Kyushu-1	41	112.0	4.48	32.5
Kyushu-2	44	102.2	4.47	30.4
Guelatao	48	124.2	4.44	26.7
Hayate*	54	99.8	4.02	21.5
Akiwase*	45	113.7	3.53	26.1

[a)] average of 1992-1994. [b)] August 28. [c)] based on the date when the three panicles emerged from flag leaf per $1m^2$. [d)] at harvest. * Japanese leading varieties.

We also found a interesting characteristic about germination in the summer-sown field test. We observed the difference among varieties and lines in the uniformity of germination. Our experimental lines were good, but some varieties were poor. Then we tried the germination test at constant temperatures of 20, 30 and 35℃, because we considered this would be due to high

temperature of soil. As a result, the difference similar to that in the field test was observed at 30℃ (Table 2). Radford and Key (1993) described that the critical temperature for differentiation of genotypic effects of germination was 30℃ and that high temperature induced a temporary dormancy or state of 'suspended animation'. Corbineau et al. (1986, 1993) also examined dormancy of oat seeds in detail. Our results agree with their results, although we have not evaluated dormancy. That is, when seeds of our lines harvested in spring are stored at room temperatures, it does not matter to sow in the next late summer. It means that non-germination habit under high temperatures disappears in a few months after harvesting.

Table 2. Germination percentage of some varieties and lines for summer-sown cultivation

Variety, line	20℃	30℃
Kyushu-1	98	88
Kyushu-2	95	94
Guelatao	96	89
Hayate*	84	21
Hayabusa (Super Hayate)*	89	0

Note: Seeds were harvested in spring 1995, stored in room after preparations. Then we started the germination test on August 29, which corresponds to the sowing date for summer-sown cultivation.
* Japanese leading varieties.

Through this breeding process, we have got to be interested in the heading and germinating characteristics of our developed lines. In fact, we obtained some lines with interesting heading habit in addition to Kyushu-1 and Kyushu-2. Hereafter, we are going to advance the breeding while examining the genetic background of heading and germination.

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The development of suitable hulless barley cultivars for southern Australia. A.J.KERRY and A.R.BARR, Department of Plant Science, Waite Campus, University of Adelaide, Adelaide, South Australia 5064, Australia.

Introduction

In September 1988, the 'Alternative End Uses of Barley' workshop was held in Adelaide. Throughout this workshop, considerable interest was expressed by local animal feed compounders in developments in Canada where two hulless barleys were released to growers.

Consequently, hulless barley breeding at the Waite Campus was initiated in 1989 primarily for feed for monogastric (pigs and poultry) animals. The major objective was to select hulless lines that expressed low beta glucan levels with agronomic and adaptation characteristics comparable to current Australian covered barley cultivars but with an expectation that a 10% reduction in yield, due to the lack of husk, would be observed. Beta glucans in barley have anti-nutritional properties when included at high levels in broiler chicken diets and to a lesser extent in young pigs. Growth of the chickens is depressed, and feed conversion efficiency is reduced. In addition, the birds produce sticky, moist droppings (Bhatty, 1986). Since the formulation of the feed objective, feed enzymes such as beta glucanases have become available and now used almost universally in barley-based diets in the poultry industry.

Traditionally, much of the barley breeding and research in Australia has been targeted at developing covered barley cultivars for the malting and brewing industries. However, at the European Brewery Convention Congress held in May 1995, a number of papers (eg., Nguyen, 1995) were presented outlining changes in processing that may have significant impact on the focus for barley breeders and researchers. A new generation of mash filters was described that could replace the traditional lauter tuns used during mashing. If such technology is adopted then quality requirements for barley may change and hulless barleys may become a viable option. Hulless barleys may also alleviate problems of disposing large quantities of 'spent' grain after the brewing process. With the possibility of increased utilisation of hulless barleys for malting, significant changes to the breeding objectives of hulless barleys in South Australia have been made.

Hence, the major objectives are to improve grain yield, agronomic characteristics, energy content and physical grain quality.

Two hulless barley cultivars have been released in Australia (Table I), namely Namoi and Morrell. When compared to Schooner, they are low yielding, small grained and high in beta glucan.

Table I: Performance of two Australian hulless barley cultivars compared to the covered standard cv. Schooner when grown in four field trials in 1994 in South Australia.

	Yield (gm/plot)	% Grain Plumpness (>2.25mm)	Extract (%)	DP	BBG (%)	Grain Protein (%)
Morrell (WA)	591	66	83.10	341	4.99	13.00
Namoi (NSW)	530	78	82.69	862	4.48	15.61
Schooner (SA)	742	98	79.11	693	3.27	9.60

This paper describes the genetic variation for two major quality (grain and malting) components of the hulless lines that are being developed in South Australia.

Materials and Methods

1. Hulless Barley Field Trials

1.1 1994 season. 577 F_6 and F_7 hulless barley lines from 23 crosses involving 14 hulless and adapted covered lines were grown in replicated yield trials at five locations around South Australia in 1994. A total of 347 hulless lines were selected from Charlick Experiment Station, Strathalbyn for grain and malting quality analysis.

1.2 1995 season. 360 F_7 and F_8 hulless lines from 23 crosses involving 14 hulless and adapted covered lines were grown at six locations around South Australia in 1995.
height and head retention prior to harvest.

2. Grain Quality Analysis

Grain Protein (GP) was measured using a Technicon 400 Near Infra-red Reflectance (NIR) Spectrophotometer. Results are expressed as percentage grain protein on a dry weight basis (Barley Quality Report Season 1994). Barley Beta Glucan (BBG) was measured using a Megazyme kit assay. Results are expressed as percentage beta glucan on a dry weight basis (Barley Quality Report Season 1994).

3. Malting Quality Analysis

30 grams of each sample was micromalted in a Phoenix Automatic Micromalting System without the use of additives. The micromalting schedule has three main stages: (i) **Steep and Air Rest**, 7:8:9:6:0.5 hours (wet:dry:wet:dry:wet) at 15°C, (ii) **Germination**, 88.5 hours at 15°C and (iii) **Kilning**, 30-40°C for 9 hours, 40-60°C for 4 hours, 60-70°C for 2 hours and 70-80°C for 4.5 hours. This program is optimised to produce malts with a steep out moisture of 42-46% and a kilned moisture of 4-5% (Barley Quality Report 1994 Season). Hot Water Extract (HWE) was determined by a rapid small scale method (Macleod *et al.*, 1991) which is closely correlated with the industry standard EBC fine grind extract method ((EBC, 1975) and (Barley Quality Report Season 1994)). Diastatic Power (DP) was measured on an extract of finely ground malt using a rapid small scale variation of a standard starch digestion followed by measurement of reducing sugars with a neocuproine/copper sulphate reagent. Diastatic Power is expressed as micromoles of maltose equivalents released per minute per gram dry weight (Barley Quality Report Season 1994). Malt Beta Glucan (MBG) was measured using a Megazyme kit assay. Results are expressed as percentage beta glucan on a dry weight basis (Barley Quality Report Season 1994). Viscosity was measured on 0.5ml of wort at 20°C using a Wells-Brookfield cone/plate digital viscometer. Results are expressed in centipoise (cP) (Barley Quality Report Season 1994).

4. Analysis of data

Grain and malting quality data for each trait was averaged over two replicates. Means and standard errors were then calculated within the breeding lines for each cross selected.

Results

Large differences were measured between and within crosses (Tables II and III) for all traits. For grain yield and plumpness, hulless crosses showed ranges of 12-22 and 6-44 percentage points, respectively, lower than Schooner. For the grain quality traits, grain protein and barley beta glucan, similar trends for all hulless crosses were observed with ranges of 3-33 and 4-22 percentages points, respectively. For the key malting traits, malt extract and DP, the crosses Chebec/ Richard, Chebec/SB 85216, Galleon/SB 85216, WI 2646/CIMMYT 42002, WI 2646/Richard and WI 2646/SB 85216 were promising with 6-9 percentage points higher malt extract and 10-22 percentage points higher DP than Schooner. Malt beta glucan and viscosity were lowest in Chebec/CIMMYT 42002, Chebec/Richard, Skiff/CIMMYT 42002 and WI 2646/Richard crosses (Table IV).

Discussion

Results from 1994 and 1995 field trials show encouraging genetic variation for grain yield and plumpness, grain and malting quality traits in hulless barley lines developed at the Waite Campus. Future crossing strategies will be aimed at extending and expanding the genetic basis for all these traits as well as incorporating scald and Cereal Cyst Nematode (CCN) resistance. Further improvements to hulless barleys will be achieved by obtaining germplasm from a wide range of sources including breeders lines and introductions requested from other barley improvement programs and recycling hulless barley lines developed at the Waite Campus and using them as parents.

Data from 1994 malting analysis and 1995 field trials combined with 1995 disease testing (scald, CCN) have been used to select the best lines for field trials in 1996. Six lines have been selected from the 360 sown in the 1995 season on the basis of grain yield and plumpness, malt quality and scald and CCN resistance. These lines have been promoted to SARDI Primary (Stage 3) trials for the 1996 growing season.

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Table II: Grain yield and grain plumpness of 312 hulless lines from 10 crosses compared to covered standard cv. Schooner when grown at six locations around South Australia in 1995.

Cultivar/cross (#sel^n/cross in parenthesis)	Grain Yield (gm/plot)	Standard Error	Grain Plumpness (%>2.5mm)	Standard Error
Schooner (n=1)	845	-	92.4	-
WI 2646/CIMMYT 42002(n=28)	699	50.00	86.0	7.05
WI 2646/Richard (n=28)	714	63.07	62.0	18.49
WI 2646/SB 85216 (n=25)	663	42.72	77.7	9.73
Galleon/CIMMYT 42002 (n=34)	687	42.42	72.7	12.51
Galleon/Richard (n=23)	721	36.46	62.2	17.68
Galleon/SB 85216 (n=12)	704	46.29	51.5	17.25
Chebec/CIMMYT 42002 (n=30)	662	47.12	75.6	12.27
Chebec/Richard (n=14)	707	50.27	65.0	13.79
Chebec/SB 85216 (n=11)	739	34.35	59.8	17.28
Skiff/CIMMYT 42002 (n=52)	700	54.05	66.1	13.77

Table III: Grain and malting quality of 312 hulless lines from 10 crosses compared to covered standard cv. Schooner when grown in field trials at Charlick Experiment Station, Strathalbyn in 1994.

Cultivar/cross (#sel^n/cross in parenthesis)	GP*	BBG*	Extract (%)*	DP*	Viscosity*	MBG*
Schooner (n=1)	12.12	3.20	79.86	589	1.78	0.72
WI 2646/CIMMYT 42002(n=28)	14.4	3.75	85.13	648	1.86	0.90
WI 2646/Richard (n=28)	13.66	3.56	85.45	715	1.85	0.79
WI 2646/SB 85216 (n=25)	14.47	3.79	85.54	688	2.05	1.19
Galleon/CIMMYT 42002 (n=34)	13.95	3.89	85.19	615	1.95	-
Galleon/Richard (n=23)	13.13	3.62	85.14	611	2.05	1.03
Galleon/SB 85216 (n=12)	12.49	3.48	87.01	587	1.91	-
Chebec/CIMMYT 42002 (n=30)	16.79	3.35	82.90	666	1.79	-
Chebec/Richard (n=14)	14.99	3.47	84.64	682	1.81	-
Chebec/SB 85216 (n=11)	16.05	3.68	82.92	700	1.91	-
Skiff/CIMMYT 42002 (n=52)	14.60	3.74	85.13	612	1.79	-

* Means within hulless crosses for quality traits shown. SE's have been omitted.

Table IV: Summary of the 1994 malting and 1995 agronomic performances of selected hulless crosses.

Cross Name	Agronomic and Malting Performances
Skiff/CIMMYT 42002	• semi-dwarf, zero head loss, small grain size • high extracts, v. low viscosity
Galleon/CIMMYT 42002 **Galleon/Richard** **Galleon/WI 2776**	• agronomically excellent at Minnipa, Weetulta and Bondleigh • short to medium height, low head loss • v. low pigment, very bright straw and kernels • high extracts and DP's
Chebec/CIMMYT 42002 **Chebec/Richard** **Chebec/WI 2776**	• disappointing - highly pigmented at some sites • medium head loss and height • high extracts and DP's
WI 2646/CIMMYT 42002	• large grain size, medium height • dark straw and kernels stained • v. high extracts and DP's
WI 2646/Richard	• smaller grain size, low head loss, >medium height • segregating for pigment • excellent extracts and DP's, low viscosity
WI 2646/WI 2776	• smaller grain size, low head loss, >medium height • segregating for pigment, excellent extracts and DP's

Somaclonal variation in field performance of progeny of protoplast-derived barley plants. M. KIHARA[1], S. TAKAHASHI[1], H. FUNATSUKI[2] and K. ITO[1],
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Introduction. In this symposium, we have reported the colony formation (Takahashi and Kaneko 1987) and plant regeneration (Sato et al. 1991) from barley protoplasts. Furthermore, we have produced fertile transgenic barley by direct gene transfer to protoplasts of cv. Igri (Funatsuki et al 1995). For the utilization of the protoplast-derived plants which have a useful introduced gene in barley breeding programs, it is necessary to investigate the nature of somaclonal variation which is caused by mutations occurring during protoplast culture and plant regeneration. Therefore, this study investigated the field performance of progeny of protoplast-derived barley plants.

Materials and Methods. Regenerated plants (designated as Pt_1) were obtained from protoplasts isolated from embryogenic cell suspension lines CA3 of cv. Igri (Kihara and Funatsuki 1994) and DLR-G1 of cv. Dissa (Funatsuki and Kihara 1994). Seeds of six Igri Pt_1 plants, four Dissa Pt_1 plants and uncultured control plants for each cultivar were sown in raising plates on December 27 in 1993 and germinated in a greenhouse. After 14 days normally germinated Pt_2 and control plantlets were transplanted to the experimental field in Nitta, Gunma. Each Pt_2 strain was derived from a single regenerated Pt_1 plant. The experiment was conducted as a randomized complete block design with three replications for each cultivar. Within a block, each strain was represented by 9 plants at 5 cm spacings with a wheat guard plant at each end of the row. Five agronomic characteristics of the four Pt_2 and four control plants randomly selected in each block were examined in the spring of 1994. Culm length was measured with the first and second longest tillers of a plant. Heading date was determined on the day when the first and second earliest spikes of each plant emerged. Fertility was calculated with two randomly chosen spikes of a plant which were bagged to promote selfing. Spike length and spikelet density were measured with two spikes on the first and second longest tillers of each plant. Analysis of variance was carried out for five agronomic characteristics represented by the average of the four plants observed as a treatment value in a replication, and the least significant differences for each characteristic were calculated to compare the field performance of Pt_2 plants with that of control plants.

Results. In the spring of 1994, five agronomic traits were examined in the

progeny of the protoplast-derived plants and in the control plants (Table 1). Analysis of variance showed that there was no significant difference among the replicates, but difference among the strains was statistically significant for the five characteristics in the two cultivars investigated. In the Pt_2 generation, culm lengths and spike lengths of all Pt_2 strains in both Igri and Dissa were significantly shorter than those of the parental cultivars. As for the heading date, those of the Pt_2 strains tended to be later than those of the control plants, although two of the six Pt_2 strains in Igri and one of the four Pt_2 strains in Dissa showed no significant differences compared with the parental controls. In the two characteristics of selfed seed fertility and spikelet density, Pt_2 strains of two genotypes showed a different tendency. In Igri, recovery of fertility was observed in Pt_2 generation, and fertilities of only two Pt_2 strains were significantly lower than those of the controls. However, fertilities of all Pt_2 strains in Dissa were significantly lower than those of the controls. On the other hand, one out of six Igri strains was not significantly different from the control plants, but three out of the four Dissa strains were not significantly different in the characteristic of spikelet density. We have statistically analyzed the field performance of Pt_3 generation in 1995, and our results have shown the tendency for the culm lengths of the Pt_3 strains in Igri and Dissa to be lower than those of control plants, and the heading dates of Pt_3 strains in the two cultivars examined tended to be later than those of control plants as observed in Pt_2 generation. Therefore,

Table 1. Field performance of progeny of protoplast-derived barley plants

Cultivar	Strain	Culm length (cm)	Heading[a] Date (day)	Fertility (%)	Spike length (cm)	Spikelet[b] density
Igri	control	66.5	5.3	96.9	8.8	5.8
	1	44.3**	7.9	88.8	3.5**	5.2**
	2	44.6**	8.8	86.5	3.8**	5.1**
	3	39.3**	11.2*	74.9	3.5**	5.1**
	4	46.7**	10.9*	81.2	5.0**	5.6
	5	36.3**	13.3**	52.0**	3.6**	5.3*
	6	37.6**	14.8**	58.1**	2.9**	4.9**
Dissa	control	78.0	18.9	84.7	8.1	6.5
	1	51.7**	26.3**	46.7**	5.2**	6.2
	2	62.3*	22.7	56.3**	6.7*	5.8**
	3	57.0**	24.8*	65.3*	5.7**	6.2
	4	58.5**	23.5*	64.6*	5.7**	6.5

*,**: Significant at 5 and 1 % level, respectively.
a: May 1 and April 1 were defined as 1.0 in Igri and Dissa, respectively.
b: Density was calculated by number of spikelets / cm.

it is certain that the changes in agronomic characteristics observed in Pt_2 generation of the two cultivars are genetic and heritable to Pt_3 generation.

Discussion. This is the first report on the field performance of the progeny of protoplast-derived barley plants, and our results show that majority of the barley plants recovered from protoplasts possess a certain kind of somaclonal variation. This fact suggests that *in vitro* environment for plant regeneration from protoplasts might be mutagenic to cultured cells of barley. It is not certain whether this variation was induced during callus induction, suspension establishment, protoplast culture and/or plant regeneration, as we have not investigated the field performance of the progeny of plants regenerated from primary calluses or suspension cells. Long-term *in vitro* culture for embryogenic suspension establishment might be associated with the occurrence of variation. In conclusion, the protoplast transformation system would be available for barley breeding, because most of the progeny of protoplast-derived barley plants grew vigorously and set seeds. However, changes in field performance might be an obstacle to the application of our transformation system to barley breeding in the future. So we should make a further effort to establish *in vitro* culture conditions that are less mutagenic to cultured cells of barley.

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GENETIC ANALYSIS OF STEM HEIGHT IN BARLEY *(Hordeum vulgare* L.)

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INTRODUCTION: The basic task of barley breeders is genetic improvement of most important traits in barley. The direction and aim of barley breeding are determined by intrnded for a specific purpose of its use. Considering this, the selection of barley today in two direction is develope. One is development of high yielding and high quality of malt barley cultivars and second is development of forage barley cultivars with high yield and quality.

The stem height is very important components of yield and lodging resistance of barley. The knowledge of genetic mechanism of expression this trait have contribute to improvement of breeding program. Therefore, the development of high yielding cultivars by breeding and increasing the quality is an important scientific task. The analysis of a diallel series of crosses has provided the breeders estimation of best combination (Hayman, 1954; Jinks, 1954). Using this method is possible conduct investigation of genetic control some traits. The diallel analysis in predicting yield of wheat were used by various workers (Paroda et al., 1974; Kraljevic-Balalic and Borojevic, 1985; Knezevic et al. 1993).

The aim of this work was investigation of mode of inheritance of stem height, gene effects and heritability in barley.

MATERIAL AND METHODS: The investigation carried out in the Centre for Small Grains Kragujevac. The four genetically divergent barley cultivar (HVW-247, Partizan, NS-293 and KG-15) were intercrossed according to scheme of diallel crosses (excluding reciprocals) and F_1 and F_2 hybrids were produced. The parent cultivars and F_1 and F_2 hybrids were grown at the same time in the randomized complite block design with three replication on the experimental field of Agricultural Research Institute "SERBIA", Center for Small Grains in Kragujevac. All the cross combination were planted by by hand in 1m long rows with 20 cm spaces between rows and 10 cm distance between each plant in the row.

The height of stem was measured on 60 plants (3 x 20 plants) for parents and F_1 hybrids and on 150 plants (3 x 50 plants) of F_2 hybrids.

The analysis of genetic components of variation was done according to Hayman (1954) and Jinks (1954). The regression analysis was done according to the method used by Mather and Jinks (1971).

RESULTS AND DISCUSSION: The average values of stem height in analyzed barley genotypes were different. The highest mean value of stem height had Partizan cultivar (x=77.1
cm) and lowest had KG-15 barley line (x=70,2 cm). The mode of inheritance was different (partial dominance, dominance, and overdominance) in prevail overdominance. In F_1 hybrids, mainly overdominance of higher parents was established and heterosis were occured. The relative heterosis varied between 3,5-19,4% (Tab. 1. and 2).

In F_2 hybrids: Partizan x NS-293, Partizan x KG-15 and NS-293 x KG-15 the overdominance was found as a mode of inheritance. At the combinations HVW-247 x Partizan, dominant mode of ineritance and in cross combination HVW-247 x NS-293 and Partizan x NS-293 the partial dominance were established. The obtained results are in agreement with results of Borojevic (1965). This author is indicated that overdominance as a mode of inheritance in the crosses of tightly different parents was established.

Genetic components of variation and environmental components of barley stem height variation were estimated (Tab. 3). The dominance components (H_1=158.2 and H_2=156.9 at the F_1 hybrids and H_1=102.1 and H_2=73.9 at the F_2 hybrids) was higher than additive components (D=8.56 at the F_1 and D=7.8 at the F_2 hybrids).

The value of F (interaction of additive x dominance effect) for the stem height in both hybrid generations (F_{F1} =8.6 and F_{F2} =6.4) was positive indicating that in the parent the dominant genes were in exess of the recessive genes.

The value of ratio $H_2/4H_1$ in both F_{F1} and F_{F2} hybrids to deviate from its expected value of 0.25. Obtained values $H_2/4H_1$=0.24 at the F_1 and $H_2/4H_1$=0.18 at the F_2 hybrids showing assimetry of gene distribution at loci indicating dominance for stem height in barley. The frequency values (u=0.59 at the F_1 and u=0.76 at the F_2) of dominant an recessive (v=0.40 at the F_1 and v=0.23 at the F_2) alleles are confirmed preponderance of dominant genes.

The value of the mean dominance degree (H_1/D) in both hybrid generations indicating overdominance in the inheritace of stem height. The ratio Kd/Kr was more than unity in both hybrid generations (Kd/Kr =1.26 at the F_1 and Kd/Kr=1.06 at the F_2) for stem height indicating that dominant genes are in preponderance of dominant genes in all parents.

The value of heritability (42%) indicated that on expression of stem height environmental factors have influence. The similar results obtained Powel et al. (1989), Vasquez and Sanchez-Monge (1989), Ore (1990).

The obtained results for F_1 and F_2 generation have confirme regression Vr,Wr analysis (Fig. 1 and 2). The regression coefficient of Wr on Vr was significantly different from unity in F_1 generation (b_{F1}=0.220+0.440) indicating presence of interallelic interaction. In F_2 generation (b_{F2}=0.850+0.340) the regression slope was significantly different from zero and not significantly different from unity indicating the absence of non-allele interaction.

The regression line cut the Wr axiss below the point of origin indicating overdominance for stem height of barley. The distribution of points along the expected regression line indicating arrangement of dominant and recessive gene. Arrays (NS-293) and (Partizan) were nearer to the point of origin possessed an excess of dominant genes for this trait. Arrays (HVW-247) and (KG-15) possesing more recessive genes.

The additive and nonadditive genes have impact in controlong of stem height of barley. The mode of inheritance have varied (partial dominance, dominance, overdominance)

depends from cross combination. This is in agreement with results Grib (1985), Lalic et al (1984), Vasquez and Sanches-Monge (1989).

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Table 1. Mean values for stem height in barley (parents and F_1 and F_2)

Parent and hybrids	F_1	F_2
HVW-247	75.68	75.68
Partizan	77.07	77.07
NS-293	72.53	72.53
KG-15	70.16	70.16
HVW-247 x Partizan	83.07^{sd}	79.12^{d}
HVW-247 x NS-293	77.53^{d}	76.30^{pd}
HVW-247 x KG-15	77.84^{sd}	74.45^{pd}
Partizan x NS-293	77.40^{d}	78.44^{sd}
Partizan x KG-15	80.84^{sd}	82.48^{sd}
NS-293 x KG-15	85.16^{sd}	73.34^{sd}
LSD :0.05	3.30	3.96
LSD :0.01	4.52	5.42

Table 2.The mode of inheritance, apsolute and relative value of heterosis

F_1 hybrids	Mode of inheritance	Ha	Hr(%)
HVW-247 x Partizan	overdominance	6.96^{**}	8.80
HVW-247 x NS-293	dominance	3.42^{**}	4.60
HVW-247 x KG-15	overdominance	4.20^{*}	6.70
Partizan x NS-293	dominance	2.60	3.58
Partizan x KG-15	overdominance	7.23^{**}	9.80
NS-293 x KG-15	overdominance	18.32^{**}	19.40
LSD :0.05		3.30	
LSD :0.01		4.52	

DOMINANT GENE CONTROLLING HIGH SEED FERTILITY OF RECIPROCAL TRANSLOCATION HETEROZYGOTE

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INTRODUCTION. Translocation lines are useful for genetic, cytogenetic and breeding studies (Ramage, 1964). Since a single reciprocal translocation heterozygote show semi-sterility, its use is not practice in breeding program. We obtained reciprocal translocation line revealed high seed fertility of heterozygote. In this report, we present a dominant gene controlling high seed fertility of reciprocal translocation heterozygote.

MATERIALS AND METHODS. Air dried seeds of genic male sterility, originally developed by Falk and Kasha (1982), were exposed to 25kR of ^{60}Co gamma-rays by 1kR/h and planted in field. 58 crosses were made between 10 male sterile plants (M_1) and various multiple marker lines. F_1 (M_2) plants grown till maturation were harvested and measured seed fertilities with eye based on four classes, i.e., full fertility (more than 90%), partial sterility (70 to 90%), semi-sterility (30 to 70%) and sterility (less than 30%). Pollen mother cells of F_1 plants were examined cytologically to determine metaphase configrations. Selections for plants were carried out on seed fertility and chromosome pairing at metaphase I of meiosis were examined.

As for the selected plants, F_2 (M_3) seeds were sown on nursery bed by 120 seeds per line, following after dividing to plump and shrunken seeds. Young plants from plump seeds and shrunken seeds were transplanted in field and greenhouse, respectively. Young spikes of plants in selected lines were collected and fixed by Farmer's solution for the observation of chromosome pairing at metaphase I of meiosis. Seed fertilities of self-pollination were measured in one spike per plant and those of open-pollination in three spikes per plant. A reciprocal translocation line between chromosome 4 and 6, γ MSF2-224 (Kurauchi, et al. in preparation), was used as the control of seed fertility.

RESULTS AND DISCUSSION. Five plants had one quadrivalent and five bivalent chromosome in meiotic cell among 73 F_1 plants between male sterile line and multiple marker line. Number of plants

belonging to full fertility, partial sterility and semi-sterility was two, two and one, respectively. In F_2 generation, one line from fully fertile plant (BH26 ; cross combination was sex-msg6 / sh-yh) was examined for chromosome pairings at MI of meiosis and measured seed fertilities. 22 of F_2 plants had chromosome configration, 1IV+5II, while 28 plants showed 7II ; then this segregation ratio was fit to 1:1 ratio (χ^2 = 0.72, .25<P<.50). Seed fertilities of translocation heterozygotes with one quadrivalent and five bivalent chromosomes was varied from 3.5 to 75.5 per cent. The average seed fertilities of them was 66.9 percent and the mode was 75 per cent. The seed fertilities of the control line, γ MSF2-224, was ranged from 17.2 to 79.1 per cent, the average seed fertilities of them was 51.3 per cent and the mode was 60 per cent.

Table 1. Numbers of plants for three classes of seed fertility in F_2 generations of translocation lines

Line number	Translocated chromosome	Chromo-some pairing	Numbers of plants of Male sterility 0～35%	Semi-sterility 35～65%	Partial ster. Full fertility 65～100%
γ MSF2-224	4 , 6	7II	17		14
		1IV+5II	5	26	7
Total			22	26	21
BH26		7II	1		27
	? , ?	1IV+5II	1	4	17
		unknown	16	8	33
Total			18	12	77
BH27		7II	1		23
	? , ?	1IV+5II		8	18
		unknown	27	10	15
Total			28	18	56

Seed fertilities were grouped into male sterility (less than 35 %), semi-sterility (35 to 65 %) and partial sterility or full fertility (more than 65 %) as presented in Table 1. Seed fertilies of BH26 were composed of 12 semi-sterile, 77 non-semi-sterile and 18 complete sterile plants being male sterile. Among the plants with a quadrivalent chromosome, one plant was male sterility, four plants were semi-sterility and 17 plants were partial sterility or full fertility. From these results, we built up a hypothesis that a dominant gene controlling seed fertility of translocation heterozygote was induced by gamma-ray treatment.

♀ \ ♂	NA	Na	TA	Ta
NA	NN AA H	NN Aa H	NT AA H	NT Aa H
Na	NN Aa H	NN aa H	NT Aa H	NT aa S
TA	NT AA H	NT Aa H	TT AA H	TT Aa H
Ta	NT Aa H	NT aa S	TT Aa H	TT aa H

N:Normal chromosome, T:Translocated chromosome,
H:High fertility, S:Semi-sterility, A:Gene controlling high seed fertility of translocation heterozygote,
a:Gene controlling semi-sterility of translocation heterozygote,

Fig. 1. Expected genotypes with high seed fertility gene and chromosome constitution in F_2 generation

Based on this hypothesis, we expected F_2 genotypes on high seed fertility gene (Fig. 1). If high seed fertility of translocation heterozygote was controlled by single dominant gene, expected segregation ratio of seed fertility would be seven non-semi-sterility to one semi-sterility. Segregation ratio of seed fertility of BH26 was 77 non-semi-sterile plants to 12 semi-sterile plants. This ratio fitted to the expected ratio 7 : 1 (χ^2=0.18, .75<P<.90). On the other hand, segregation ratio of seed fertility of γ MSF2-224 was 21 non-semi-sterile plants to 26 semi-sterile plants. This ratio did not fit to the expected ratio 7 : 1 (χ^2=78.79, P< .01) but fit to 1 : 1 ratio (χ^2=0.71, .25<P< .50). These results support above hypothesis.

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Barley and oat breeding for quality and disease resistance in Estonia.
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Most widely grown cereals in Estonia are barley and oat. The acreage of barley was 191 400 and oat 35 300 ha in 1995. It makes up 63% and 12% of the total cereal cultivation area, respectively. Barley and oat are mainly used for feed. 15 000 t of barley is needed for malting and same amount for food. 12 000 t of oat is used for producing flakes. Good quality of malting barley and food oat gives a chance to produce them for export. To enhance the competition ability of our cultivars, improving grain quality and disease resistance possess a key role in barley and oat breeding.

The basic requirements for malting barley are: germination more than 95%, protein content 9,0...12,0%, grading at last 90% (kernels large than 2,5 mm), moisture content 11,0...14,0%.
The Jõgeva Plant Breeding Institute has released in 1989 a malt barley cultivar 'Elo' ('Triumph' x 'Lofa'). 'Elo' has turned out to be successful in the trials of European Brewery Convention and meet all requirements of malt barley. Especially good is extract yield (more than 81,5%) and diastatic power (more than 300 WK). A negative feature of cultivar 'Elo' is small kernel (1000 kernel weight less than 40 g).
To improve quality parameters of malting barley, best results has been obtained by crossings with cultivar 'Triumph'. There are used many cultivars ('Hylkema', 'Roland') to increase 1000 kernel weight. The problem is to breed out malting cultivars with low ß-glucan content.
Most widely spread diseases of barley in Estonia are scald (*Rhynchosporium secalis),* and barley stripe (*Helmitosporium gramineum (Drechslera graminea*)). We have tried to find out an initial material for resistance breeding to these diseases, but we have not been a success, up to the present.

The main requirements for grain quality of oat cultivar used for flakes production are large 1000 kernel weight (38g min.), high level of volume weight (560 g/l min.), low hull content and the hulls will have to be easily separated from kernels. In addition, the grains will have to be free from damages, caused by diseases. The main oat diseases spreading in Estonia are crown rust (*Puccinia coronata)* and stem rust (*Puccinia graminis)*. In some years oat crops are moderately damaged by septoria (*Septoria avenae)* and leaf blotch (*Drechslera avenae)*. Only a very susceptible cultivars are attacked by loose smut (*Ustilago avenae)*. The increasing nutritional value of oat is contributed to raising protein and oil content of grains.

In 1993, by the Jõgeva Plant Breeding Institute was released an oat cultivar 'Jaak' ('Fraser' x ('Vigor' x 'Seisukindel')). 'Jaak' has big 1000 kernel weight - more than 40 g. The hull content and the volume weight are in a medium level - hull content remains in limits 24,5...25,5% and the volume weight varies between 545...565 g/l. The hulls are easily separated from the kernels. 'Jaak' is resistant to loose smut, medium resistant to crown rust and medium susceptible to stem rust. The crude protein content of the grains of 'Jaak' is about 13,5% and oil content about 4,5%. 'Jaak' has a high yield capacity. The cultivar 'Jaak' is used for oat flakes producing in Estonia.

The oat cultivar 'Alo' ('Leanda' x 'Lody'), developed at the Jõgeva Plant Breeding Institute, is in cultivation in Estonia since 1986. 'Alo' is resistant to crown rust, stem rust and loose smut. The 1000 kernel weight of 'Alo' is between 34...36g. 'Alo' has a high protein and oil content - 13,5 and 6,0 %, respectively. Hull content and volume weight are on the same level to those of 'Jaak'. 'Alo' is cultivated for feed only.

At the Jõgeva Plant Breeding Institute there have been released oat and barley cultivars with high grain quality and good resistance to diseases, but the breeding work will continue to develop new cultivars with improved characteristic's to satisfy the demands of industry.

BREEDING BARLEY FOR ALUMINUM TOLERANCE IN BRAZIL. E. MINELLA, M. S. SILVA, Embrapa-Centro Nacional de Pesquisa de Trigo, Cx. Postal 569, Passo Fundo-RS, 99001-970, Brazil.

INTRODUCTION

Limited amounts of malting barley have been produced in Brazil since 1930. Average production over the last five years was around 108,000 metric tons per year, supplying one third of the amount needed by the malting industry. The malt produced domestically meets around 40 % of the quantity used for beer production. Although pursued, self-sufficiency has not been achieved mainly because the yield and quality can vary substantially over years and production zones, leaving the industry with no guarantee of a consistent supply of malting quality barley. Losses in yield and quality are associated with adverse climatic conditions and foliar diseases. Low seed set, usually associated with high grain protein content (> 12 %) occurs frequently, being one of the major causes of the instability of yield and malting quality in Brazil. Reduced seed set, due mainly to male sterility, is caused by drought associated or not with heat, during the gametogenesis and fertilization phases of plant development. Barley higher sensitivity to soil water deficit as compared to wheat, triticale, rye and oats, has been associated with its shallower root system developed under the soil conditions of southern Brazil. Reduced root growth has been associated with the high sensitivity of currently grown varieties to toxic levels of aluminum (Al) in the soil and/or subsoil. Acid soils, originally high in exchangeable Al, predominate where barley is grown and, even though they have been surface limed, the Al remaining in the subsoil functions as a chemical barrier to the root growth of sensitive genotypes. Unable to penetrate into the soil profile for water and nutrient uptake, the crop becomes more vulnerable to losses even during short periods of drought stress. Since liming the subsoil is unfeasible, the use of Al tolerant varieties able to develop a deeper rooting system than current ones seems the most appealing approach to reduce the yield losses resulting from drought.

RESEARCH WORK

The objective of this paper is to report the research effort being made at Embrapa in Passo Fundo, Rio Grande do Sul to improve barley for Al tolerance. The work underway has two major goals. The first is to transfer the gene (s) of winter barleys from USA, reported to have a higher degree of tolerance than the most tolerant Brazilian lines (Minella,1989 and Minella & Sorrells, 1992), to current high yielding, disease resistant and malting quality cultivars and lines. The second and most important goal is to find and/or develop germplasm with a higher degree of tolerance than the one already available in cultivated barleys. This goal is being pursued through searching for additional sources of tolerance in both untested cultivated and wild barleys as well as for transgressive segregates in progenies from crosses among tolerant parents from diverse origins. On the search for new sources of tolerance, lines of cultivated barley reported in the literature to have some degree of tolerance and other untested germplasm are under screening and, starting this season, the USDA-SGC accessions of **H. vulgare** subsp. **spontaneum** and a few genotypes of **H. stenostachys** collected in Brazil will be screened. In looking for more tolerant transgressive segregates, we are presently screening the populations of random inbred lines derived from the crosses between the Brazilian lines Antarctica 1, FM 404 and PFC 7802 and Dayton (CI 9517) and, hybrid populations derived from other crosses among tolerant lines of different origins. By using these populations, it is also hoped to establish the relationship between the results from the field tests and those obtained by the hematoxylin staining technique.

The screening is performed in both field and greenhouse, using a soil naturally acid and high in exchangeable Al. In order to account for natural variability in the field regarding the Al level, tolerant and sensitive indicator genotypes, are replicated several times in a set of genotypes under testing. Aiming at avoiding misclassification, a genotype is scored in the field only when it can be replicated. Screening in the greenhouse is either done in pots or in boxes, using a layer of 0,20m of a soil collected in the field and homogenized for uniformity of the Al level. Scoring in the field is based on top growth and on the Al toxicity symptom development relative to those of the checks and/or parental genotypes of known reaction to Al. In the greenhouse, scoring is based on relative top and root growth in unlimed/limed soil. Selection in the field and in the greenhouse is performed using Al levels similar to those found in commercial barley fields.

PRELIMINARY RESULTS

In the screenings performed so far in Al toxic soil, Dayton has shown better root and top growth than the most tolerant Brazilian lines, confirming earlier results obtained by the hematoxylin staining technique (Minella, 1989). Random inbred lines from the Dayton/Antarctica 1 cross that scored the same as Dayton in nutrient solution, were also superior to Antarctica 1 in top and root growth when tested in the soil.These findings, indicate that the tolerance of Dayton can be easily transferred to the target Brazilian germplasm.

Homozygous lines derived from the Antarctica/Dayton cross and backcrosses, will be used to determine under field conditions the agronomic significance of the tolerance of Dayton combined with that of the adapted Brazilian line.

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Pleiotropism of minute gene (*min*) of barley. T. Morikawa and E. Gomi, College of Agriculture, Osaka Prefecture University, Sakai, Osaka 593 Japan.

Introduction. It is coined as mixoploidy that cell populations whose component cells differ in their chromosome numbers, irrespective of whether these numbers are euploid or aneuploid (Nemec, 1910). This phenomenon is often induced by stress from spontaneous mutation, high temperature and chemical reagent in plant (Takahashi *et al.*,1955) Morphology of the minute homozygous plant showed drastic dwarfness as less than 10 cm in plant height. The gene also pleiotropically controlled mixoploidy in root-tip cells. A lot of evidences of minute plants in various cereal crops are reported, but the mechanism is still unknown. The minute plant is suitable for studying organization of cell membrane during cell division. In this study, we compared seed weight, frequency of mixoploidy emergence and tissue culture response between the different genotypes to describe the pleiotropism of the minute gene.

Materials and Methods. The mutant line (OUL 921) of cultivated barley, *Hordeum vulgare* cv. 'Kairyoubouzu' which is heterozygous at *min* locus was used in this study. This mutant gene was spontaneously occurred in the hybrids between hulled and naked barley cultivars (Takahashi *et al.* 1955). Genotype of the plantlets could be determined by measuring plant height in 5 days after germination. The plantlet whose plant height was higher than 5 cm could be determined as tall homozygote (+/+) or heterozygote (+/*min*). The minute homozygote (*min*/*min*) was determined when the plantlet was shorter than 5 cm. Observation for somatic chromosome was carried out according to the method described by Morikawa and Leggett (1996). Immature seeds from the middle florets of each ear were harvested in fourteen days after flowering. The basal MS medium (pH5.8) supplemented with 30g/l sucrose, 2g/l gerangum and 2.0 mg/l 2,4-D was used to induce calluses from immature embryos.

Results and Discussion. The minute gene (*min*) is referred as recessive for plant height. So, it is very difficult to distinguish the genotypes of tall phenotypes in an early growing stage. We preliminarily compared dry seed weight of the homozygote (+/+) and the heterozygote (+/*min*) of tall plants by utilizing progeny test. The frequency distribution was drawed, in which 129 homozygous and 157 heterozygous seeds were weighted. Seed weight of the progeny of tall homozygotes showed small variance (S^2=7.82). The progeny of tall heterozygotes was expected to segregate the genotypes as tall

homo-, hetero- and minute homozygotes = 1:2:1. And it resulted in large variance of seed weight as S^2=16.64. This result indicated that seed weight of the minute homozygote is possibly lighter than those of the tall homozygote and heterozygote. And the *min* gene possibly affects endosperm development and resulted in light seed weight. Dry seed weight differences were tested statistically by utilizing progeny test. Table 1 shows comparison of dry seed weight between tall phenotypes (+/+ and +/*min*) and minute plants(*min*/*min*) from five parental lines. All lines showed that means of seed weight were statistically different between the two phenotypes. In order to clarify relationships between the genotypes and frequency of mixoploidy, somatic chromosome numbers of root-tip cells of tall homozygous (+/+), tall heterozygous(+/min) and minute homozygous (*min*/*min*) plants were examined. The frequency distribution and rage were shown in Table 2. In the tall homozygotes, euploidy cells (2n=14) were 88.4% of the total observed 844 cells. And hypoaneuploid (8.2%) and tetraploid cells (1.4%) were often observed. In the tall heterozygotes, euploid cells were 79.5% and hyperploidy cells (2n=more than 60) were also identified. The frequency and rage of aneuploid and polyploid cells were much higher than those of the tall homozygotes. In the minute, the frequency of aneuploid cells was quite lower than the other two genotypes. The rage of chromosome number was widely distributed from 2n=7 to 195. The typical abnormality was hypoaneuploid cells (24,1%) caused by chromosome losses. In this study, high frequency of chromosome losses and polyploids were especially observed in the tall phenotype. From the evidence of mixoploidy in the tall homozygous (+/+) and heterozygous (+/*min*) plants, the minute gene is thought to affect wild gene to induce mutation when the *min* gene is heterozygous at *min* locus. The chromosome abnormality of minute plants was thought to be accelerated under callus culture condition.. In order to clarify the expression of the *min* gene under culture condition, the growth rates of calluses were compared between the selfed progeny of tall homozygote (+/+) and heterozygote (+/min) for 4 months. Fresh weight of callus of each genotype was measured every month when replacement of culture medium was carried out (Table 3). Mean fresh weight of calluses in tall homozygote was statistically heavier than that of heterozygote during one to three months after callus induction. After four-month-culture, however, the difference of mean fresh weight was not significant between the two genotypes. This result indicated that abnormal mixoploid cells caused by the gene expression of *min* were gradually eliminated during cell culture. So far, regenerated plants of minute homozygote was not obtained.

Table 1. Comparison of dry seed weight between tall (*+/+* and *+/min*) and minute (*min/min*) plants of barley

Parent line	Phenotype	No. of seeds	Seed weight(mg) Mean	S.D.	t-value
1	tall	34	23.71	3.01	6.34***
	minute	15	18.00	2.85	
2	tall	15	20.67	3.99	2.59**
	minute	10	17.40	2.22	
3	tall	43	24.93	3.83	3.53***
	minute	7	20.14	3.24	
4	tall	15	18.68	3.33	2.78*
	minute	8	15.00	2.83	
5	tall	16	18.13	2.34	5.68***
	minute	8	13.50	1.60	
Total	tall	123	22.93	4.29	6.14***
	minute	48	16.94	3.30	

Table 2. Frequency distribution of somatic chromosome number in the root-tip cells of tall homo-, hetero-and minute homozygotes of barley

Genotype	No.of root-tips	Chromosome number(2n) 13<	14	15~27	28	29~55	56	57~69	70	70>	Total cells
+/+	31	69 (8.2)	746 (88.4)	14 (1.7)	12 (1.4)	3 (0.4)					844
+/min	30	94 (11.1)	672 (79.5)	22 (2.6)	23 (2.7)	29 (3.4)	2 (0.3)	3 (0.4)			845
min/min	4	19 (24.1)	28 (35.4)	14 (17.7)	2 (2.5)	7 (8.9)		4 (5.1)	1 (1.3)	4 (5.1)	79

Number in parenthesis is percent.

Table 3. Growth rate of the calluses from immature embryos in the selfed progeny of tall homozygote(*+/+*) and heterozygote(*+/min*)

Months for culture	Parent genotype	No. of calluses examined	Fresh weight of calluses (g) Mean	S.D.	t-value
1	*+/+*	64	0.248	0.132	2.51*
	+/min	49	0.192	0.104	
2	*+/+*	42	0.369	0.168	2.68**
	+/min	36	0.280	0.124	
3	*+/+*	34	0.449	0.218	1.08*
	+/min	23	0.400	0.122	
4	*+/+*	11	0.880	0.360	0.89NS

Inheritance of Russian wheat aphid resistance in spring barley germplasm line STARS-9577B. D.W. MORNHINWEG, D.R. PORTER, and J.A. WEBSTER, USDA-ARS, Plant Science and Water Conservation Reasearch Laboratory, 1301 N. Western, Stillwater, OK, USA.

Introduction. The Russian wheat aphid (RWA), *Diuraphis noxia* (Mordvilko), is a devastating pest of barley grown in the intermountain regions of the western United States. All barley cultivars presently in commercial production are susceptible to RWA feeding damage. Typical RWA feeding damage to the leaf results in characteristic longitudinal white, yellow, or red chlorotic streaks with convolute rolling of the leaf. Rolling of leaves reduces photosynthetic area, provides an optimum environment for aphid reproduction, protects the aphids from contact insecticides and natural predators, reduces stand, and at the heading stage, can prevent spike extrusion, resulting in obstruction of flowering and decreased seed set.

A damage rating scale for wheat and barley seedlings has been developed by Webster et al. (5) based on visual rating of the amount of chlorotic leaf tissue, due to either leaf streaking or spotting, and on the amount of leaf rolling. On a scale of 1 to 9, resistant seedlings have a chlorosis rating of 1 to 3, moderately resistant to moderately susceptible seedlings have a chlorosis rating of 4 to 6, and susceptible seedlings a rating of 7 to 9. All available accessions of *Hordeum vulgare L.* in the USDA National Small Grains Collection have been screened as seedlings for RWA resistance using this scale. Forty-five lines have been identified as resistant and an additional 61 as moderately resistant to moderately susceptible (1).

Genetic diversity has been shown to exist for RWA resistance in wheat, where a total of 6 genes (*Dn1* to *Dn6*) have been identified that confer some level of RWA resistance (4). Mornhinweg et al. (2) reported the high level of RWA resistance in barley germplasm line STARS-9301B to be under the control of two genes (*Dnb1* and *Dnb2*). Analysis of data from the F2 and BC to both parents suggested recessive epistasis of a dominant gene (*Dnb2*) on an incompletely dominant gene (*Dnb1*). Robinson et al. (3) reported a single dominant gene that controlled a moderate level of RWA resistance in barley line S13. It has yet to be determined if Robinson's single dominant gene is the same as *Dnb1* or *Dnb2*. The search for a barley germplasm line with a high level of RWA resistance and simple inheritance goes on, as does the search for genetic diversity.

Materials and Methods. Crosses were made between Morex, a susceptible malting barley cultivar, and STARS-9577B, a newly released RWA-resistant germplasm line. Genetic analysis was performed on 18 parents, 18 F1, 18 reciprocal F1, 200 F2, and 150 BC to each parent, as well as 300 F2-derived F3 families.

Results and Discussion. The number of homozygous resistant and homozygous susceptible F2-derived F3 families indicated multiple gene control. Chi-square analysis of the F2 and BC to either parent suggested RWA resistance in STARS-9577B to be under the control of two dominant genes with recessive epistasis.

Conclusions. RWA resistance in STARS-9577B, measured by chlorosis damage ratings following RWA infestation, appears to be under the control of two genes. Probable gene action is two dominant genes, with recessive epistasis. This inheritance differs from that of barley germplasm line STARS-9301B, indicating that genetic diversity does exist for RWA resistance in barley.

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Analysis of oat and barley plant breeding data using AGROBASE™ 96 for Windows©. D.K. MULITZE, Agronomix Software, P.O. Box 67, Portage la Prairie, Manitoba, R1N 3B2, Canada.

Introduction. Oat and barley breeding programs, not unlike many other plant breeding programs, require the management and analysis of plant breeding data. Cereal breeders often perform early generation, yield trial, genotype x environment, and genetic analyses. A comprehensive, integrated, user-friendly, PC-based software package developed for the specific needs of plant breeding would be invaluable to the plant breeder.

Materials and Methods. The Microsoft® FoxPro® database language was used to develop a plant breeding software package. While a database language does not have some of the scientific functions found in other computer languages, the functions required for computing probability values for the F and t distributions, for example, were developed in native FoxPro code. FoxPro routines were developed for various G x E, genetic, replicated and non-replicated yield trial, and other analyses using algorithms published in scientific and statistical publications. Since Microsoft Windows® is the most widely used operating system for personal computers, and an increasing number of plant breeders are using local area networks (LANs), a Windows single or multi-user-based software package was developed.

Results and Discussion. AGROBASE 96, developed in FoxPro 2.6, is a Microsoft Windows application that requires Windows 3.xx or Windows 95, for both single-user or LAN processing environments. AGROBASE 96 uses standard Windows pushbuttons, icons, spinners, and other controls for easy and intuitive manipulation of files and data. Analyses are quickly performed by setting options in just one or two screens. Statistical and quantitative genetics analyses currently available in AGROBASE 96 for oat or barley breeding programs are as follows:

(1) Yield trial analyses of variance: Conventional analyses of variance for complete block experimental designs using linear models for main effects, interactions, and nesting are supported, as well as for square and rectangular lattices. A batch analysis facility expedites the automatic analysis of hundreds of trials with variables from any number of database files. A breeder could potentially analyse all his/her trials with the click of a button in the BATCH command screen. Entry means may be passed to means files for final reports or G x E analyses. Randomizations for the following experimental designs are supported in AGROBASE 96: completely randomized, randomized complete block, square and rectangular lattice, generalized lattice, Latin square, Latin rectangle, split-plot, split-split-plot, split-block, factorial, and group-balanced block.

Alternatively, yield trials may be analysed by nearest-neighbours methods (Stroup and Mulitze, 1991; Stroup et al. 1994; Ball et al. 1993) to eliminate the confounding effects of field trends and establish a more likely genetic ranking. Trends may be estimated in "east-west" or "north-south" directions. Trends and unadjusted/adjusted residuals may be displayed to verify the analysis. Nearest-neighbours analyses often reduce CVs, increase

precision, and sometimes salvage trials that might otherwise be rejected due to a high CV. A table of means shows the comparative ranks and adjustments from a classical RCBD or nearest-neighbours analysis. Trials need not have been previously randomized for a nearest-neighbours analysis. For more optimal analyses, future trials may be randomized in AGROBASE 96 for a RCBD layout such that no entry is beside another entry more than once in the "east-west" direction.

(2) Genotype x environment analyses: To assess the relative performance and interaction of genotypes across environments, the following analyses or statistics are currently available in AGROBASE: (a) ecovalence (Wricke 1962) as each genotype's contribution to the G x E interaction sum of squares (b) cultivar performance (Lin and Binns, 1988) as a measure of yield and stability relative to the highest mean at each environment (c) stability variance (Shukla 1972) (d) crossover/noncrossover interaction test for pairs of entries (Baker 1988) (e) non-parametric rank difference and variances (Huehn and Nassar, 1989) based on the change in ranks of genotypes across environments (f) stability regression (Eberhart and Russell, 1966) (g) percent of trial mean, or percent of a check or group of checks. Additional analyses are planned for future versions of AGROBASE.

(3) Non-replicated early generation trials analyses: Early generation material may be planted as a modified augmented design (Lin and Poushinsky, 1985) in row-column layout to account for field soil heterogeneity and provide a more accurate estimate of yield. Both modified augmented designs type 1 and type 2 may be randomized and analysed. Up to 2,940 genotypes (including checks) can be included in a type 2 design as a 14 x 14 row-column layout with 15 sub-plots per whole plot. Moving means analysis will be developed in a future version of AGROBASE as an alternative to the modified augmented design analysis.

(4) Analysis of segregating populations: Pedigrees can be generated (Purdy et al., 1968) for F1 hybrids as derived from crossing block files, and then advanced using single seed descent, bulk, or modified bulk selection methods for as many generations, locations and populations as required. Data from populations may be advanced through generations to monitor the change in correlation of traits, response to selection, or the changing frequency of populations or parents in crosses. Rank data may be analysed with the Kruskall-Wallis, Mann-Whitney, or Wilcoxon's signed ranks tests. Histograms may also be generated to characterize data from segregating populations.

(5) General and specific combining ability: Diallel analysis can analyse crosses for general and specific combining ability (Griffing, 1965; Singh and Choudhary 1976), fixed or random effects, with or without subsampling, using parents, F1s, and reciprocals for methods 1 through 4.

(6) Least squares evaluation of parents for crossing: For a given set of parents with data on key traits in a breeding program , all possible mid-parent values for single crosses and single backcrosses between two parents, and three- and four-way crosses are compared (Baker, 1984). Primarily additive genotypic effects are assumed in the cross

prediction.

(7) Other analyses: Regression, correlation, analysis of covariance, Hotelling's multivariate T and Student's T, Chi-square, Friedman's test for the 2-way classification, and probit analysis are also supported.

In addition to the above analyses, AGROBASE 96 provides full support for field books, labels, bar codes, data logger and other file format import/export, field planting plans, reports, file and data management. All stages of a cereal breeding program are supported in AGROBASE 96.The author may be contacted by phone (204-857-833), fax (204-239-0147), or e-mail (mulitze@agronomix.mb.ca).

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UTILIZATION OF CLUSTER AND DISCRIMINANT ANALYSIS FOR PARENTS SELECTION IN BARLEY

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INTRODUCTION

In a breeding programme the first step is evaluation of genetic variation in breeding population and the second step is to choose the parents for crossing blocks .Clearly the good parents must be sufficiently different genetically so that transgressive segregation can occur in their crosses(Knott, D. R.1987).

Multivariate cluster analysis is carried out to categorize genotypes based on their traits. The objectives of this paper are evaluation of genetic diversity of 42 barley genotypes for some morphological and phenological traits and classification of these genotypes for utilization in choosing parents for crossing programme.

MATERIAL AND METHODS

42 barley genotypes (Hordeum vulgare) were evaluated at Sararood Rainfed Agricultural Research Station in the west part of Iran in 1992-93.. The following traits , which are presented in table 1 ,were recorded .

Table 1 : Recorded traits and their measuring units

Recorded traits	Unit
Grain yield (GY)	*Ton/Ha.*
Days to heading (DH)	*Days number to 50% head emergence*
Days to maturity(DM)	*Days number to 50% maturity*
Filling period (FP)	*Days between DH & DM*
Frost damage (FD)	*Percent of plot damage by frost*
Lodging (LG)	*Percent of plot lodging*
Spike shattering (SS)	*percent of spike shattering*
Plant height (PH)	*Centimeter*
1000-kernel weight (TKW)	*Gram*
Growth habit (GH)	*Scored from 1 to 5**

Note: * 1= spring and 5= winter

Cluster and Discriminant analysis was carried out on the means value of these genotypes for cited traits using standard statistical packages.

RESULTS AND DISCUSSION

Four groups of genotypes were detected by cluster analysis using Ward method. For determination of differences among the traits of genotypes belonging to differences groups (D'Antuono, L.F. and A. Pavoni. 1993) , discriminant analysis was carried out by STATGRAPH package . The first three function were significant for discrimination of the

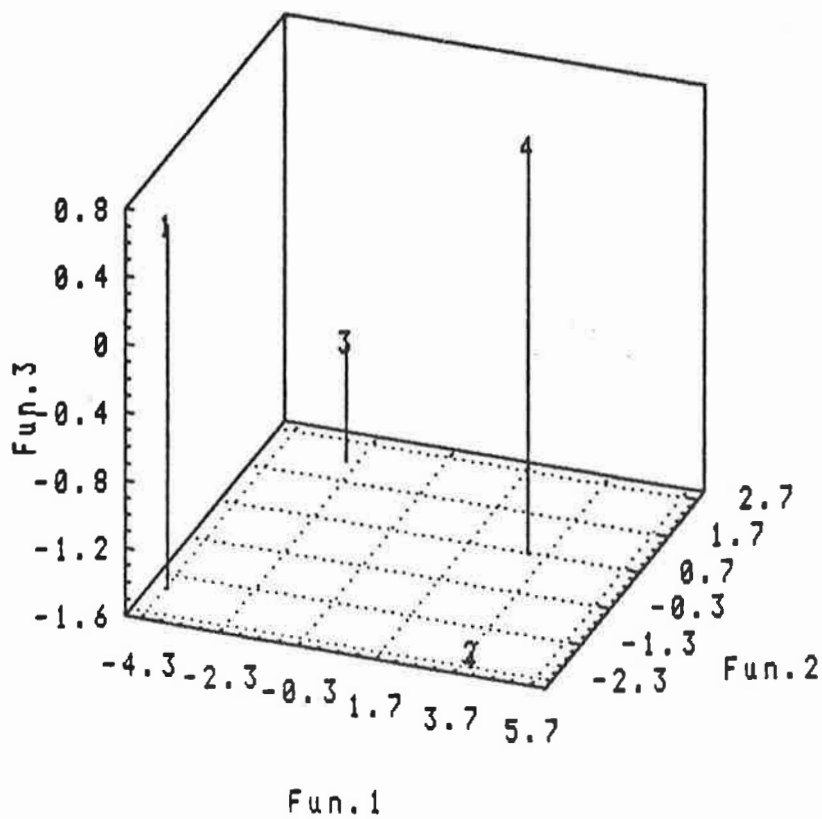
groups and their correlation coefficient to the 10 traits , which were used for discriminant analysis are reported in table 3. Classification results indicated that grouping was done correctly .

Table 3. The first three functions which are significant for discrimination of the groups and their correlation coefficients to the 10 traits .

Traits	Func.1	Func.2	Func.3
GH	0.819*	0.186	0.101
LOG	-0.273	0.530*	0.444
SH	0.093	-0.342*	0.313
FD	0.032	0.183*	-0.004
DM	0.004	0.339	-0.029
DH	-0.001	0.024	0.455*
YLD	0.051	0.323	-0.427*
TKW	-0.077	0.099	-0.361*
PH	-0.099	0.021	0.329*
FP	0.004	0.101	-0.259*

The first function was positively correlated to GH. The second function was positively correlated to LG , DM and negatively correlated to FD and SS . The third function was positively correlated to DH and PH and negatively correlated to GY, TKW and FP. The layout of the groups in the space of the three discriminant functions is presented in figure 1. In this figure groups 1 and 3 are clearly separated from groups 2 and 4 by function 1 .

Figure 1. The layout of the groups in the space of three discriminant functions.



CONCLUSION

If improvement for GH is the objective of breeding programme, it is better to choose parents from those groups which are genetically separated . For example , in this case , parents should be selected from groups 1 or 3 and 2 or 4 (fig.1) . If improvement for lodging resistance and spike shattering and frost resistance are the objective parents should be chosen ,based on function 2 , from groups 2 and 3. If improvement for early heading, increasing kernel weight , plant height and filling period are the objectives , parents should be chosen from groups 1 or 4 and 2 ,based on function 3 (fig.1)

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The change of populations productivity in common spring barley hybrids (F1-F25) in the process of their microevolution. G.F. Nikitenko, The Agricultural Research Institute of Non-Chernozem Zone of Russia, p\o Nemchinovka-1, Odintsovskiy District, Moscow Region, 143013, RUSSIA.

The problem of hybrid breeding is very actual and interest to it is understandable. Further progress in breeding will take place with wide use of hybridization method which gives an opportunity to combine desirable features and characteristics of their parents to a new hybrid organism or receive new qualities which crossing parents do not have.

But as the all world breeding experience shows it is very seldom possible to obtain all planning complex of features and characteristics combined in one hybrid organism. As a rule only a small their part is successful according to the task and the breeding direction even of very grounded crossing combinations. As CIMMIT statistics shows only 0,1% of hybrid combinations carried out in system gave start for new varieties (1).

From one hand the analysis of all aspects of this phenomena gives an opportunity to confirm that in its basis is still insufficient knowledge of elementary processes which take place into real hybrid populations during their evolution. From another hand it is deficiency of testing technology and evaluation hybrid populations. All it does not allow the breeder to direct a forming process to desirable direction. It tells about efficiency of hybrid method.

We carried out many years trials (1965-1995) in this direction. These trials consisted of analysis of genetically consequence for a long time influence of natural selection for hybrid population. The dependence of productivity level of different hybrid generations from speed population homogeneity was observed. Also the influence of abiotic factors for speed, direction and character forming process was studied.

Intervariental hybridization was carried out for it. We used different morphobiological features and characters for hybridization of parental forms. The cycles of the crossing of 1965, 1970, 1979, 1990 were carried out. Special method was used for study population of simple hybrids. It allowed to study and value many generations of one hybrid combination at the same time in one field experiment. The method of our investigations and the result we received for three cydes of crossing (1960, 1965, 1979) we accounted in our previous papers (2, 3).

It is known that to receive necessary results when hybridization method used some need to perform quite a big value of crossing. The scale of the following work with hybrids (study, mark, reject as defective etc.) depends on it directly. The productivity is a main feature which is used as a rule from complex features and characters during selection and reject as defective. Breeder has to use very hard reject as defective for hybrid material from early generation because number of crossing combinations as a rule is very much, but financial-technical possibilities are very limited. Breeder does it to hope that desirable genotypes will select and preserved in this process.

Our many years trials convincing showed that probability of selection and preservation such genotypes is very small. Productivity of early generation hybrids (F1-F4) of 21 crossing combination was more less than the same for their parents. According to this they have to be rejected as defective genotypes. But the same genotypes in later generations (F8-F15) surpass initial forms in productivity for more than -20-30%.

As our results show productivity of simple hybrids raise during their process of microevolution from early to late generations. Inside population interaction of genetically non-homogeneous segregates is the main reason which determines the size and speed in-

creasing of hybrid population productivity in microevolution process. The interaction leads to increase of segregates high reproductive capacity in population in part of frequency characteristic of the population in later generations. In this case the whole population productivity increases.

This conformity to natural laws confirmed by our result of tests and by four crossing cycle evaluations of common hybrids. It allows to make the essential change in the present breeding process technology which directly connected with rational use of potential genetic resources of hybrid populations: 1. to study and evaluate many generations of hybrids from one crossing combination in the same place and at the same time; 2. to make prognosis of breeding value for the received hybrids safely and exactly; 3. to raise effectivity and reliability of elite plant selection.

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CHARACTERISATION OF THE GENE FOR NAKEDNESS IN OATS (*Avena sativa* L). H.J.OUGHAM, J.VALENTINE, and G.LATIPOVA*, Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, Dyfed, SY23 3EB, UK.
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The naked-grained character in the cultivated hexaploid oat, *Avena sativa*, is controlled by a dominant gene, *N-1* (Simons et al.,1978). The effect of the gene is to produce a thin papery lemma from which a naked or hulless caryopsis threshes free, unlike in conventional oats in which the groat (kernel) is surrounded by a thick fibrous hull. Other apparently pleiotropic effects of the gene include multiflorous habit and long rachillas.

As well as its high potential for the study of a unique system of developmental regulation in the small grain cereals, the naked oat gene is a valuable means of drastically altering the potential of the oat crop worldwide (Valentine,1995). By virtue of high content of oil and essential amino-acids, naked oats have a higher nutritive value than any other cereal. A naked oat breeding programme was started at IGER (then WPBS) in 1971. The first naked oats were commercialised in 1989 and now occupy about 4% of oats in the UK via largely unique specialised markets with a high potential for penetration into new, general animal feed and industrial markets.

We sought answers to the following questions.

Are different sources of the gene at the same locus ? (i) We grew 50 plants from each of three F2 populations involving different sources of nakedness. The parental lines were Pendragon and a sister line tracing to Chinese/Laurel, the source of many naked oat varieties (Valentine, 1995); a line containing Cc6333, of unknown origin, found by Boland (1972) to have very high expression, a line containing Uspekh from Usbekistan, and 41/5 from Australia containing Vicar derived from a natural mutant of a Canadian husked oat. 10 plants of each parent were also grown. The average degree of lemma lignification and %naked grains of each plant was assessed. It was observed that the degree of lemma lignification, the morphological character which confers nakedness, has a wider range than % naked grains. However, the latter is a more objective measure and is easier to measure. 41/5 had the poorest and most variable expression of nakedness (8.5/100 hulled grains), followed by Pendragon (1/100) and its sister line (1.7/100), with the Uspekh derivative (2/1000) and the Cc6333 derivative (1/1000) having the highest levels of naked expression.

Irrespective of the degree of nakedness of parents or the measure of expression used, all F2 plants had the naked phenotype. The gene for nakedness in all these crosses is at the same locus. The degree of variation in the F2 was broadly associated with that of the parents. It is not possible to tell from these results whether it is the potency of different alleles or the action of modifier genes that affected the degree of naked expression but the use in breeding of the non-Asiatic source of nakedness (41/5) does seem inadvisable.

At which stages in development do naked and hulled oats diverge? Near-isogenic hulled and naked lines of the winter cultivar Bulwark were developed. The source of nakedness was Cc6333. Plants of each type were vernalised and then grown in a growth cabinet at 20 C and 16h light/ 8h dark. At weekly intervals from 16 days after transfer, apices were examined under a stereoscopic microscope. In the hulled spikelets, the committment to two-floret was made early in development. The rachilla subtending meristematic tissue which in naked oats gave rise to floret 3 onward continues to extend but the tissue withered to give a vestigial structure.

Lignin deposition was visualised using phloroglucinol from four weeks after transfer to maturity, removing soluble lignin precursors by thorough rinsing with ethanol. Lignin did not accumulate in either genotype until the lemma had reached its maximum size, just prior to panicle emergence. The mature hulled lemma is uniformly stained across the lower two-thirds. In naked oats, lignification is largely confined to the vascular bundles. The pattern of lignification, and indeed the morphology of the lemma, is very similar to that of the subtending glumes. Surpisingly, paleas from both types are similar in size and, despite their thin papery texture, exhibit strong lignin staining.

Are there correlated differences in gene products (mRNA and proteins)? In parallel to the above, buffer- and detergent soluble proteins and mRNA translation products were separated by SDS-PAGE (for full description of methods, see Ougham, Latipova, and Valentine, in press). Only at 45 days after transfer to 20 C did a difference in polypeptide profile become apparent, as a 41.5 kDa protein much more abundant in hulled than naked spikelet tissue. At this stage, the band may represent an enzyme required for lignin synthesis or deposition. There were no visible difference between naked and hulled oats in the complement of translatable mRNAs throughout.

Why do diverse compact-panicled dwarfs suppress nakedness? The naked gene also interacts with compact-panicled dwarfs. Both the Milford gene (Atiyya and Williams, 1976) and the NC2469-3 (*DW-7*) gene (eg our unpublished data) completely suppress naked expression. Since the former gene is GA-sensitive (Borner, personal communication), and the latter GA-insensitive (Mia, 1984), and open-panicled *DW-6* and *DW-8* dwarfs do not suppress nakedness, it appears that it is compactness rather than dwarfness that actually inhibits naked expression. Since the gene for shortness is linked to the gene for compact panicles with a recombination frequency of 0.08+-0.01 (Federizzi and Qualset, 1989), we predict that populations of a few hundred plants will be necessary to obtain dwarf open-panicled naked oats in crosses between compact-panicled dwarfs and naked genotypes.

The glume-for-lemma hypothesis for mode of action of the gene which explains its diverse effects. We propose the following. In naked oats, glume development fails to throw the molecular switch committing the next bract pair to develop as lemma and palea. Instead a further glume or glume-like structure is produced. In hulled oats, lemmas inhibit further floral development, so conversely in naked oats there is reduced inhibition. The rachillas of naked oats, which like panicle branches have pedicel-like swellings at the point of lemma attachment, extend

according to the developmental pattern of the panicle branches subtending glumes. Since compact-panicled dwarfs have short panicle branches, their suppression of the naked character is consistent with this hypothesis.

The naked gene is therefore likely to be a homeotic gene, i.e. one which specifies organ identity. The gene is noteworthy in that it produces functional fertile florets and is of economic significance. In comparison, *Hooded,* another dominant developmental mutation in a cereal, causes the awn of barley to elaborate one or more extra rudimentary florets, the first of which is of inverse polarity. In many homeotic genes studied, mutant phenotypes result from alterations to non-coding regions of the genes, mostly affecting introns. The tissue specificity of gene expression rather than a protein product is affected. Homeotic gene products are likely to be very low in abundance, probably accounting for the lack of detectable differences in mRNA translation products in our study. As a result, a sensitive subtractive hybridisation or PCR-based technique, or alternatively transposon tagging, would be required in order to identify the gene product or isolate the gene. The gene is a possible target of transformation.

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Production of haploids in lines of barley-wheat hybrids with restored fertility.
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Introduction. Haploid production of wide hybrids permits to accelerate formation of homozygous recombinant lines. In previous work we reported about production of barley-wheat hybrids, *H.vulgare* × *T.aestivum* and *H.geniculatum (H.marinum subssp.gussoneanum)* × *T.aestivum* (1,2). The fertility of these hybrids was restored as a result of backrossing hybrids to wheat or colchicine treatment of plantlets regenerated in callus tissue induced from young inflorescenses (3). In this work we present our data of haploid production in lines of barley-wheat hybrid progenies as compared with common wheat.

Material and Methods. The plant material used in this study consisted of 7 lines of BC progenies of barley-wheat hybrids and cultivars of *T.aestivum* – Saratovskaya 29 (Sar), Pyrotrix 28 (Pyr), Mironovskaya spring (Mir), Novosibirskaya 67 (Nov) and one line of *Zea mays*. The designation of lines are as follows: L–1 (2n = 42), L–2 (2n = 44), L–3 (2n = 46) – F_5BC_1 of *H.geniculatum* × *T.aestivum* (Pyr) × Pyr; L–4 (2n = 40+2t) – of F_5BC_2 *H.geniculatum* × *T.aestivum* (Pyr) × (Pyr) × Nov; L–5 (2n=42) – F_5 of *H.geniculatum* × *T.aestivum* (Pyr) amphiploid; L–6 (2n = 42) – F_3BC_3 of *H.vulgare* (Nepolegayushchy) × *T.aestivum* (Sar) × Mir × Mir × Pyr; L–7 (2n = 42) – F_3BC_3 of *H.vulgare* (Nepolegayushchy) × *T.aestivum* (Sar) × Mir × Mir × Sar. Plants were grown in greenhouse. Two experiments for haploid production were carried out and repeated three times. Results were summarized. Experiment 1 involved the production of androgenic haploids. Spikes with anthers containing pollen grains at mid-uninucleate stage were stored at 4° C for 7–9 days. Anther culture was placed in tubes containing potato II medium (4) solified with 0.9 % Difko Bacto Agar, with 0.75 mg/l 2.4–D and 90 g/l sucrose. The culture was incubated in darkness at 29.5° C. Developed pro-embryos were transferred to tubes with 0.25 mg/l kinetin and 0.5 mg/l IAA. In experiment II plants of all hybrid and common wheat genotypes were emasculated and pollinated by pollen of *Zea mays*. Each flower at 2 and 3 days after pollination was treated with solution of 2.4–D (100 ppm). Embryos were removed at 16–18 days following pollination and cultivated on MS medium (5) with 0.1–0.25 mg/l kinetin and 0.25–0.5 mg/l IAA. Green plantlets were grown in pots with vermiculite.

Result and discussion. The result of anther cultivation showed different reaction of genotypes on these conditions (Table 1). Significance of percentage of responded anthers was higher for L–6 and L–7, compared with other hybrid lines and cultivars of wheat. The plants of these lines, unlike the lines L–1 – L–5, had dehiscent anthers *in vivo* and high level of stained pollen. As a result of anthers cultivation, L–6 showed highest level of pro-embryos and regenerants development including green plantlets per 100 cultivated anthers. The 44-chromosome line L–2 which had incomplete dehiscent

anthers *in vivo* did not produce androgenic plants. The level of androgenic plants formation including the green plants in self-fertile lines of L–3 (2n = 46) and L–4 (2n = 40+2t) was at the same level as in wheat cultivars. Green plantlets of L–1 – L–5 were weak and sterile. Among the plants of L–6 and L–7 which came to head, about 35 % plants set seed and had 42 chromosomes in somatic cells. A number of gametoclones with alteration in plant height, spike length and vegetative period were isolated among the self-pollinated progenies of 42-chromosome androgenic plants of L–6 and L–7. In experiment II all genotypes had about the same reaction of flowers pollinated with *Zea mays* to treatment with 2.4–D (Table 2).

Table 1. Results of anther cultivation

Genotype	Number of anthers	% Anther response	Pro-embryos / 100 anthers	Regenerants / 100 anthers	
				Green	Albino
L–1 (2n = 42)	414	4.8 ± 0.9	20.3	0.5	8.3
L–2 (2n = 44)	148	2.6 ± 0.7	6.5	0	0
L–3 (2n = 46)	107	1.9 ± 0.6	26.2	4.7	16.8
L–4 (2n = 40+2t)	567	3.5 ± 0.2	14.1	0.7	1.6
L–5 (2n = 42)	492	6.0 ± 1.0	25.8	7.7	10.1
L–6 (2n = 42)	729	13.7 ± 1.2***	217.1	23.0	122.2
L–7 (2n =42)	295	29.4 ± 2.6***	46.4	6.1	11.5
Pyr	837	8.1 ± 0.9	98.8	4.8	49.7
Sar	587	5.1 ± 0.9	33.6	5.7	8.6
Nov	432	3.9 ± 0.9	39.3	5.3	10.1
Mir	328	4.3 ± 1.1	8.3	1.2	1.2

*** The difference is significant at 0.01 % level compared with other genotypes

However, only part of caryopses contained embryos. The percentage of embryos was significantly higher in caryopses developed in lines L–1, L–5, L–6 and L–7 than in caryopses developed in wheat cultivars. It is possible that 2.4–D provided a suitable background for embryos development from these hybrid genotypes. As known from results of experiment I, development of androgenic embryos on medium with 2.4–D was also more effective for hybrid lines L–6 and L–7, compared with wheat. Zygotic haploids were treated with colchicine. Dihaploids of hybrid genotypes produced in experiments I and II were involved in genetic and breeding investigations.

Table 2. Results of hybridization of hybrid lines and wheat with *Zea mays*

Genotype	Pollinated flowers	% Developed caryopses	% Caryopses with embryos	% Haploid plants
L–1 (2n = 42)	238	57.9 ± 4.2	13.2 ± 2.9***	2.6 ± 1.0
L–2 (2n = 44)	262	64.5 ± 6.1	3.2 ± 1.3	0.4 ± 0.4
L–3 (2n = 46)	136	35.4 ± 3.4	0	0
L–4 (2n = 40+2t)	196	39.5 ± 5.0	3.1 ± 1.8	1.4 ± 0.8
L–5 (2n = 42)	134	52.9 ± 8.5	8.2 ± 2.3*	5.9 ± 2.0*
L–6 (2n = 42)	196	32.9 ± 3.4	7.3 ± 1.8*	5.2 ± 1.7*
L–7 (2n = 42)	232	47.3 ± 3.3	6.8 ± 1.7*	6.8 ± 1.7**
Pyr	2161	38.0 ± 1.0	2.5 ± 0.3	0.9 ± 0.2
Sar	450	57.1 ± 2.3	2.6 ± 0.7	1.3 ± 0.5
Nov	151	39.7 ± 3.9	1.3 ± 0.9	1.3 ± 0.9
Mir	570	46.5 ± 2.1	0.5 ± 0.4	0.2 ± 0.1

*, **, *** The difference is significant at 0.5, 0.1 and 0.01 % levels, as compared with wheat cultivars

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Influence of Hordeum vulgare and Secale cereale on intergeneric crossability and viability of barley × rye hybrids. L.A.Pershina, L.I.Belova, O.M.Numerova and V.K.Shumny, Institute of Cytology and Genetics Siberian Department of the Russian Academy of Sciences, 630090 Novosibirsk, Lavrentiev Ave. 10, Russia.

Introduction. A number of hybrid combinations between different species of *Hordeum* and *Secale* were obtained (1). Hybrid plants of some of these combinations produced on the basis of wild species of barley or rye were viable and showed ability for fertility restoration after colchicine treatment (2) or after backcrossing to rye (3). As regards the crosses between *H.vulgare* and *S.cereale* there are no problems to produce the hybrid seeds. However, hybrid embryos and seedlings of *H.vulgare* × *S.cereale* had poor viability (2). This investigation was carried out to study the influence of environments and genotypes of *H.vulgare* and *S.cereale* on crossability, development of embryos and hybrid seedlings using barley and rye genotypes originating from different regions.

Material and Methods. The next winter varieties of *H.vulgare* (2n=14) were used as maternal forms: Viner, Nepolegayushchy, Omsky 85, Moskowsky 121 (Russia), Sofia 4, Galina (Bulgaria), line 319 (Byelorussia). The sources of pollen were rye cultivars (2n=14) from Europe (Tiroler, Tesovne, Somro, Vyatka), Asia (Onokhoyskaya, Dzyanantina), samples of local rye genotypes from Ukraine, Leningrad region, North Osetiya, Armenia, Kazakhstan, Tadjikistan, Krasnoyarsk region, Buryatia, Amur region, one sample of local rye from Brazil. Crosses were carried out in greenhouse three times in different years during March and April (twice), November and December. On the second and third day following pollination a small drop of GA-3 solution (100 ppm) was applied to each floret. The embryos were excised from developing seeds at 9-13, 16-18 and 20 days after pollination. These embryos were placed on 14-days endosperms of *H.vulgare* transferred on each of the following media: Norstog (4), Kruse (5) with amino acids, MS (6) with 0,25 mg/l kinetin and 0.5 mg/l IAA. The phenotypes of seedlings were studied beginning with development of the first leaf. Root tips for somatic chromosome counts were prepared according to the Feulgen technique. The data for 12 hybrid combinations produced between barley cultivars Galina, Sofia 4; L-319 and the next rye cultivars: Onochoyskaya, Buryatskaya, Amurskaya, Tadjikskaya were analysed using two-factorial analysis of variance to determine the effect of the environments and genotypes of barley and rye on variability in the percentage of seed and embryos developed. Student's criterion was used in assessing the statistical significance of difference between cross combinations in percentage of seed, embryos, viable and necrotic hybrid seedlings obtained for all of the studied hybrid combinations.

Results and discussion. On the whole 21,992 florets of barley were pollinated with rye. Mean value of seed developed was 12.6%. From 2,783 seeds, 1,020 seeds (36.6%) had embryos (from globular stage to embryos with signs of differentiation). The differences between genotypes of cross combinations with extreme values of percentage of seed developed (Galina × Onokhoyskaya: 6.5±0.6%; L-319 × Buryatskaya: 28.5±1.2%) and embryos (Galina × Somro and Viner × Leningradskaya: 0; Sofia × Kazakhstanskaya:

17.1±4.3%) were highly significant (P<0.001). The analysis of variance (Table 1) showed significant effect of barley genotypes on percentage on seed and embryos developed.

Table 1. Analysis of variance.

Source of variation	df	Percent seed MS	Percent embryos MS
Genotype of barley (B)	2	464.08**	90.56**
Environments (Env.)	2	1016.79**	22.10
B × Env	4	45.93	3.67
Error a	27	74.10	12.31
Genotype of barley (B)	2	463.51*	91.82**
Genotype of rye (R)	3	138.90	40.87**
B × R	6	109.5	20.91*
Error b	24	130.0	5.75

Significant at *P<0.05; **P<0.01

Effect of rye genotypes was significant only for percentage of embryos at the 1.0% level. Effect of three environments was significant only for percentage of developed seeds while showing nonsignificance for percentage of developed embryos. Different character of an influence of environments and genotypical factors on two analysed crossability traits between *H.vulgare* and *S.cereale* can indicate to independent genetical control of seed and embryo development. The frequency of seedlings developed as a consequence of embryo cultivation depended on the age of embryos and the medium (Table 2). The cultivation was more efficient for 16-18 days embryos on Kruse medium with amino acids. The best result in percentage of seedlings developed in embryo culture was obtained for hybrid combinations of barley cultivar Moskowsky × rye (38.09±7.49), the lowest result was for barley cultivar Viner × rye (12.2±2.67). As a result of embryo cultivation, the 225 seedlings developed. The 216 seedlings had stiff hairs on leaf surface. This trait is typical of rye. Seedlings of *H.vulgare* × *S.cereale* inherited this trait, haploids formed as a consequence of elimination of rye chromosomes had not such hairs. Thus, it was possible to analyse the phenotype of hybrids beginning with emergence of the fist leaves. It was discovered that the lethality of hybrid seedlings *in vitro* and *in vivo* was caused by one reason. The development of hybrid seedlings was delayed, leaflets necrosed and young plantlets died irrespective of medium composition in tubes or growing conditions in pots. From 225 analysed hybrid seedlings, 118 seedlings with 1 leaf died on agar medium and 87 seedlings as plantlets with 3-4 leaves died in pots. Only 11 hybrid plants came to head. From 9 haploid seedlings, two seedlings reached the heading. In our previous research some of the rye genotypes used in this work were involved in crosses with wild barley, *H.jubatum* and *H.geniculatum (=H.marinum ssp.gussoneanum)*. As a result of these crosses the viable hybrid plants

Table 2. Percent of seedlings developed *in vitro* depending on age of embryos and medium.

Age of embryos, days	9-13	16-18	20
	0.7 ± 0.4	24.7 ± 1.5***	10.4 ± 2.1

*** The differences compared with 9-13 and 20 days significant at the 0.1% level.

Medium	Norstog	Kruse + AC	MS
	1.8 ± 1.0	24.9 ± 2.1***(*)	19.4 ± 1.5

(*),***The differences compared with MS medium significant at the 5% level, with Norstog medium significant at the 0.1% level respectively

without signs of necrosis were produced (3). Our data showed that genes controlling hybrid necrosis in *H.vulgare* × *S.cereale* have wide distribution among the genotypes of cultivated barley and rye analysed in this work because all hybrid combinations of *H.vulgare* × *S.cereale* had plantlets with hybrid necrosis. It is possible that the mechanism of action of genes of hybrid necrosis in *H.vulgare* × *S.cereale* is similar to that in hybrid combinations between wheat and rye (7). Thus, hybrid necrosis is one of the most strong barriers of incompatibility between *H.vulgare* and *S.cereale*. The display of this trial depends on influence of barley and rye genotypes.

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Mutator-induced variability in barley. A.R.PRINA, S. MALDONADO[1], M.C. ARIAS, N. COLOMBO, R.D. RIOS, A. ACEVEDO, M. OTEGUI[2]. Instituto de Genética "Ewald A. Favret" (IGEAF), CICA, INTA, Castelar, Argentina, [1]) Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Instituto de Recursos Biológicos (CIRN), Argentina. [2]) CONICET, Fac.de Ciencias Naturales y Museo, UNLP, Argentina.

Introduction. After over fifty years of barley mutation induction, nowadays, there is a great amount of information concerning the use of artificially induced variability in barley genetics and breeding. Nevertheless, little is known about mutants originated in genetically unstable genotypes (GUGs). We isolated several GUGs after mutagenic treatments, bearing in mind that a better characterization will make them useful tools to be used as novel and more specific sources of biodiversity. Most of these GUGs are at present only roughly characterized by the types and frequencies of macromutations they have induced, which show they have particular and repeatable spectra of variability. Our most advanced studies are being performed on the GUG denominated chloroplast mutator (CpM) (Prina 1992), which corresponds to a nuclear gene (Cpm/cpm) whose mutant allele expresses as recessive. CpM-genotype produces mainly striped seedlings of diverse chlorophyll deficiency (CD) types, with maternal inheritance. Some morphological changes have been observed in association with the CDs (Prina, in press); nevertheless, cases of non-CD-associated morphological changes or sterility have seldom been observed and, so far, none of them has proved to be inherited. Recently, some CpM-induced cytoplasmic lines (CLs) have been described and genetically characterized, showing different patterns of variegation and breeding behaviour (Prina in press). From these results, it was possible to hypothesize about the existence of chloroplast (cp) transposons and also about the influence of some mutant cp-genes on plant morphogenesis and cp-response to developmental information. In this report, we present our current investigations on CpM-induced variability, regarding light and electron microscopy, pigments spectrophotometry and cpDNA molecular analysis.

Materials and Methods. Experimental material consisted of four CLs, obtained after several generations of natural self pollination and individual plant selection of viable CpM-induced CDs, their hybridization as female with normal genotypes and further selection of genetically stable families carrying only one type of CD (see Prina, in press).
The analyzed seedlings were grown under controlled conditions, in long days or darkness and at diverse temperatures between 14 and 28° C.
For microscopic analysis, the leaves were fixed in glutaraldehyde, post-fixed in osmium tetroxide, dehydrated and included in Spurr's resin. Sections for light microscopy were stained with toluidine blue and fuchsin acid, sections for electron microscopy were stained with lead citrate and uranyl acetate.
For pigment spectrophotometric analysis, etiolated and non etiolated leaves were ground in aqueous acetone solution

(Kahn et al 1976, Maclachlan and Zalik 1963) . Extracts were clarified by centrifugation before the analysis which was carried out with a Beckman DB-G spectrophotometer. Etiolated leaves were pretreated in Sørensen buffer phosphate, with and without δ-aminolevulinic acid (Nielsen, 1974).
Chloroplast DNA analysis was performed by non-radioactive RFLP (Hoisington *et al.*, 1994), using three cp specific probes with total DNA digested with PstI. The probes consisted of internal gene fragments of psbC and 23S rDNA (probes Pst14 and Xho17a, b, from Marano and Carrillo, 1992) and psbA (Ríos et al, 1995).

Results and Discussion. Electron microscopic analysis showed differences between the cps of the CLs and the cps of the controls, regarding the *grana* and the stromatic membranes orientation, cp size and shape, the presence of starch and/or stromatic vacuoles. On the other hand, no abnormalities were observed in the mitochondria. The most marked differences were observed in the cps of CL1, which was previously described as a discontinuous or positional variegated CD of normal-green/ albino type (Prina, in press). CL1 showed two different cp-types, distinguishable from each other even with light microscopy. One of them was a fairly normal cp-type and the other, several times smaller, severely vacuolated and showing a completely abnormal thylakoid organization. Their distribution depended on cell position, being the two types sometimes observed together in the same cell. Due to previous genetic results, (Prina, in press) we interpret this as a consequence of a different cp response to epigenetic information rather than genetic mosaics or true mixed cells.
Pigments analysis revealed that the homogeneous *viridis* mutant CL3 (Prina, in press) showed the most conspicuous diminution in chlorophyll content, markedly changing chlorophyll *a/b* ratio. Meanwhile, CL1 had different chlorophyll *a/b* ratio between the top and the bottom of the first leaf blade. CL3 mutant features were mainly expressed at higher temperatures (28°C), while CL3 seedlings looked almost normal at 14°C. On the contrary, CL1 and CL4 had maximum expression at lower temperatures (14°C). Spectrophotometric analysis of seedlings grown in darkness did not show any difference from the controls, suggesting the four analyzed CLs are involved in processes occurring after protochlorophyllide synthesis.
Chloroplast DNA analysis has recently been started. So far, no polymorphism has been detected, indicating that there are not large structural variations in the genome regions studied (ca. 42 % of the cp-genome).
It is worthwhile mentioning that several other CD types have been isolated from CpM genotype, and are at present in the process of crosses and selection in order to obtain pure and stable CLs (see Material and Methods). We also want to prove that CDs are not the only variability originated by CpM. In this respect, we are carrying out experiments concerning the selection for drug tolerance. We have started experiments under field conditions applying a sublethal dosis of atrazine on approximately 6.000 tillering plants. After two

rounds of selection, we have at present 127 progenies to be tested for atrazine tolerance.
To our knowledge, the CpM herein studied is the only GUG inducing a wide spectrum of cytoplasmically inherited CD types described in monocots. We consider CpM a very valuable source of variability of the otherwise highly conservative cp-genome. Moreover, due to its specific action of mutation induction, it greatly facilitates the selection and the analysis of the cp-mutants, which are obtained in an stable nuclear genetic background.

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Epigenetic variability and inheritance of complex characteristics of barley.

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Introduction. Genetic control of farming, morfological and biological characteristics of plants has always been an object of special attention of biologists and selectionists. Being complex by their nature these characteristics are the final results of forming volumetric structure of multicell organisms and populations. Knowledge of epigenetic processes is highly important for understanding and controlling their variabilities. (Litun and Proskurnin, 1992).

Material and Methods. The study was carried out on a specially bred for this purpose genetic material of barley: lines, hybrids and model genetic populations were used. Individual variability and state of genetic organization of characteristics were the object of the investigation. The lines having different levels of development of complex characteristics were crossed.

Results and Discussion. Experimental improvement of significant specificity of plants with their level and character of epigenetic variability and heredity of complex characteristics as compared to animals and other organisms was received. One of the main reasons of its plant's specific modular type of its organization, diachronic type of development and peculiarity of the living form due to the static way of living. Plant's complex farming characteristics, productivity among them, have coefficient of variability greater than 50% while for animals with their unavy organization and synchronous development this is less then 10%.

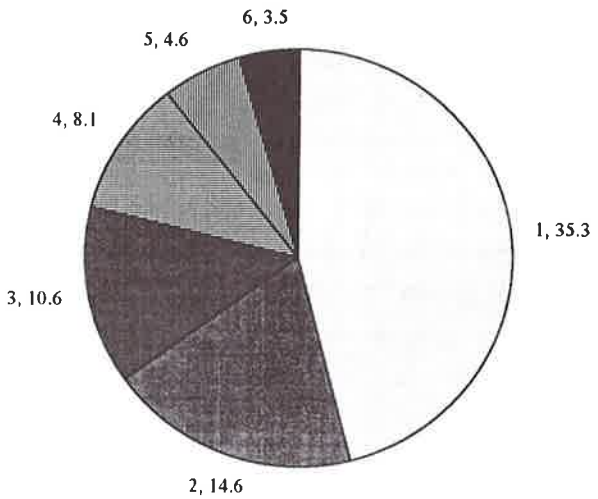
There 2 possible levels of studiying the epigenetic heredity and variability according to plant's biology. The first one is related to archetype studies of organization structure of particular plant forms.

The first one is related to archetype studies by organizational structure in certain plant forms. Ontogenesis of plant represents a stage-by-stage formation of organization and reflect specificity of embrional branch development as well as vegetative, reproductive spheres and generation of new seeds. The state of this organization at a certain moment of subprogram of organism's development may be considered as a factor reflected in the archetype of the whole system, in availability of specific plan of forming volumetric structures (which are characterized for some individual plant forms) and a factor providing a system control of complex characteristics.

That is why complex characteristics reflect inner functional peculiarites characterizing a particular organization of a system and they can be used for evaluating and classifying according to their by archetypes. System analisys results approved multifactorness of barley, namely, availability of different subprogrames of its development which leads to the correlation between separate groups of complex characteristics.

The given scheme shows six factors contributing to the total variability of barley plant (the first figure stands for factor number / name, the second one - its share / percentage).

The percent of total variance of factors, which determinate populatic variability of plants.



1. Forming of reproductive and generative plant spheres;
2. Differentation of structure elements of vegetative and reproductive spheres during geterotrophic nourishment period;
3. Forming micropopulation;
4. Forming the upper level of vegetative sphere;
5. Forming phytomers of the middle level;
6. Forming vegetative sphere during the geterotrophic nourishment.

Such analysis enables to classify initial and selection material according to archetype and to form the base collections of plant's genetic resources.

The second level conserns solving selection problems on productivity and other complex characteristics within the frame of the chosen archetype. In this case genetic organization of the characteristic becomes an object of research. Epigenetic process in the chain of heredity characteristic's display is characterized by hierarchy corresponding to different levels of morfogenetic processes and structures. That's why the peculiarities of the system effects for each such level should be taken into account when solving selection problem. The authors (Litun, 1984; Proskurnin and Litun,1991; Litun, 1991) proposed the procedure based on module of characteristics. Such procedure enables to explain the peculiarity of forming the characteristic in the terms of functional organization of a particular object and to define technological solutions of selectionists (Litun 1991).

Thus, in case of plants with their specific in development organization types and their static way of living, the knowledge of genetic control and genetic organization of complex characteristics becomes for us a question of a great importance. Genetic organization of a complex characteristics is determined by interrelation and arragment of genetic processes on the level of the whole system and that's why the epigenetic heredity and variability reflect the specific state of self-organization, self-development and self- regulation processes - that is the general state of inner processes in the system.

The study of epigenetic variability and heredity of complex characteristics is possible only from the standpoint of system researches.For this purpose the authors propose the strategies of two level system analisys: analisys of archetype and that of specificity of characteristic's organization and its state.

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Study on gene location of Monoecious form of Barley.

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INTRODUCTION. The history of breeding monoecious form barley, his importance for breeding and genetic study was shown in our previously paper (1). In this paper we publish the first hand data on study of monoecious gene location on chromosome. For this proposal we used the set of seven chromosome tester system what was kindly supplied to us by Prof. Yasuda.(2)

METHOD AND MATERIAL. We use monoecious form 031 for crossing with seven chromosome testers separately and reciprocally crossing also was done. Then in F1 all plants have been carefully observed for elimination of no hybrids by dominant marker gene in each case. Then in F2 all plants for different combinations have observed and recorded for future genetical analysis.

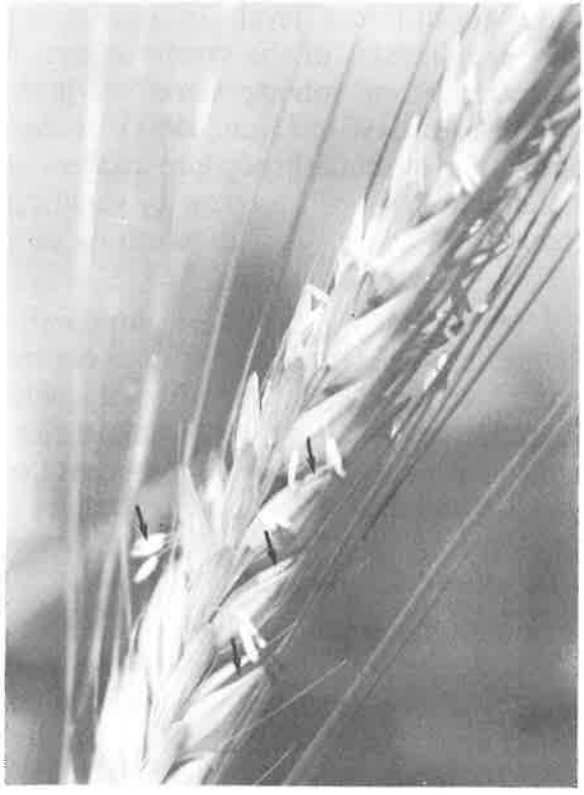

Fig. 1 Typical spike monoecious 031 , the arrow shows the anthers from lateral male flowers are pollinates on central female flowers

RESULTS AND DISCUSSION. After careful observation on all plant F1 of different combinations we received the hybrids for all combinations except tester chromosome number 6, where we can not got hybrid seeds for missing in flowering time and bad development flowers of Chromosome tester number 6. The results placed in Table 1.

Table 1 Number of hybrid plants for different combinations

combinations	Chromosome tester 1-7 for crossing with monoecious						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Monoecious x chrom. tester 1-7	9	3	8	6	3	-	-
Chrom. tester 1-7 x Monoecious	-	35	4	-	22	-	18

In the F2 three duplicated samples have been carefully observed and recorded, the data are placed in Table 2.

Table 2 Segregation of monoecious form in F2 in different combinations

combinations	N0. plants	Chromosome tester 1-7 for crossing with monoecious						
		1	**2**	**3**	**4**	**5**	**6**	**7**
Monoecious x chrom. tester 1-7	Total	83.6	83	79.6	80	104.6	-	-
	Monoec.	11	8	12	16.6	31.3	-	-
Chrom. tester 1-7 x Monoecious	Total	-	106	39.6	-	88.3	-	95
	Monoec.	-	18.6	3	-	36.7	-	17

From these results we can see the combinations of Chromosome tester number 5 is very tightly correlated with Monoecious genes. More detail data about this are placed in Table 3 and 4. The specific white glum marker at spiking time located at Chromosome 5 , it is recessive gene and the monoecious gene also is the recessive gene. Both of these genes are closely linkaged.

Table 3 Segregation of F2 plants for combination Chromosome tester 5 x monoecious

green glum	N0.plants	white glum			green glum		
		No. plants	Fertile	Mono	No. plants	Fertile	Mono
1	83	27	5	22	56	37	19
2	88	21	8	13	67	55	12
3	94	30	9	21	64	41	23
Total	265	78	22	56	187	133	54
Average	**88.5**	**26**	**7.3**	**18.7**	**62.3**	**44.3**	**18**

Table 4 Segregation of F2 plants for combination Monoecious x Chromosome tester 5

duplicates	N0.plants	white glum			green glum		
		No. plants	Fertile	Mono	No. plants	Fertile	Mono
1	118	25	11	14	93	74	19
2	88	20	5	15	68	53	15
3	108	40	18	22	68	59	9
Total	314	85	34	51	229	186	43
Average	**104.6**	**28.3**	**11.3**	**17**	**76.3**	**62**	**14.3**

From data in Table 3 and 4 we can see both white color glum and Monoecious are recessive genes and tightly linkaged between them. White glum with more Monoecious then Green color glum and these difference are statistically significant. Based on these data we can speculate what the monoecious gene is located at Chromosome 5.

REFERENCE. (1) Shao Qiquan, B. Harvey, B. Rossnagle, S Jana and Li Ansheng 1991 New Monoecious Form Barley *Genetic manipulation in Plants* Vol. 7, Number 2, pp.95-100
(2) T. Kinishi, J. Hayashi, I Moriya and R. Takahashi 1984 Inheritance and Linkage studies in Barley "Berichte des Ohara Instituts fur Landwirtschaftliche Biologie Okayama Universitat" Bd.XVIII, Heft 4 S. 251-264, Marz 1984

BREEDING OF NAKED SEED COMPACT SPIKE OAT

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INTRODUCTION. The grains of oat are very nutritional and good for health in meaning reduce TG contents in blood . But current used varieties in China are low yield for their soft and high steams, and spike structure is also too extended with less seeds. For improve it we introduced indirectly through VIR from New Zealand a variety Ohau v mutica A.L. (Dr. Soldatov from VIR kindly supplied this form). Ohau with short strong steam, compact spike, more grains per spike more yield, but the grains are huked. For the food we desirable to have naked seed form.

METHOD AND MATERIAL. We take the current used best naked seed varieties in production to crossing with Ohau to combine the best characteristics in one new variety. The crossing was done in 1994 spring , for speeding the breeding process we use greenhouse in Beijing location to have two-three generations in one year. Now we have F5 planted in March of this year. In F4 we got some ideal line with naked seed and high yield.

RESULTS AND DISCUSSION. The data about original material for crossing are placed in

Table 1. Morphological characteristics of original parents

	Height of plants in cm	length of spike in cm.	Weight of steam in mm	length of second branch in mm.	leaf type	huked or naked	color of grains	color of spike	lagging	No. grains per spike
ohau	94.8	21.2	5	98.2	Erect	huked	yellow	yellow	-	104.6
Ym2	117.8	26.4	3.3	150.8	prostrate	naked	yellow	yellow	+	47.4
Ym4	110.8	29.6	3.3	160.4	prostrate	naked	yellow	yellow	+	55

From the data in Table one we can see Ohau is the best from many angles to estimate but it is huked . In the same time Ym2 and Ym4 have higher plants, thinner steam, longer second branches and less grains per spike, but it is naked seed form. Making crossing between these forms we try to combine the best characteristics from them in one variety.

Table 2 Segregation of F2 plants from hybrid combinations between Ohau and Ym2, Ym4

combinations	type of spikes compact or laxis	No. of plants	Height of plants in cm	length of spike in cm.	length of second branch laxis cm.	huked	naked	color of grains	color of spikes
ohau x Ym2	laxis	29	93.2	16	4	7	16	yellow	yellow
	compact	28	29	14.6	1.86	3	20	yellow	yellow
Ohau x Ym4	laxis	24	102.6	22.8	5.8	8	16	yellow	yellow
	compact	36	85	16.7	3.34	8	23	yellow	yellow

from segregated F2 and so on we strictly select on compact spike, low height of plants and more grains per spike. Now we have F4 plant lines with naked seed, no high plants , more grains per spike.

Table 3 Characteristics of new compact spike naked seed oat

combinations	N0. of plants	Height of plants in cm	length of spike in cm.	length of second branch	type of spikes compact or laxis	huked seeds	naked seeds	color of grains	color of spike
ohau x Ym2	40	84.8	30.8	10.6	compact	0	40	yellow	yellow
ohau x Ym4	42	83	31.4	11.8	compact	0	42	yellow	yellow

The new for naked seed oat with compact spike no high plants and good yield.

Fig. 1 Plants of new naked seed compact spike oat in experimental field

Recurrent selection in barley using genetic male sterility: evaluation of S1 progenies of the first and second cycle. M.C. SANGUINETI, S. CONTI, E. NOLI, S. SALVI AND R. TUBEROSA. Department of Agronomy, University of Bologna, Via Filippo. Re 6, 40126 Bologna, Italy.

INTRODUCTION. Recurrent selection (RS) is a cyclic breeding method which allows the gradual increase in frequency of favourable alleles in plant populations. RS has been seldomly utilised in self-pollinated crops because of the difficulties in making large number of crosses in the recombination phase of the selection cycle (Hallauer, 1985). Genetic male-sterility is a powerful tool to reduce such intermating limitations and has been successfully applied in different crops (Doggett and Eberhart, 1968; Abdalla et al., 1989; Burton et al., 1990). This system can be efficiently utilised if the male-sterility gene is tightly linked to a morphological marker detectable before flowering. In barley, the recessive *msg6* gene, which induces the inability of the pollen grains to germinate (Ahokas, 1975), is tightly linked (recombination frequency < 1%) to *sex1*, a gene determining shrunken endosperm. The *msg6-sex1* system allows a pre-sowing selection of male sterile plants. Falk et al. (1981) developed a genetic stock (GB C695) including the *msg6* and *sex1* genes linked in coupling phase to be used for breeding purposes. This germplasm was crossed to elite genotypes well adapted to northern and central Italy in order to develop a base population to undertake a recurrent selection program in which the intermating procedures are facilitated by genetic male-sterility (Conti et al., 1992). In this study we report the results of the first two cycles of recurrent selection.

MATERIALS AND METHODS.

Population development.

- Two mating design (A and B) were utilised: A) seven six-row varieties (Barberousse, Eldorado, Etrusco, Gerbel, Jaidor, Plaisant, and Thibaut) and a breeding line (IABO14), chosen on the basis of their agronomic performance, were crossed according to a partial diallel scheme (12 crosses); B) each of the eight elite genotypes was crossed to the GB C695 population.
- The crosses of the A group were mated to those of the B group which did not share common parent; 24 double crosses were obtained (first backcross to transfer *msg6-sex1* system in the base population).
- Double crosses were grown and a within family selection for disease resistance was performed; only the shrunken seeds were then utilised. The deriving S_1 male-sterile families were crossed, according to a balanced scheme, to the hybrids of the A group with unrelated parents (second backcross); 24 six-way crosses were obtained (base population). In this step, the A group hybrids having Thibaut as one parent were replaced by the same cross combinations with Pirate.
- In the base population (C_0), 216 plants were selected for heading date, kernel weight, and powdery mildew resistance.
- The corresponding 216 C_1S_1 families were tested in a replicated field experiment; 25 families were then selected.
- In order to facilitate the intercrossing, the remnant seed of each of the selected S_1 families was sown in two rows: one containing male-sterile plants (from shrunken seeds) and the other fertile plants (from normal seeds). Genotypes derived from different six-way hybrids were crossed by hand.
- Individual selection was performed within each cross: 207 S_0 plants were chosen and the corresponding C_2S_1 families were tested in a replicated field trial.

Response to selection.

In 1990-91 the 216 selected C_1S_1 families and the ten parents (checks) were evaluated in a field trial carried out near Bologna (Po valley), according to a randomized complete block design (two reps). In each block, the parental lines, with the exception of Thibaut and Pirate, were included twice. The plot consisted of a single 3.5-m-long row. Rows were spaced 0.5 m apart (350 viable seeds m^{-2}). The 207 S_1C_2 selected families were tested in an analogous trial, with three replications, conducted in 1993-94. Heading date, plant height, lodging at harvest, powdery mildew and stem rust resistance, grain yield

and kernel weight were recorded on a plot basis in both trials. Analysis of variance was computed for each experiment. Broad sense heritability of cycle 1 and 2 was calculated on a family mean basis, using the variance components. The response to selection was estimated as [(mean value of selected population - mean value of parental lines) / mean value of parental lines] * 100.

RESULTS AND DISCUSSION. Highly significant differences among genotypes were detected for all the investigated traits, with the exception of lodging at harvest, in both experiments (data not shown). The response to the two selection cycles are reported in table 1.

Table 1 - Responses of the investigated traits to two cycles of recurrent selection.

TRAITS	C_1S_1		C_2S_1	
	Responses	h^2_B	Responses	h^2_B
Grain yield (%)	13.5**(1)	0.40	15.1**(2)	0.29
Kernel weight (%)	-3.5**	0.78	0.3	0.85
Heading date (d) (3)	-1.9**	0.83	-3.3**	0.88
Plant height (%)	1.5*	0.49	2.2*	0.46
Lodging at harvest (%)(3)	3.2	0.11	3.0	0.14
Powdery mildew (%)	-35.5**	0.26	-55.3**	0.73
Stem rust (%)	0.0	0.21	-7.8**	0.40

(1)*, **: comparison between parents and C_1S_1 significant at P 0.05 and 0.01, respectively.
(2)*, **: comparison between parents and C_2S_1 significant at P 0.05 and 0.01, respectively.
(3) Differences between the mean values of selected population and parental lines.

A sizeable response to selection was observed in C_1 for grain yield (+ 13.5%). This result should mainly represent a correlated response because selection was performed on an individual basis for highly heritable traits such as disease resistance, earliness, etc.. Such a response could also be ascribed to heterotic effects probably enhanced by the low seed density (Hayes and Foster, 1976). A further, though limited, response for grain yield was obtained in the second selection cycle. Among the C_2S_1 families, the 13% exceeded the most productive parent, while none was equal or inferior to the less productive parent. Differences in kernel weight did not account for the yield increase observed in both cycles; in fact a small but significant reduction (- 3.5%) for this trait was evidenced in C_1 while no appreciable differences were found in C_2. However, these results should be considered positively because all the S_1 families were segregating for shrunken kernels which are lighter than the normal ones, while GB C695 was the only parent showing such a segregation. It is noteworthy that a high genetic variability for this yield component is still present in the selected population. As compared to the checks, the S_1 families were characterized by greater earliness (-1.9 and -3.3 d in C_1 and C_2, respectively), slightly taller plants and higher disease resistance. In particular, noticeable decreases in powdery mildew susceptibility were observed in C_1 and C_2 (-35.5 and -55.3%, respectively). These findings can be related to the effectiveness of selection, which, in turn, was due to the large variability available in the base population (the parental line Eldorado shows a high degree of resistance) and to the high inoculum of powdery mildew in the selection fields as a consequence of the presence of susceptible lines artificially inoculated with this pathogen. The good agronomic performance of many C_2S_1 families suggests that a sufficient degree of recombination was obtained and hence this population can be utilised as source to select pure lines.

The heritability values computed in the second cycle suggest the possibility of further genetic gains for almost all the considered traits.
Significant correlations were detected between grain yield and kernel weight (0.22), heading date (-0.17), plant height (0.24), and powdery mildew and stem rust susceptibility (-0.15 and -0.25, respectively). In general the correlation pattern between all the investigated traits (data not shown) can be considered favourable for breeding purposes.

CONCLUSIONS. The first two cycles of RS were effective in increasing grain yield, earliness and disease resistance; the high genetic variability still present in C_2 suggests that further genetic gains are possible in case RS is continued. The high agronomic performance of many C_2S_1 families and the correlation pattern indicate that valuable pure lines can be selected from C_2.

Acknowledgements. This work was partially supported by Ministero dell'Università e della Ricerca Scientifica e Tecnologica.

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INITIAL MATERIAL FOR EFFECTIVE BARLEY BREEDING ON THE NORTH CAUCASUS. **V.M. Shevtsov, Barley Breeding Department, Research Institute of Agriculture, 350012, Krasnodar, Russia.**

Introduction. Many factors, including advanced methods, the diversity of germplasm, volume of breeding nurseries, breeder's skill and others, determine success in practical breeding. It is not easy to isolate the pure effect of every component. Variability in the initial material is undoubtedly a very important prerequisite for a good result. It can be achieved through introduction, mutagenesis and recombination. Many well known breeders and geneticists have recommended several approaches to dealing with initial germplasm. One of the outstanding breeders academician P. Lukyanenko (1973) preferred to cross parents of ecologically and geographically distant origin (1). A prominent barley scientist D. Rasmusson (1992) emphasized the value of crosses with high and stable expressions of agronomic, physiological and morphological characters, because "...good performance breeds good performance" (2). A deep connoisseur of barley breeding for stressful conditions. S. Ceccarelli (1983) has shown the importance of landraces in low yielding environments (3). The goal of this study was to assess different approaches in selection of parental forms for hybridization, taking into consideration the origin of 16 winter and 6 spring barley varieties by our Institute.

Materials and Methods. During 30 years 9632 crosses of winter barley have been studied. About 13,000 different parental forms, including released varieties from Europe, America, Asia and Africa, locally developed varieties, landraces, and promising lines of hybrid and mutant origin, have been involved in hybridization. Usually the F_2 bulk is tested in a check nursery in plots of 5 m^2, and the F_3-F_7 bulk in the preliminary trials in plots of 25-40 m^2 at 2-3 geographical sites. Earlier we started making spike selections from the F_2-F_3 and now usually from the F_5-F_7. The average annual volume of breeding material contains 48,000 families (head rows) in pedigree nursery, 3000-3600 lines in the check nursery, 800-1200 lines in preliminary trials and 100 varieties and lines in advanced trials. Concerning experimental mutagenesis, annually seeds of 8-10 promising lines are treated with 2-3 chemical mutagens: nitroso-ethyl-urea, ethylene-imine, or nitroso-methyl-urea in 6 dosages (3 concentrations and 2 durations). Spike selections are made from M_2. The volume of mutant material in early nurseries is about 10% of that of hybrid origin and in preliminary and advanced trials - 30-40%.

Results and Discussion. The analysis of the origin of commercial varieties has shown that out of 13,000 parental forms only 17 winter and 7 spring varieties have contributed for practical results. Some of them were parents of many varieties. The original germplasm was involved in parentage of the first variety and lately through its derivatives in many others. A cold tolerant facultative barley Kruglik 21 was one of the parents of Start variety, which served as a parent of four other varieties. A dense-headed winter barley of Chinese origin is in the parentage of 5 varieties. Among varieties, which left an evident trace in barley breeding, are Beta 40 (Hungary), french variety Ager which was used in developing 3 new varieties, and respectively Start (locally developed) - 4, Zavet (local) -4, Harrison (USA) - 3, Paoly (USA) - 3, Radical (local) - 2, Vavilon (local) - 1.

The long-term data confirm the old conclusion that local germplasm is a main source of good adaptation. All 20 varieties out of 22 released have in their parentage locally developed varieties or lines and only winter barley. Meteor=Fogel Zanger Gold (Germany) x Harrison (USA) and spring barley Krasnodar 35=Spartan (USA) x Braune (Germany) were obtained by crossing American and German varieties. While talking about more effective approaches in the use of germplasm, it is reasonable to note that a principle of crossing ecologically geographically distant forms is very important. On the background of this approach, two principles in parent selection are worthy mentioning: a) **biological remoteness** and b) **morphological contrast**.

Biological remoteness, when spring type, facultative and winter type are used, permits enhancement of genetic divergence in connection with a different response to thermo- and photo-period, related to plasticity, broad and narrow adaptation. A spring barley Nutana 27 and facultative Kruglik 21, Beta 40, Zavet, Odessa 31 can be found in the parentage of 10 released varieties.

The principle of morphological contrast in choosing parental forms has proven successful, especially when six-rowed, lax-headed and dense-headed subspecies have been crossed. Our significant achievements are connected with the late dense-headed model and the first cross between a Hungarian variety Beta 40 and Chinese local variety with compact heads became a turning point in winter barley breeding in the North Caucasus. All varieties, which greatly contributed to a sharp yield increase in the farmers' fields, Zavet, Novator, Cyclon, Radical, Vavilon, Bastion and Kozir belong to this parallelum type with the up-right plant habit, stiff straw and very dense plant stands. Rather rough comparison of selections, made from F_2-F_7 speaks in favour of more late generations F_5-F_7, because 2-3 years of testing segregating populations in preliminary trials in different ecological zones and during different seasons gives decisive information to discard poor combinations. The pure lines, selected from the remaining more adapted populations, as a rule, were highly plastic with good indices for stable productivity. Selections from early generations of F_2-F_3 are more expensive. In order to find out a proper way for mutant application in practical breeding all mutants induced during 30 years, were grouped into two classes: 1) **micromutants** - forms without drastic differences from an initial genotype, 2) **macromutants** - forms with visible phenotypic changes which can be easily fixed in the field or laboratory. The number of lines, saved after their evaluation in advanced and official trials, has proved that selections from the class of macromutants were more effective. Some pure mutant lines with favourable combinations of traits conditioning good agronomic performance with resistance to biotic and abiotic stresses became commercial varieties: early spring varieties Temp and Mamluk, cold tolerant winter barley Debut and newly developed facultative barley Secret. The choice of some unique mutants as parents for crosses, considering their biological and morphological contrast, generates an explosive flash of variability, including economically valuable transgressions.

On the basis of waxless mutant K-57M17 the early variety Skorohod has been developed. The pedigree of the first variety with increased protein content Novator includes a mutant form K-31M15. Cold tolerant variety Radical inherited resistant to powdery mildew from a mutant 52M1. Early mutant variety Temp was a good parent for developing spring barley Kaskad and Perlom due to its high plasticity and resistance to net blotch. One of the very productive winter barley

varieties, Vavilon carries in its pedigree a mutation for gigantism K-31M13. The recently released winter barley varieties Bastion = Radical x Vavilon and Kozir = Radical x K-253 combine all advantages of parental forms, carrying mutant genes: cold tolerance, resistance to lodging, and high yielding capacity.

Table 1. Performance of winter barley varieties Bastion and Kozir in advanced trials. Krasnodar

Variety	Cold* tolerant, %	Resistance to: **) score, 1-9 Net blotch	Crown Rust	Yield, kg/ha 1990	Average 1990-1995
Radical-check	88.5	6	5	8950	5440
Bastion	90.7	8	7	9870	6280
Vavilon-check	23.2	5	7	9630	5120
Kozir	40.4	8	8	10340	6540
L S D-05	9-11			480	340-480

* percent of surviving plants after freezing in chambers.
** score: 1 - very low resistance, 5 - moderate, 9 - very high

The above data show a great potential for transgressive and mutational trends in barley breeding. Many untapped reserves remain in handling the initial material for further improvement of the barley plant.

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Continued commercialization of the doubled-haploid (bulbosum spp.) method in barley variety development: 1996 progress report from the Nairn Research Laboratory. L.P. Shugar and M.J. Etienne, Nairn Research Lab., W.G. Thompson & Sons Limited, R #1, Ailsa Craig, Ontario N0M 1A0, Canada.

Introduction. The utilization of doubled-haploidy is increasing. Gene mapping, cloning and marker assisted selection demand 100% homozygosity. Many breeding programs around the world are now using some form of DH line production in barley and wheat; female fertilization by bulbosum and maize respectively, anther and microspore culture in both for experimental and varietal development purposes. DH production makes a lot of common sense from a purely basic research point of view: no residual heterozygocity and clearer and faster results and conclusions within granting periods. Varietal developers enjoy the DH method because large blocks of favourable gene combinations can be fixed, selections in nurseries are stable and non-segregating and the time from cross to market is advantageous compared to more traditional methods. As with any technique however, there are a few disadvantages. Doubled-haploidy can be more expensive (labour and time consuming). D.H. requires the use of plant rooms or greenhouses for large programs and breeders must choose parents wisely and introduce new genes carefully. Some genotypes are less compatible with bulbosum - that can lead to inefficient use of inputs.

As you are aware, Pierre Devaux of Florimond Desprez is presenting a paper on the use of the doubled-haploidy technique in barley on Saturday and Choo et al. will be reporting on a number of D.H. lines that were recently supported for registration in eastern Canada in 1996, in the new *Barley Newsletter*. This poster presentation intends not to duplicate their efforts but to describe our varietal development success at the Nairn Research Lab. using the D.H. (bulbosum) method in barley.

Results. Our first varieties, since we purchased the doubled-haploid facilities in 1984, were supported for registration in Canada in 1988 and our progress is reported to 1996. 17 of the 19 barley varieties developed at Nairn are D.H. F1 derived and two are from a modified bulk selection methodology (Table 1). Since D.H. (bulbosum) is a 25+ year old technique, we have skipped the methods section and hopped right into the results.

We have modified the earlier described method since 1984. We do not emasculate the F1's before pollination. We just cut the tops off the female florets and pollinate on the same day. Selfing percentage is about 06% and they can be picked off the spikes before embryo rescue, 14-15 days after pollination. Instead of cutting the fertilized culms and tillers and placing in a nutrient solution, we grow the fertilized F1's in a special "nursery room" along with the rescued cultured embryos. This room is brighter (1000+ ft. candles compared to 800 in the growth rooms) and kept aseptic. The bulbosum is continuously selected for vigour, good pollen harvest and low hybrid counts with vulgare.

All our F1's are produced indoors in the winter so that our staff spends more time outside evaluating populations during the growing season, not performing lab procedures. No matter what technique is used, nothing supercedes dynamic field evaluation and care, respect and understanding among research personnel. Declare a goal and go after it!

Table 1. List of varieties registered in Canada or marketed in the USA from the cereal varietal development program at W.G. Thompson & Sons, Nairn Research Laboratory, 1988-1996.

YEAR	VARIETY	METHOD	PEDIGREE	MARKET
1988	Craig (2R)	D.H.	Rodeo/ Aramir	Ontario
	Etienne (6R)	mod. bulk	Perth/R10-501	Ont., Que.
1989	Winthrop (2R)	D.H.	Micmac/B7735-5	Que., AB.
	Bounty (6R)	mod. bulk	M66102/Bonanza//Jotun/ Conquest/3/Perth	USA-Wis.
1990	Lester (2R)	D.H.	UPBS60/UPBS66//Rodeo	Ontario
1991	Ontario (6R)	D.H.	OB150-29//Vanier/Laurier	USA-NY.
1992	TB891-6 (6R)	D.H.	Vanier/Keystone//BT421/3/ Vanier/Laurier//Perth	Ontario
1993	Prospect (2R)	D.H.	Rodeo/TR462	Sask.
	Bronco (6R)	D.H.	Vanier/Laurier//Perth/3/Leger	Sask.
1995	Sandrine (6R)	D.H.	Mingo/OB339-1//QB203.4	Ont., Que.
	Beluga (6R)	D.H.	unknown female//Mingo/ OB339-1	Ont.,Que.
	McGregor (6R) winter type	D.H.	Tapir/Wisor	Ontario
1996	Grant (6R)	D.H.	P885-4 (Keystone/Laurier/2/ Vanier/Keystone/3/Leger)/ P854-35 (Vanier/2/Keystone/ Laurier/3/Loyola/York//Peguis)	Ont., Que.
	Belmore (2R)	D.H.	Winthrop/Lester	Ontario

In 1996, W.G. Thompson & Sons received registration support for four 2-rowed spring barleys and one 6-rowed winter barley (all D.H.). They will be described further when registration is finalized. We have also bred two oats and seven winter wheats since 1988.

Identification of maintainer and restorer lines for heterotic hybrids in barley. D.SINGH, Department of Plant Breeding,CCS Haryana Agricultural University, Hisar 125004, INDIA

Introduction. Discovery of male sterility gene msg1 (Suneson, 1940) and development of Balanced Tertiary Trisomic (BTT) system of hybrid seed production in barley (Ramage, 1965) opened new vistas for the production of commercial hybrids. Soon it was realized that the system has inherent weaknesses which hamper its practical use besides the susceptibility of hybrids (Hembar, Amy and Rolle) to diseases and lodging. The development of workable CMS lines msm1 and msm2 by Ahokas (1979, 1982) and IA and 2A by Minocha and Sidhu (1988) has renewed the interest of barley breeders in the development of hybrid barley. However, the success of development of commercial hybrid barley will mainly depend on adequate heterosis and natural outcrossing.

Materials and Methods. CMS lines msm1 and msm2 each with 100 pollen parents and 1A and 2A each with 60 pollen parents were crossed to identify good maintainer and restorer lines in the year 1992. The hybrids thus produced were grown in single row of 1.5 m length with pollen parents during 1993. Five ear heads in each entry were bagged with parchment bags and seed setting was calculated. In the year 1994, sufficient seed of 11 hybrids each with 1A and 2A line was produced and evaluated along with pollen parents in the year 1995 in a trial having four replications. The data was recorded on 10 plants selected randomly from each entry and replication for characters tillers/plant, grains/ear, 1000 grain weight and grain yield/plant. Per cent heterosis for each character was calculated on standard check, BH75 and significance was tested by t test as per Wynne *et al*. (1970). The extent of out crossing was observed in 1994, 1995 on CMS lines with pollen parent BH342 (Anther extruder) sown in 1:1 ratio in a four replicated separate experiment planted in isolation. In both the experiments, a single row of 2.5 m length for each entry was sown with row to row and plant to plant distance of 30 cm and 5 cm (near crop condition), respectively.

Results and Discussion. Out of 100 genotypes tested 44 were found to be maintainers of sterility in both msm1 and msm2 and only eight pollen parents (BH75, BH85, RD2035, DL100, DL550, PL172 , BH230 and Karan19) could restore >85% fertility in msm2 line only. BON (LRA) - 10 was the only pollen parent which restored fertility in both msm1 and msm2. Cytoplasm of msm2 was to be reported more sensitive to restore fertility than cytoplasm of msm1 (Singh, 1996). The rest of the pollen parents (47) were found to be partial restorers. Among the partial restorers there may be semi-dominant single gene restorers. In some instances, if not in

all, partial restoration depends on more than one allele or gene (Ahokas, 1979). In IA and 2A, most of the pollen parents restored complete fertility. Pollen parents Amber and K1144 in 1A and BH85, BH91, DL200 and Jyoti in 2A maintained sterility. For hybrid seed production, the seed set on the sterile parent should not be less than 50 per cent (Scholz & Kunzel, 1973). The out crossing recorded on msm1, msm2, 1A and 2A CMS lines during 1994, 1995 (Table 1)

Table 1: Out crossing (%) in CMS lines with BH 342 (Anther extruder)

Year	CMS lines	Out crossing(%)	
		Range	Mean
1994	msm1	3.6 - 27.3	12.8
	msm2	4.4 - 32.8	16.2
1995	msm1	3.1 - 59.3	16.7
	msm2	3.3 - 65.6	20.0
	1A	2.4 - 33.3	17.7
	2A	4.2 - 43.8	20.8

is too low than the percentage required for economic hybrid seed production. So there is a need to increase cross pollination through the incorporation of allogamy characters in parents. Higher out crossing through increased anther extrusion, anther length and stigma size could be achieved by induced mutation (Hentrich & Schreiber, 1991) and inducing short awned in female parent (Scholz & Kunzel, 1987). The hybrids which exhibit 20-30% heterosis for yield are considered to be economical to compensate the seed production cost of the hybrids (Kim & Rutger, 1988). Out of 22 hybrids evaluated, only 10 hybrids showed significant heterosis for grain yield over standard check BH75(Table 2).

Table 2 Heterosis (%) for grain yield and its components over the standard check BH75

Hybrids	Tillers/ plant	Grains/ ear	100 grain weight	Grain yield/ plant
IA X RD2501	9.2	5.6	40.8*	44.1*
IA X BH85	17.1	11.1*	26.0*	30.5*
IA X RD2528	22.4	12.5*	21.8*	35.9*
IA X RD2052	23.6*	-5.6	38.5*	25.7*
IA X DL88	36.8*	1.4	39.8*	49.0*
2A X BH 85	14.4	-5.6	26.0*	28.2*
2A X RD2528	28.9*	2.8	35.0*	26.2*
2A X RD2052	13.2	-1.4	28.8*	29.6*
2A X DL88	38.2*	5.6	32.0*	55.3*
2A X BH75	44.7*	6.9	28.2*	53.4*

*Significant at 5% level

The heterosis was attributed to tillers/plant and 1000 grain weight. In the present study, three hybrids namely 1A X DL88, 2A X DL88 & 2A X BH75 exhibited high significant heterosis for tillers/ plant as well as for grain yield/plant. These hybrids may be exploited for commercial hybrid seed production.

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Genetic analysis of biological yield, harvest index and grain yield in six-rowed barley. I. SINGH, S.N. SHARMA, S.L. DASHORA and S.M. BHATNAGAR, Agricultural Research Station, Durgapura, Jaipur 302018, Rajasthan, India.

Introduction. Barley (*Hordeum vulgare* L.) is an important cereal crop in varied farming situations of India and is consumed as human food, animal feed and for malt preparation in the country. However, both area as well as production has been on decline in past one and half decade, due to several factors particularly competition with wheat and mustard crop and specifically the industrial potential of barley has not been properly exploited, which holds a good promise due to recently increased interest of industries in utilizing good quality grain for malting and brewing to compete in international market. Recently, because of liberalization of new policies and increased demand of industries, the barley breeding programme has received a new thrust. Therefore, the information about nature and magnitude of genetic components of variation for yield and its related characters is essential for planning an effective future breeding programme for tangible advancement in barley crop. An attempt was made in the present study to understand the genetic architecture of barley yield and its two important component characters viz. biological yield and harvest index through generation mean analysis.

Materials and Methods. The experimental materials comprised four genotypes, which were crossed in two combinations: B-27-15/B-11-47 (cross I) and B-31-7/B-19-89 (cross II). Seven populations (i.e., the two parents, F_1, F_2 and F_3, BC_1 and BC_2) of each cross were grown in randomized block design with three replications of 30 x 10 cm spacing under normal sown environment. Each parental, F_1 and back cross generation was represented by 2 rows and each F_2 and F_3 generation by 5 rows of 2 m length. Biological yield (g), harvest index (%) and grain yield (g) of 10 random plants in each parent and F_1, 20 plants in each back cross generation and 40 plants in each F_2 and F_3 was recorded in all the three replications of both the crosses separately. The individual scaling test of Mather, 1949 was used to detect the presence of nonallelic gene interactions. The parameters of components of variation were estimated by the joint scaling test of Cavalli (1952).

Results and Discussion. The analysis of variance revealed significant differences among the generation means for the characters studied excepting harvest index in both the crosses, indicating there was narrow range of variation among generations means for harvest index. The results of individual scaling tests viz., A,B,C, and D exhibited that one or more than one scale tests were found significant, indicating the greater role of epistatic interaction to control the inheritance of biological yield and grain yield (Table 1). The application of joint scaling test revealed that in both the crosses the chi-square values (χ^2) of 3-parameter model for these traits were significant, indicating inadequacy of this

model to explain the differences among the generation means and further confirmed the role of epistatic interactions to control the inheritance. The results revealed that 6-parameter model fitted the data in the cross I for both the characters studied. However, the chi-square values were found significant in the cross II, indicating its inadequacy and exhibited that even the digenic interaction model could not fully accounted for the differences among the generation means, this indicated the involvement of more complex interactions or presence of linkage in the inheritance of biological yield and grain yield (Table 1). The various gene effects were, however, estimated following the digenic interaction model as only three and six parameter models were compared in this study.

Table 1. Estimates of scale tests and joint scaling tests for biological yield and grain yield in barley

Characters	Cross	Estimates of scale tests				Estimates of joint scaling tests						
		A	B	C	D	(m)	(d)	(h)	(i)	(j)	(l)	χ^2
Biological yield	I	16.8	-15.8**	-6.8*	- 3.9	38.7	- 8.1**	-20.0*	-0.9	24.2**	8.4	0.3
	II	26.4**	19.8**	16.9**	-14.6**	24.4	-24.0**	51.5**	9.4**	3.3	-46.7**	22.7**
Grain yield	I	11.3	-8.2**	0.4	-1.4	18.5	-4.2**	-7.3	-1.1	16.0**	1.6	0.3
	II	0.2	1.2**	0.9**	-0.3	13.4	-0.7	17.4**	2.1	-4.5	-17.7**	19.7**

***, ** significant at 5% and 1% levels, respectively.**

The results of the estimation of genetic components of variation revealed that both additive (d) and dominance (h) gene effects were operating in the inheritance of biological yield but their relative significance and magnitudes changed with the crosses (Table 1). Of these two gene effects, either additive (d) or dominance (h) was also observed operating in the inheritance of grain yield. The results further indicated that among digenic interactions [(i), (j) and (l)], additive X dominance (j) interaction played significant role in controlling the inheritance of biological yield and grain yield in the cross I. Similarly, dominance X dominance (l) epistatic interaction was played greater role for both the characters in the cross II, however, additive X additive (i) was also contributed in the inheritance for biological yield in the cross II (Table 1). The findings suggested that digenic nonallelic interactions were responsible for the inheritance of biological yield and grain yield in barley, however, the relative importance and magnitude of these epistatic interactions changed drastically with the crosses as well as change in characters. The parameters (h) and (l) were sngnificant in the cross II for both the attributes and there signs indicated a duplicate epistasis between two genes. However, in other cases the types of epistasis could not be ascertained as either (h) or (l) or both parameters was non significant. Duplicate epistasis may restrict the expression of characters studied in early segregating generactions. Thus,

it is suggested that selection intensity should be mild in early genrations and intense in later ones, which could be more effective in the tangible advancement of barley.

The analysis of total gene effects (Table 2) revealed that epistatic effects [(i) + (j) + (l)] were much grater in magnitude than the main effects [(d) and (h)]. Thus, it is evident that epistatic interactions has a greater role in the inheritance of biological yield and grain yield. Present findings were also in line with the observation made by Tripathi and Singh (1983) and Sethi, Paroda and Singh (1986).

Table 2. Abstract table showing main effects, total of epistatic effects, fixable and nonfixable gene effects for biological yield and grain yield in barley

Character	Cross	Main effects (d)	Main effects (h)	Epistatic effects	Total gene effects: Fixable	Total gene effects: Nonfixable
Biological yield	I	- 8.1	-20.0	33.5	9.0	52.6
	II	-24.0	51.5	59.4	33.4	101.5
Grain yield	I	- 4.2	- 7.3	18.7	5.3	24.9
	II	- 0.7	17.4	24.3	2.8	39.6

Results further indicated that as a consequence of higher magnitude of nonallelic interactions, total nonfixable gene effects [(h) + (j) + (l)] were of much greater magnitude than fixable gene effects [(d) + (i)] in both the crosses of the traits studied. This indicates a greater role of nonadditive gene effects in controlling the inheritance of biological yield and grain yield. Other studies (Bhatnager and Sharma, 1995) also led the similar conclusion. Thus, nonallelic interactions should not be ignored in formulating breeding programme and the biparental approach would be extremely useful for enhancing genetic variability and creation of transgressive segregates which could ultimately help in the improvement of the yield potential in barley crop.

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STERILITY IN BRAZILIAN MALTING BARLEY CULTIVARS AND LINES. M. SO E SILVA , E. MINELLA, , Embrapa-Centro Nacional de Pesquisa de Trigo (CNPT), Cx. Postal 569, Fax 054 311 36 17, e-mail soesilva@sede.embrapa.br, Passo Fundo-RS, 99001-970, Brazil.

INTRODUCTION

Malting barley is produced in southern Brazil, in the states of Rio Grande do Sul (RS), Santa Catarina (SC) and Parana (PR) in latitudes between 24 and 31°S. Malting barley is grown in high plateau areas (500 to 1100 meters) where the average temperature and air moisture in the spring (sept-nov) are below 19 °C and 70 % , respectively. Production in the last five years is stabilized around 100,000 tons/year, which represents only one third of the amount used by the malting industry. The average grain yield has varied between 1,000 and 2,300 Kg/ha in the last decade. The most important yield limiting factors are soil and/or subsoil acidity and aluminum toxicity, extreme low (frost) or high (heat) temperatures, lack or excess of rainfall and foliar deseases. The most commonly affected yield component is grain number/m^2 associated with reduced seed set resulting from sterility.The aim of this paper is to report on the occurence of sterility in Brazil, its effects on yield and protein content, and on genotypic variability for this trait.

MATERIALS AND METHODS

Following the first report on male sterility in 1981 (Minella,1982) the sterility percent (100 - [seed set ÷ total spikelet number per spike X 100]) score has been determined for the check varieties and lines in the National barley yield trial (NBYT-official trial for variety release and recomendation) in Guarapuava,PR, in Lagoa Vermelha,RS and Passo Fundo, CNPT-Embrapa, RS.

RESULTS

The data collected so far suggest genotypic differences for this trait (Table 1 and 3). Among the genotypes grown commercially at the present time, Embrapa 43 has been the least and MN599 the most affected with maximum sterility scores, 30 and 80 %, respectively. The other varieties (BR 2, MN668 and MN656) make up an intermediate group with sterility scores between 30 and 50 % range (Table 1). The data also show that sterility scores up to 20 %, usually do not reduce yield because the grain number is compensated by the increase weight of grain set (Table 3). High sterility scores have been associated with low yield ($r^2 = - 0.864$) and with high protein ($r^2 = 0.871$). SO E SILVA et alli (1995) have also associated the reduced seed set with water deficit and the highest sterility scores occuring when the plants were water stressed between Zadock stages Z37 and Z49 (adapted by TOOTMAN & MACKPEACE, 1979). The sterility , mainly male sterility has also been associated with dry hot weather during the booting-heading stages (Minella,1982). Based on these findings, the barley breeding program at Embrapa-CNPT is working in the development of a screening method in order to select for low sterility and is also breeding for improved aluminum tolerance (MINELLA & SO E SILVA elsewhere in this volume). By combining Al^{+3} tolerance with low sterility scores in otherwise adapted genotypes it is

hoped to reduce the yield and quality losses due to drought and high temperature stresses contributing therefore for more stable malting barley production in Brazil.

Table 1. Yield, heading time, protein content and sterility scores of brazilian barley genotypes in Guarapuava, Parana, of the NBYT-1995

Cultivar/ Lines	Yield kg/ha	Heading*	Protein %	Sterility %
BR2(T)	3934	29-Aug	13.8	37.0
MN599	1665	06-Sep	19.2	74.2
MN656	3099	04-Sep	16.6	56.0
AF1343	4071	07-Sep	14.7	34.6
MN668	3278	30-Aug	14.6	36.2
MN681	4295	04-Sep	15.0	22.5
MN682	3193	07-Sep	16.8	47.8
MN686	4083	04-Sep	14.6	30.1
MN691	4113	03-Sep	15.1	34.5
MN693	2298	07-Sep	17.1	61.3
EMB43	4049	30-Aug	14.0	12.5
PFC 9201	3937	01-Sep	13.8	25.4
PFC 9202	4312	31-Aug	13.4	18.2
PFC 9205	5095	29-Aug	12.9	10.5
PFC 9210	4864	31-Aug	12.9	12.2
PFC 9215	4263	04-Sep	14.2	25.2
PFC 9216	3680	05-Sep	14.4	24.3
PFC 9219	4497	31-Aug	14.0	19.8
Average	3818		14.8	32.3

*Emergence date=7/July/95

Table 2. Correlation matrix of variables included in Table 1.

	Yield	Protein	Sterility
Yield	-	**- 0.839 ****	**- 0.864 ****
Protein		-	**0.871 ****
Sterility			-

** Significance level of coefficients at error probability of 1 percent.

TABLE 3. Yield, protein content and sterility scores of brazilian barley genotypes in Lagoa Vermelha e Passo Fundo, Rio Grande do Sul, of the NBYT-1992

CULT./ LINES	LAGOA VERMELHA			PASSO FUNDO		
	Yield Kg/ha	Protein %	Sterility %	Yield Kg/ha	Protein %	Sterility %
ANTARCTICA (T)	2125	11.8	29.3	4822	8.8	13.7
MN599(T)	1284	13.2	60.0	4858	8.9	10.6
ANTARCTICA 6	2103	12.3	36.0	4205	9.1	10.7
MN607	966	14.5	75.0	4525	9.7	7.2
BR2	2734	11.6	21.2	4567	8.9	6.5
AF279	1462	12.5	50.3	4928	8.3	7.3
AF288	2139	12.1	33.0	4411	8.7	9.7
MN642	959	12.5	72.4	4792	8.7	13.3
MN649	1838	13.0	36.5	4920	8.7	4.9
MN656	1969	11.8	40.6	4575	8.6	10.0
MN657	2180	11.7	41.5	4968	9.0	6.1
MN662	3029	11.0	18.3	4768	8.7	6.1
MN666	2201	11.0	19.4	5202	9.1	5.6
MN667	3033	10.0	6.6	4513	8.4	5.6
PFC85104	3402	10.0	4.5	4433	8.3	5.3
PFC85106	2152	12.2	26.4	4641	8.6	4.6
PFC85107	3613	10.2	9.0	4878	8.5	5.1
PFC86104	2911	10.4	11.3	5603	9.5	7.1
AVERAGE	2228	11.8	32.9	4756	8.8	7.7

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Winter barley lines selected under high and low input conditions. A.M. STANCA, G. DELOGU and L. CATTIVELLI, Experimental Institute for Cereal Research, Section of Fiorenzuola d'Arda, I-29017, Via S.Protaso, 302, Fiorenzuola d'Arda (PC), Italy

Introduction. A winter barley breeding program for high yield has been developed to compare the effect of selection applied at the same location under two different management conditions: with (high input) and without (low input) fungicide, herbicide and fertilizer treatments. This is to evaluate if the selection procedure undertaken in the two agronomic conditions is efficient to evidenciate new genotypes with better performance for low or high input conditions.

Materials and Methods. The lines were selected from four F_2 populations. The single crosses were performed in the spring of 1990 by using as parents high yielding winter genotypes for the six-rowed and two-rowed crosses. The crosses were identified by the following cross number: FO 2197 (six-rowed): (advanced line Fior synth 1 x Trebbia); FO 2194 (six-rowed): (Robur x Vetulio) x Jaidor; FO 2210 (two-rowed): (Fior 100 x Arda) and FO 2211 (two-rowed): (Fior 100 x Baraka).
Starting from the F_2, visual field selection was performed at Fiorenzuola d'Arda (Italy). 2000 F_2 seeds per each cross were splitted in two parts: 1000 viable seeds were sown on one row at a spacing of one seed per 10 cm in normal field conditions (here considered high input.); the other 1000 seeds were sown in an adjacent field in low input conditions in which all agronomic practices were the same as in high input except for fungicides (seed borne and foliar disease control), herbicides and fertilizers (N_2-P_2O_5-K_2O) applications.
For each population, either in low or high input, 50 plants have been visually selected for agronomic traits (winter hardiness, heading time, plant height, seeds/spike, powdery mildew and *Pyrenophora graminea* resistance).
1992-93. The 50 F_3 progenies selected in low and high input conditions per each of the four crosses have been sown in field under low and high input conditions respectively in small plots (8 rows of 1 m length) of 160 spaced seeds. Visual selection has been applied by using the same criteria as in F_2. 15% of the total progenies has been selected on the basis of the agronomic traits and by taking into account uniformity for plant height and heading time.
1993-94. Two replicated yield trials have been established by using the 58 F_4 progenies (29 visually selected in low and 29 visually selected in high input conditions). Trials have been carried out in the same farm in low and high input conditions by using a randomized block (3 replications) design with plots of 6 m^2. Grain yield and other agronomic traits have been scored. The same progenies have been grown in a nursery in low and high input conditions. In 1994-95 the 58 F_5 progenies have been evaluated in the same conditions as in F_4 in replicated yield trials.
Details of the experiments. One trial was established in a field at low input conditions in which wheat was the previous crop and fertilizers, herbicides, fungicides for seed or foliar diseases were not applied.
A second trial was established in high input conditions and it was characterized by: previous crop: wheat; fertilizers pre-sowing: N_2 30 kg/ha; P_2O_5 100 kg/ha K_2O 100 kg/ha N_2-top dressing: 70 kg/ha (50% at the end of January and 50% at the end of March)

herbicide treatment: Bi-Hedonal (2,4D + MCPA) 2 kg/ha fungicides treatment: Vitavax Flo (Carboxin + Thiram) for seed borne disease control; Triadimefon + Carbendazim (Bayleton) for desease control before heading.

Results. The data of the replicated yield trials have been statistically analyzed and the results (Fig. 1) show that the visually selected lines either selected in low or high input conditions perform exactly the same. In fact the best lines (11 for each group) with an index of productivity above the 5% more than the mean values gave the same results either when selected in poor or fertile conditions.
From the 11 visually selected lines in low input conditions well adapted at high level of productivity in both agronomic conditions, four belong to the cross FO 2197 (six-rowed), five to the cross CO 2194 (six rowed), two to the cross 2211 (two-rowed) and no one to the cross FO 2210.
From the 11 lines visually selected in high input conditions well adapted in both agronomic conditions, five belong to the cross FO 2197 (six-rowed), two to the cross FO 2194 (six-rowed), one to the cross FO 2210 (two-rowed) and three to the cross FO 2211 (two-rowed).
The interaction (genotypes x environment) was very low in comparison with the other components of variance as the lines and the growing conditions showed more variation. The best results have been obtained, as average, with the two six-rowed crosses and the lines from the cross FO 2197 gave the highest values in the two environmental conditions (Fig. 2).
The results presented here indicate that the selection carried out in the same site in low and high input conditions has given the same effect. The yield reduction in low input condition is about 40% less than that harvested in normal conditions (Fig. 1). The selected lines even if derived from modern varieties, show a good adaptation when evaluated in dramatic condition.
Although grain yield loss was consistent in low input conditions in comparison to the high input conditions, it is evident that the best progenies (two rowed or six rowed) selected under low or high agronomic conditions express the genetic potentiality at high level of grain yield and show a high level of adaptation to cope with contrasting agronomic conditions.
This exercise indicates that the selection for low input condition in a target area can be done with the same efficiency by selecting the progenies in low or high input conditions. The breeder can choose where it is more confortable and less expensive.

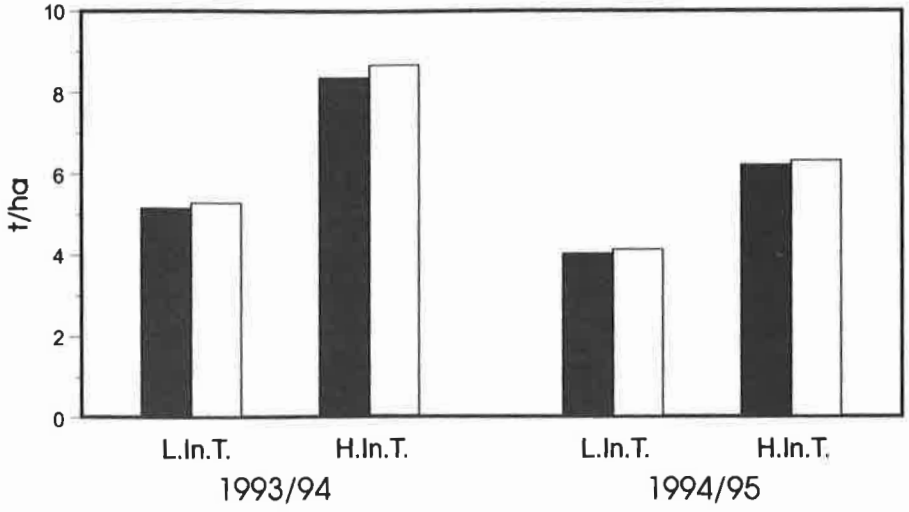

Fig. 1. Average grain yield (t/ha) of the progenies visually selected under low □ and high ■ input conditions and grown in L.In.T. (low input trials 1994 and 1995) and H.In.T. (high input trials 1994 and 1995)

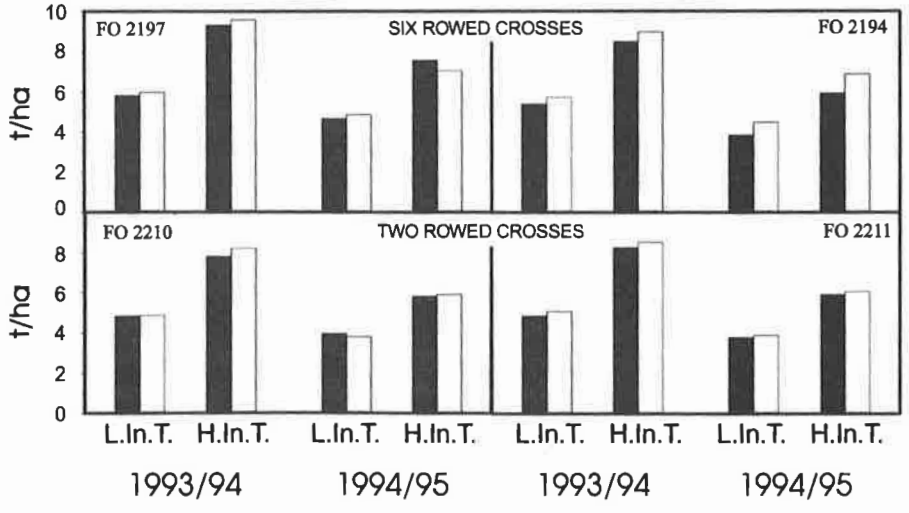

Fig. 2. Average grain yield, for each cross, of the progenies visually selected under low □ and high ■ input conditions and grown in L.In.T. (low input trials 1994 and 1995) and H.In.T. (high input trials 1994 and 1995).

Production of doubled-haploid lines from Brazilian barley cultivars and hybrid F_1 populations. A.L. STIVAL, M.I. MORAES-FERNANDES, G. ARIAS and M.SÓ E SILVA, Centro Nacional de Pesquisa de Trigo, Embrapa, Caixa Postal 569, Passo Fundo, RS, 99001-970, Brazil.

INTRODUCTION

In vitro production of doubled-haploid plants allows the obtention of pure lines without the numerous cycles of inbreeding needed by conventional breeding programs. In barley, many studies have been carried out with a few varieties, like "Igri" which presents an extraordinary androgenetic capacity. The development of protocols specifically adjusted for highly responsive cultivars is considered inconvenient for breeding purposes that usually require hybrid materials. Along with wheat and oat, barley is one of the crops cultivated during the cold season in the southern region of Brazil. Its production is mainly consumed by breweries. Since 1993 anther culture of Brazilian varieties and F_1 populations of barley is being employed at the National Wheat Research Center (EMBRAPA - CNPT), Passo Fundo, RS, Brazil. The goal is to accelerate the breeding program and the strategy is to obtain homozygous lines using material that combines good androgenetic capacity with important agronomic characters (high productivity, quality and disease resistance). This paper reports the results of three years of research on barley anther culture at EMBRAPA - CNPT.

MATERIALS AND METHODS

The experiment was conducted in Passo Fundo, located at 28° 15' S, 52° 24' W, 687 m. Plants were cultivated in pots in the screenhouse with ambient temperature and light during the winter. Spikes containing pollen at the mid-uninucleate stage were collected and kept during 10 days at 4 °C in the dark. Distance between the base of the flag-leaf and the anterior leaf indicated the stage of pollen development. After pre-treatment in the cold anthers were placed in Petri dishes containing basic N_6 medium (Chu, 1981) supplemented with 2 mg/l NAA and 1 mg/l BAP. After seven days in culture some microspores divided, forming two identical nuclei. Multicellular pollen grains were visible after 14 days (Fig. 1A). They contained 8 to 12 cells, divided by cell walls but still enclosed into the exine (Fig. 1B). When the exine breaked it set free globular structures (Fig. 1C). After 30 days in the dark at 25 °C the first androgenetic structures bursted out on the surface of the anthers (Fig. 1D). Histological analysis showed the presence of typical embryos, with shoot and root meristems (Fig. 1E). Regeneration of plants through embryogenesis is very desirable since it reduces the percentage of albinism and the occurence of chromosomic abnormalities. Embryos germinated regenerating green and albino plantlets in the same medium used for induction (Fig. 1F). Green plantlets were placed into test tubes containing P-2 medium (Chuang, 1981) supplemented with 1 mg/l AIA for rooting. Acclimatization was done in pots containing vermiculite and Hoagland's nutritive solution. Before transferrence to soil, ploidy level of the plants was determined through chromosome counts on root tips. Haploid plants were treated with colchicine for chromosome doubling. Doubled-haploid plants were grown under controlled temperature (18 ± 2 °C) in growth chambers until grain formation.

RESULTS AND DISCUSSION

Since 1993 more than 30,000 anthers have been cultured and 996 green plants obtained from different F_1 populations, lines and varieties (Table 1). The regeneration varied from a minimum of 0.1 to a maximum of 8.3 green plants per 100 cultured anthers, with an average of 5.3. In general, hybrid material showed better responses than pure lines or varieties. The cultivar BR 2 and the line PFC 9104 showed the highest percentage of green plantlet regeneration in the case of homozygous materials. PFC 9104 descends from "Igri", a model variety for anther culture studies in barley due to its high responsivity. Androgenetic capacity of genotypes was influenced by growth conditions of the donor plants. PFC 87143 (Gimpel), for example, is reported to be highly androgenetic in Germany. Possibly because of the extreme climatic conditions of crop cultivation in south Brazil, this characteristic was not observed in the cultivar itself, being expressed only in the F_1 progeny of crosses made between PFC 87143 and other lines better adapted to local conditions. About 55 % of the plantlets showed spontaneous doubling of the chromosomes avoiding the need of colchicine treatment. Due to the homozygosis every plant represents a potential line which can be directly evaluated and selected for a number of important agronomic traits, eventually leading to the release of a new cultivar.

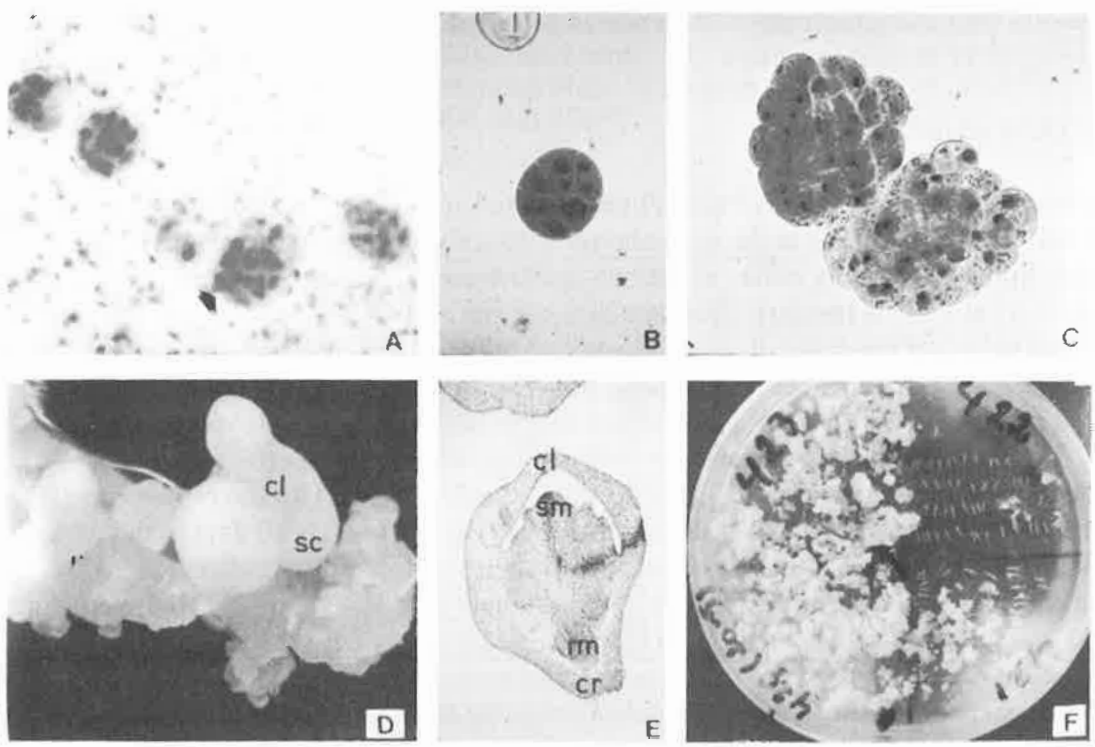

Fig. 1: A. Multicellular pollen grains (arrow) inside the tissue of an anther which has been cultured for 14 days. x 78,75. B. Multicellular pollen grain still enclosed into the exine. x 200. C. Globular structures formed after the rupture of the exine. x 200. D. Androgenetic embryo on the surface of an anther (sc: scutellum; cl: coleoptile). x 25. E. Longisection through an embryo showing typical bipolar organization (sm: shoot meristem; rm: root meristem; cr: coleorhiza). x 78,75. F. Regeneration of green (arrow) and albino plantlets.

Table 1. Anther culture response of cultivars, lines and F_1 hybrids of barley

CULTIVARS / CROSSES	Anthers	Green plants			Albino plants		
	No.	No.	%		No.	%	
MN 668/PFC 9104	1579	131	8.3	A	85	5.4	AB
BR 2/MN 607	1229	69	5.4	AB	27	2.2	BCDEF
PFC 87143/PFC 9202	3167	142	4.5	AB	100	3.2	ABCDEF
BR 2/PFC 9104	1408	61	4.4	AB	52	3.7	ABCDEF
PFC 9104/PFC 9134	1043	42	4.3	AB	29	2.8	ABCDEF
MN 607/PFC 9104	886	36	4.2	AB	35	4.0	ABCDE
PFC 9216/PFC 87143	1330	52	3.9	AB	63	4.7	ABCD
PFC 9211/PFC 87143	1821	64	3.5	AB	45	2.5	BCDEF
PFC 87143/PFC 9216	1961	66	3.4	AB	35	1.8	BCDEF
PFC 9237/KRONA//PFC 9216	1928	62	3.2	AB	92	4.8	ABC
AF 279/PFC 9104	983	30	3.0	B	24	2.4	BCDEF
MN 656/PFC 9104	1448	43	2.9	B	40	2.8	ABCDEF
PFC 9112/PFC 88154	1234	29	2.5	B	31	2.5	ABCDEF
PFC 9301/PFC 9216	1489	36	2.4	B	74	5.0	AB
BR 2	521	10	1.9	B	0	0.0	F
PFC 9104	730	14	1.9	B	28	3.8	ABCDEF
PFC 9215/PFC 87143	1788	30	1.7	B	45	2.5	ABCDEF
PFC 9301/PFC 9201	619	10	1.6	B	15	2.4	BCDEF
PFC 9463	720	10	1.4	B	0	0.0	F
PFC 86104/PFC 9104	1280	15	1.2	B	49	3.8	ABCDEF
PFC 9470	724	8	1.1	B	0	0.0	F
PFC 85107/PFC 9104	1137	11	1.0	B	32	2.8	ABCDEF
KHATARINA/PFC 85107//PFC 9248	1139	9	0.8	B	10	0.9	CDEF
PFC 9464	548	4	0.7	B	2	0.4	DEF
PFC 9477	996	5	0.5	B	64	6.4	A
MN 668	558	2	0.4	B	2	0.4	EF
PFC 85107	646	1	0.2	B	12	1.9	BCDEF
DEFRA/MN 607	1210	3	0.2	B	9	0.7	CDEF
PFC 87143	732	1	0.1	B	0	0.0	F
Totals	34854	996	5.3		1000	5.0	

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Expression of barley marker genes in wheat-barley hybrids.

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Introduction. Barley is a potential source of useful genes for wheat improvement. F_1 hybrids between wheat and barley (Fedak 1980; Islam et al. 1981; Sethi et al. 1986; Koba et al. 1991; Taketa et al. 1995) and six of the seven possible wheat-barley chromosome addition lines (Islam et al. 1981) were produced. In wheat-barley hybrids, isozymes and storage proteins of the barley parent are generally expressed, enabling the determination of their chromosomal locations. In contrast, the phenotypes of wheat-barley hybrids are similar to those of the wheat parents. For example, two-rowed ear type and covered karyopsis of the barley parent, which are each controlled by a single dominant gene, are not expressed. This may suggest that expression of some barley genes is suppressed in the genetic background of wheat. For successful transfer of barley genes to wheat, information on the mode of expression of various barley genes in the wheat genetic background is important. In barley, many morphological marker genes with known chromosomal locations are well documented (Tsuchiya 1983). In the present study, barley accessions carrying such marker genes were crossed with wheat, and their expression in the hybrids was studied.

Materials and Methods. A Japanese common wheat (*Triticum aestivum* L.) cultivar 'Shinchunaga' was crossed as the female parent with five accessions of wild and cultivated barley (*Hordeum spontaneum* C. Koch and *H. vulgare* L., respectively) carrying morphological marker genes listed in Table 1. Crossing, embryo culture and C-banding were conducted as previously reported (Taketa et al. 1995). Since elimination of barley chromosomes frequently occurs in wheat-barley crosses, expression of barley genes was studied only in the plants which were confirmed by C-banding to retain barley chromosomes with marker genes.

Results and Discussion. In all cross combinations, F_1 hybrids which retain barley chromosomes with morphological marker genes were obtained. Two recessive barley genes, i.e., *gl-3* (glossy seedling-3, chromosome 4) and *o* (orange lemma, chromosome 6) were not expressed in the F_1 hybrids carrying respective genes (Table 1). Expression of the genes for brittle rachis (*Bt-Bt2*) was examined in a disomic addition line of chromosome 3 from wild barley (*H. spontaneum*). The wheat-wild barley addition 3 showed tough

rachis. Suppression of these genes may be caused by interactions with homoeoalleles of the wheat parent.

The dominant barley genes for leaf blade pubescence (*Pub*, chromosome 3), hairy leaf sheath (*Hs*, chromosome 4), hooded lemma (*K*, chromosome 4) and black lemma (*B*, chromosome 5) were expressed in the F_1 hybrids carrying respective genes (Table 1). The F_1 hybrids with the *Pub* or *Hs* gene showed weaker hairiness than the parental barley accessions. Expression of the *B* gene was proportional to its dosages and hybrids with two representatives of chromosome 5 showed stronger coloration than those with a single chromosome 5. Expression of the hooded gene on chromosome 4 was largely affected by the presence or absence of barley chromosome 6. In 28-chromosome hybrids with full complement of the barley genome, hooded lemma was clearly observed. However, in a 27-chromosome hybrid lacking barley chromosome 6, only traces of hoods were observed on distal part of some awns. Recently, the hooded gene was shown to be a homoeobox gene, which is overexpressed in inflorescences (Muller et al. 1995). Barley chromosome 6 may carry a modifier which enhances the expression of this gene.

The barley genes expressed in the hybrids will be useful markers for the manipulation of barley chromosomes in the genetic background of wheat. Also, the two expressing dominant genes on barley chromosome 4 will be helpful to clarify the orientation of this chromosome, which is one of controversial issues of barley cytogenetics.

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Table 1. Expression of barley morphological marker genes in the hybrids with a Japanese common wheat cultivar 'Shinchunaga'

Marker gene (chromosome)	Barley accession [1]	Gene expression [2]
Recessive gene		
gl-3 (4)	OHL056	-
o (6)	OUL139	-
Dominant gene		
Pub (3)	WMD	+
Bt-Bt2 (3)	OUH602	-
Hs (4)	OUJ064, OUL056, WMD	+
K (4)	OUL056, OUL139, WMD	+
B (5)	OUH602, OUL139, WMD	+

1) OU:Accession number of Barley Germplasm Center, Okayama University, WMD:Wolf's mutiple dominant stock (Wolf and Franckowiak 1991).

2)+:Expressed, -:Not expressed.

Using diallel analysis and male sterile elite germplasm in barley to determine the best parents for crossing in a breeding program. M.C. THERRIEN and S.K. PLETT, Brandon Research Centre, Box 1000A, RR#3, Brandon, MB, R7A 5Y3 and Limagrain International, Willmar, Minnesota, USA.

Introduction. Traditionally, breeders choose parents in their crossing program based on parental agronomic performance for important quantitative traits such as yield. Transfer of agronomically important quantitative traits is problematic due to the many cross combinations that must be produced to obtain the desired recombinants. This is a costly, albeit necessary, undertaking in a breeding program. In cross-pollinated crops, such as corn, identification of best recombinants for yield is facilitated by the outcrossing nature of the crop. Many cross combinations can be obtained with relatively little effort and a parent's potential for producing the desired recombinants can be fairly estimated using diallel analysis and the estimation of General Combining Ability (GCA; Baker, 1978). If this approach could be practically implemented in a self-pollinated crop, such as barley, then relatively large quantities of crossed seed can be obtained at a relatively low cost and the best parents could be chosen to maximize yield potential in a hybrid, and save time and effort in a breeding program. The use of genic male-sterility in barley affords the opportunity to partially simulate the outcrossing nature of corn and the application of diallel analysis to barley. We present the results of an experiment outlining this potential.

Materials and Methods. Four cultivars of barley, namely Manley, Deuce, Lacombe, and Conquest, were chosen for their large range in yield response under growing conditions in Brandon, MB. These were designated as the 'male parents'. A total of seven locally-adapted elite lines of male-sterile barley were chosen from an elite population developed over a 10-year period and derived from a male-sterile composite cross population produced by R.T. Ramage, Arizona State University, USA. These were designated as the 'female parents'. Near-isogenic male-fertile sibs were also available from these elite lines and served as the second parent in a cross. Since only the female parents were male-sterile, crosses were unidirectional with male parents as the sole pollen source. Spikes of male parents in full anthesis were placed in close proximity to spikes of female parents at the equivalent stage and allowed to cross-pollinate under sealed glassine bags for 6 hours. This procedure was repeated for all 28 possible cross combinations. Crossed F1 seed was harvested at maturity and sown in the field, along with parent lines. Plots consisted of three sub-plots. Each sub-plot consisted of a double row 3 m long and 30 cm spacing each containing the male parent, the F1, and the near-isogenic male fertile sib of the female parent. Main plots were arranged in a four-replicate Randomized Complete Block Design (RCBD). Plots were harvested for grain yield at maturity. Yield was calculated on a tonne per hectare basis and data subjected to a partial diallel analysis in the manner of Gardner and Eberhart (1966), with General Combining Ability (GCA) estimates determined for each parental genotype..

Results and Discussion. Table 1 shows the parents and successful cross combinations. Seed set was not sufficient to provide for all 28 cross combinations, which is a limitation for diallel analysis in male-sterile barley and did not allow for determination of Specific Combining Ability (SCA). However, sufficient numbers of crosses were obtained to estimate GCA for the 11

parents used in this study. Table 2 shows the results of the diallel analysis for yield. GCA estimates were significant for yield performance for the four male parents. In this case, Manley was identified as the best recombinant male parent for yield, although GCA values were similar for each of the four male parents. More diverse germplasm would likely have shown a broader range of responses. Only one female parent, EX344, had a significant GCA. This is of interest, as the 7 female parents are derived from basically the same population, but do not necessarily produce the same yield response. Thus, it is important to use more than one source of male-sterile parent. The ease of producing F1 hybrids via the male-sterile system makes diallel analysis possible, as well as practical, in a barley breeding program. Thus, many potential parents can be screened with relative ease to identify superior germplasm and the progeny of the best cross combination(s) have already been initiated for further selection and evaluation.

Table 1. Parents used in partial diallel analysis and F1 progeny obtained.

Parent	Cross Code	Type	Cross	Cross Code
Manley	P1	Male Parent	EX340/Manley	P5XP1
Deuce	P2	Male Parent	EX340/Deuce	P5XP2
Lacombe	P3	Male Parent	EX356/Lacombe	P6XP3
Conquest	P4	Male parent	EX356/Conquest	P6XP4
EX340	P5	Female parent	EX345/Manley	P7XP1
EX356	P6	Female parent	EX345/Deuce	P7XP2
EX345	P7	Female parent	EX345/Lacombe	P7XP3
EX350	P8	Female parent	EX345/Conquest	P7XP4
EX396	P9	Female parent	EX350/Manley	P8XP1
EX355	P10	Female parent	EX350/Deuce	P8XP2
EX344	P11	Female parent	EX396/Lacombe	P9XP3
			EX396/Conquest	P9XP4
			EX355/Lacombe	P10XP3
			EX355/Conquest	P10XP4
			EX344/Manley	P11XP1
			EX344/Deuce	P11XP2

Table 2.Mean yield, percent heterosis, and General Combining Ability (GCA) for 11 parental and 16 F1 barley genotypes derived from male-sterile crosses.

Line	Yield T/ha	Significance[1]	Heterosis %	GCA %	Significance[2]
P1	4.97	e	---	16.2	**
P2	5.01	e	---	18.1	**
P3	5.63	d	---	13.1	**
P4	4.38	f	---	11.7	**
P5	6.07	cd	---	4.3	NS
P6	5.05	de	---	4.1	NS
P7	6.50	c	---	1.9	NS
P8	4.30	f	---	2.4	NS
P9	4.45	f	---	2.0	NS
P10	5.62	d	---	1.8	NS
P11	7.12	b	---	10.1	**
P5XP1	5.50	d	-9.4	----	--
P5XP2	6.48	c	6.8	----	--
P6XP3	6.36	c	13.1	----	--
P6XP4	6.22	c	23.2	----	--
P7XP1	6.15	c	-5.3	----	--
P7XP2	7.28	b	12.0	----	--
P7XP3	6.91	b	6.3	----	--
P7XP4	6.75	c	3.8	----	--
P8XP1	8.14	a	63.8	----	--
P8XP2	5.86	d	16.9	----	--
P9XP3	7.65	b	35.9	----	--
P9XP4	4.79	e	7.6	----	--
P10XP3	6.95	b	23.6	----	--
P10XP4	4.55	f	-19.1	----	--
P11XP1	7.36	a	3.4	----	--
P11XP2	6.24	b	-12.3	----	--

[1]Values followed by the same letter are not significant at 5% level.

[2]** = significant from 0 at 1% level; NS = Not Significant.

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Development and characterization of doubled haploid progeny of a multiple recessive and a multiple dominant genetic marker stock in barley, with an update on related marker stock development. R.I. Wolfe, P.M. Hayes[1], L. Shugar[2], K. Sato[3], J. Costa[4], and C. Jobet[1]; Field Crop Development Centre, AAFC, 5030 50 St., Lacombe, AB Canada T4L 1W8, [1]Dept. of Crop and Soil Science, Oregon State Univ., Corvalis, OR 97331 USA, [2]W.G. Thompson & Sons Ltd., RR#1 County Rd. 19, Ailsa Craig, ON Canada N0M 1A0, [3]Research Institute for Bioresources, Okayama Univ., Kurashiki, 710, Japan, and [4]Dept. Of Agronomy, Univ. of Maryland, College Park, MD 20742 USA.

Introduction. Progress has been made on a set of multiple dominant and recessive genetic marker stocks developed by the senior author and reported by Wolfe and Franckowiak (1990). The original aim was to produce a master dominant and a master recessive, plus seven multiple recessive stocks, one for each chromosome, combining a genetic male sterile, a dwarf, and a surface waxy mutant. The incorporation of seven genetic male steriles was successful. A dwarf was incorporated for all seven chromosomes, but the one used for chr. 2, *mnd,,c,* may not be on this chromosome (Personal communication, J.D. Franckowiak.). Surface waxy mutants for five of the seven chromosomes were incorporated. In addition, recessive alleles of several genes not in the master recessive have been incorporated. Except for chromosome 4, to get the complete set of recessive alleles for a chromosome, two or more stocks are needed. The stocks for Chromosome 1 have ***msg10***, ***br***, and ***cer-a(gs3)***; Chr. 2, ***msg2***, ***(mnd,,c)***, ***gs6***, and in addition, ***e***, and ***li***, not in the master recessive; Chr. 3, ***msg5***, ***uz***, and in addition ***als***. Chr. 4 has ***msg24***, ***ert-i***, ***gl***, and ***yh***, in a recessive background similar to the master recessive (The recessive gene, ***al***, in the master recessive, is not in this stock); Chr. 5, ***msg***, ***ert-b***, ***cer-e***, and in addition, ***trd***, and ***nec,,_*** (a necrotic spotting on the leaves); Chr. 6, ***msg36***, ***l9***, ***gs4***, and in addition, ***uc2***, and ***sex1***; Chr. 7, ***msg19***, ***ert-g***, and in addition, ***cs***, a recessive streaking of lower leaves. This mutant sometimes kills all but one of the tillers, allowing it to grow larger than normal, thus the name "corn stalk."

The aim of this study was to produce doubled haploids from a cross between the master recessive and dominant.

Materials and Methods. We produced doubled haploids (DH) from the master recessive and master dominant stocks, using the *Hordeum bulbosum* method. A DH selection of each was entered in the Plant Gene Resources of Canada collection, as PGR27409, and PGR27408. These two lines were crossed, and 107 DH progeny produced from the F1. Ratios have been fit to the data to determine segregation and linkage.

Results and Discussion. The doubled haploid lines from this cross segregate for a minimum of 19 observable genes. These were checked for correspondence with a 1:1 ratio (Table 1). Fourteen were not significantly different from this. The hood, *K,* segregated 1:3. It apparently does not express in the short awned phenotype, *lk2*. To obtain the *Lk2:lk2* ratio (Table 1), the hooded lines were included with the long awns. The blue aleurone, when not masked by the black and/or purple lemma and pericarp, *B* and *Pe2,* seems to be controlled by at least two genes, both of which are needed to produce the blue color. The ratings for purple lemma and pericarp were taken on ripe heads. The ratio

Table 1. Genetic ratios for a number of genes segregating in doubled haploid progeny of a cross between a master recessive and a master dominant genetic stock of barley.

Gene	Description	Loc.	Ratios[z]
Wx wx	Normal starch, waxy	1S	54:48 ns
N n	Hulled seed , hulless	1L	53:54 ns
Lk2 lk2	Normal awn, short	1L	58:47 ns
Re2 re2	Purple lemma & pericarp, non-p.	2L	13:40 **
V v	Two-row, six-row	2L	49:57 ns
Zeo zeo[y]	Dense headed dwarf, normal	2L	42:63 ns
Wst,,k wst,,k	Normal, juvenile white stripe	2L	50:50 ns
Pub pub[y]	Pubescent flag leaf blade, non-p.	-	46:54 ns
Al al	Normal lemma, albino	3S	58:45 ns
Bt bt	Brittle rachis, tough	3	27:34 ns
Hs hs	Hairy lower leaf sheaths, non-h.	4S	42:61 ns
K k	Hooded awn, non-hooded	4L	34:70 ns
B b	Black lemma & pericarp, non-b.	5L	43:61 ns
O o	Normal lemma, orange	6L	61:44 ns
S s	Long haired rachilla, short h.	7L	64:40 *
R r	Rough awn, semi-smooth	7L	43:29 ns
Bl,,_ bl,,_[y]	Blue aleurone, non-blue	-	20:40 ns
Gh gh[y]	Long glume hairs, short g.h.	-	61:41 ns
Vb vb[y]	Strong vein barbing, weak	-	55:47 ns

[z] The ratios tested are 1:1, except for *K k,* and *Bl,,_ bl,,_,* which are 1:3.
[Y] The symbols for these genes have been chosen for convenient use in this poster.

should improve when we can observe color development just prior to the development of the black pigmentation in ripening heads. The hairy sheath gene, *Hs hs,* should be checked again. Some of the smooth-awned lines, *r,* may have been misclassified. We have not characterized this gene in hooded plants. Rachilla hair length, *S s,* showed an excess of the dominant phenotype. The variation in glume-awn hairs, *Gh gh,* showed some interaction with *Zeo zeo* (Table 2). More work will have to be done on this interaction to see if *Gh* can be determined reliably in the *Zeo* background. Brittle rachis, *Bt bt,* data were taken only on the non-*Zeo* plants. *Bt* did not express in its dense-headed phenotype. It was a surprise to us that the master dominant was carrying this characteristic. Table 2 lists some of the linkages found, and illustrates interaction between *Zeo* and *Gh.* The *n lk2* and *s r* linkages in this material are similar to those found by Jin et al. (1992).

We have a challenge in this material to distinguish between unusual random segregation, linkages, pleiotropy, differential viability, multiple rather than single gene effects, and the masking of the effects of one gene by another. Some of these problems will be mitigated when the master dominant and selected recessives are crossed to material under investigation, as not all the genes will be segregating in the progeny of a single

cross. Backcrossing to the recessive will also help overcome some of these problems.

These data will be subject to revision as more is learned about the material. Hopefully most current problems of scoring and interpretation will be overcome. We feel these stocks will be useful for research and the teaching of genetics.

Table 2. Linkages and interactions between several genes in doubled haploid progeny of a cross between a master recessive and a master dominant genetic stock of barley.

Genes	Parental AA	Parental BB[Z]	Non-Par. AB	Non-Par. BA	Total	Linkage
Wx/N	31	29	19	23	102	41% ns
Wx/Lk2	31	23	23	25	102	47% ns
N/Lk2	48	43	4	10	105	13% **
Re2/V	13	26	0	14	53	26% **
Re2/Zeo	6	23	7	17	53	45% ns
Re2/Wst,,k	6	17	7	23	53	57% ns
V/Zeo	24	39	24	18	105	40% ns
V/Wst,,k	26	30	20	24	100	44% ns
Zeo/Wst,,k	31	41	9	19	100	28% **
Re2/Pub	6	18	7	22	53	55% ns
Wst,,k/Pub	31	34	19	14	98	34% **
Pub/Al	25	24	30	21	100	51% ns
Pub/Bt	9	19	15	17	60	53% ns
Al/Bt	24	27	7	4	62	18% **
S/R	37	17	11	5	70	23% **
Zeo/S	24	23	16	39	102	54% ns
Zeo/Gh	15	16	25	46	102	70% ** reverse
S/Gh	49	27	14	12	102	25% **
R/Gh	36	20	5	8	69	19% **

[Z] A represents the dominant alleles, B the recessive.

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Section XI: Physiology

Different planting periods as a strategy for stabilize grain yield production in oat.

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Introduction. One of the main aspects in growing oat (*Avena sativa* L.) in the South Brazil is the planting date due to the high interaction between plant and environment conditions. A proper planting date may result in more stable and safe grain yield together with a better grain quality (Almeida et al. 1995). According to Sorrells & Simmons 1992 the growth of oat is influenced greatly by environmental factors such as available water, temperature, solar irradiation, and soil physical and chemical conditions. Those factors may have less or more influence at different times during the growing season. The oat growing region in Southern Brazil is characterized as subtropical climate, with a well-defined cold season. During the growing season, which comprehends winter and spring in the South hemisphere, the average temperature from May to November, in the average of the 19 years, is 15 ° C. In June and July, especially in the high plateaus, in addition to light and sporadic snowfall, frosts are frequent. The amount of rainfall from May to November, in the average of the 19 years, is 1123.9 mm. The main objective of this research was to determine the most appropriate planting period for two different oat genotypes, in which they can express the maximum grain yield potential in the Southern region of Brazil.

Materials and Methods. The experiment was developed during four years, from 1992 to 1995, at FAPA`s research station. A split plot design was used with four replicates, where planting date was installed in the main plot and genotype in the sub-plot. Although planting dates had a slight variation among the years, they can be classified in four main planting periods: from a 15 to 30 May date (first planting period); from a 1 to 15 June date (second planting period); from a 15 to 30 June date (third planting period) and from a 1 to 15 July date (fourth planting period). Two genotypes were commonly tested along the years: UFRGS 7 and UPF 7. No fertilizer was broadcasted before planting. The four year experiments were sown with an experimental nursery planter Hege. Plots were six rows five meters long with rows 0.17 meters apart. The amount of seed used was 315 seeds per square meter. An average of 20 kg ha^{-1} of nitrogen was broadcasted as a side dressing during tillering stage of the plants. Disease control was done whenever it was necessary. Other agronomic measurements like test weight, plant height and days to maturity were done. Yield and agronomic data were statistically analyzed to determine whether planting date significantly influences any of these traits among the years.

Results and Discussion. Analysis of the grain yield stability has revealed that there is no regular pattern over the years. During the 1992 experiment, there was just one strong frost, which happened on July 19 (-1.8 °C). There was no drought period during that growing season. Both genotypes showed the same pattern of grain yield among the planting periods, with a slight reduction in the third planting period. On the other hand, during 1993 growing season seven strong frosts occurred: 1 June (-1.0 °C), 19 June (-2.5 °C), 14 July (-5.0 °C), 15 July (-2.0 °C), 31 July (-4.1 °C), 1 August (-3.3 °C) and 3 August (-2.2 °C). In addition, a drought period was observed during August in that year. The grain yield of the first planting period was significantly lower than the other three

planting periods for both genotypes. This was probably due to the frost damages and drought effects. During the 1994 experiment, a sequence of five strong frosts occurred on the following dates: 26 June (-5.4 °C), 27 June (-2.0 °C), 9 July (-6.0 °C), 10 July (-3.4 °C) and 4 August (-1.6 °C). A drought period was observed again during August in that year. Contrasting with the 1993`s results, the grain yield of the first and second planting periods was significantly higher than the third and fourth planting periods for both genotypes. Finally, in the 1995`s growing season, no frost has occurred. However, a drought period was observed again during August 1995. There was no significant difference among the planting periods, indicating that a drought period, without frost may occur without lowering grain yield. With exception of the 1993`s experiment, test weights average values were significantly superior in the first planting periods of the 1992`s, 1994`s and 1995`s experiments. It was also observed during the four year experiment, that as the planting period was progressively delayed, plant heights were shorter and days to maturity were reduced for both genotypes.

Conclusion. In conclusion, there has been great weather variability among years in the conditions of South Brazil. Therefore, it is difficult to indicate a particular planting period for a particular genotype. Although the response of planting period has varied over years, planting different genotypes in different planting periods is one of the most appropriate and cost free management strategies for achieving stable oat yields in Southern Brazil.

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THE VARIATION OF ENDOGENOUS HORMONES OF BARLEY ANTHER IN THE PROCESS OF COLD PRETREATMENT

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INTRODUCTION. It has been reported that cold pretreatment can largely improve barley anther response frequency. But little information was available to account for the mechanism of cold pretreatment. We assayed the variation of endogenous hormones of barley anther in the process of cold pretreatment using ELISA(Enzyme Linked Immunabsorbent Assay) method in order to inquire into the effect of cold pretreatment in barley microspore development from gametogenesis to sporogenesis.

MATERIALS AND METHODS. Spikes of barley in which the pollen was in the mid to late uninucleate stage were harvested and then cold-pretreated at 4 ℃ in a refrigerator for 0,7,14,21.28 days respectively. We assayed endogenous hormones of barley anther using ELISA method.

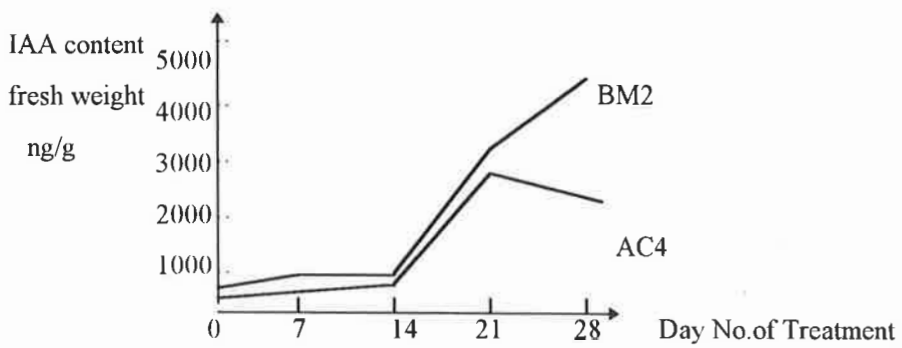
RESULTS AND DISCSSION.

Table1. Response Frequency of Barley Anther After 0,7,14,21,28 Day Cold Pretreatment

Genotype	Days of Cold Pretreatment				
	0	7	1.4	21	28
AC4	1.1	1.3	8.2	37.6	38.2
BM2	3.6	8.8	9.3	36.2	30.8

From the data of table 1, we can see that three-four week cold pretreatment is necessary to barley anther culture.

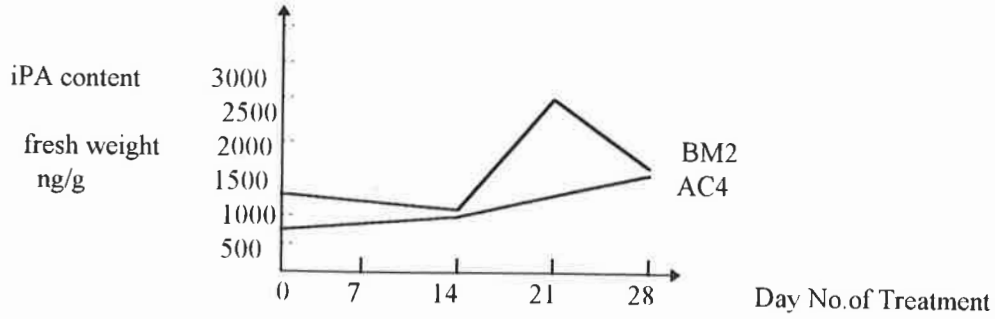
Figure1. Variation of Endogenous IAA



IAA content in anthers of BM2 and AC4 increase during the cold pretreatment.

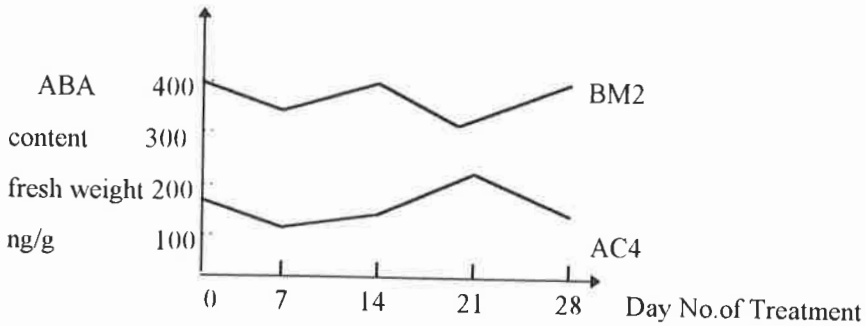
It increased slowly in first two weeks. When it goes to the third week, the content of IAA in BM2 and AC4 increases apparently.

Figure2. Variation of Endogenous iPA



The content of iPA in anthers of AC4 and BM2 is different at beginning and there is also difference on dynamic curves of cold pretreatment time, the variation of iPA is the greatest from the second to the third week, particularly in BM2.

Figure 3. Variation of Endogenous ABA



The content of endogenous ABA varied stable with the time. There was quite difference of ABA content between the two genotypes. ABA content of BM2 is much higher than that of AC4

There have been many articles about cold pretreatment. From the data of these acticles we can see that three - four week's cold pretreatment is essential to most barley genotypes. In our experiment the variation of IAA, iPA increased violently at the third week. So we suggested a probable mechanism of cold pretreatment: It changes the endogenous IAA and iPA in anthers and promotes

pollens to develop from gametogenesis to sporogenesis. In anther culture, it appears to be the increasing of response frequency.

In tissue culture, different types and supplements of exogenous hormones are very important. A great deal of work has been done in this field and many experience have been known. Relatively, the research about endogenous hormones is less than that of exogenous hormones in plant tissue culture. If we study exogenous hormones in coordination with endogenous hormones, it will be probably easier to regulate plant tissue culture artificially at hormone level.

Development of barley and wheat genotypes as a function of soil acidity. J.R. BEN; G. ARIAS. EMBRAPA, National Wheat Research Center (CNPT), P. O. Box 569. 99001-970 Passo Fundo, RS, Brazil.

Introduction. The production of barley and wheat in acid soils of southern Brazil is limited by their susceptibility to Al. The acid tolerance of cereal species is not the same and barley is more sensitive than wheat (Minella, 1989). The objective of this experiment was to evaluate the behavior of barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), and durum wheat (*Triticum turgidum durum*) in relation to soil acidity conditions.

Material and methods. The experiment was carried out in a greenhouse at EMBRAPA-CNPT, Passo Fundo, RS, Brazil, in a dark red dystrophic latosol (Haplorthox). The soil was treated with four lime levels: 0, 25, 50 and 100 % of the recommended rate to reach pH 6.0 (12.6 t/ha). Plastic pots containing 5 kg of soil were used. The fine milled lime and the air-dried soil were mixed and incubated at a moisture level of approximately the field capacity for three weeks prior to fertilizer application. All the treatments received the same NPK fertilization. The soil pH values in water varied between 4.4 without liming, and 5.7 in soil limed with 12.6 t/ha. Exchangeable Al varied from 2.33 in unlimed soil to 0.05 $cmol_c/L$ soil (Table 1). The genotypes tested were the Brazilian tolerant wheat, cv BR 35 and the susceptible Mexican cv Anahuac, durum wheat cv Altaika, the Brazilian barley cvs. FM404, MN599, Volla and the barley lines PFC8026, FC85104 and Volla/Dayton (Minella, 1989). Plant height, as well as shoot and root dry matter were determined at anthesis.

Results and discussion. The sensibility to acid soil of the susceptible wheat genotype Anahuac, durum wheat (cv Altaika) and all barley genotypes, was indicated by the low dry matter production in unlimed soil, in relation to the production in limed soil (Table 2). The tolerant wheat BR35 produced in acid soil 41 % of the dry matter produced in limed soil. The susceptible cv Anahuac reached in acid soil 4 % of the production in limed soil whereas the durum wheat, cv Altaika, 1 % only. The barley genotype FM404 produced in the no-lime treatment 14 % of the dry matter produced in the 12.6 t/ha treatment. The most susceptible line, PFC8026, produced at the no-lime rate only 3 % of the dry matter produced at the highest lime rate. The height of plants was also affected by soil acidity.

Conclusions. Barley genotypes were much more susceptible to Al than tolerant wheat BR35. The results show a better adaptation of Brazilian barley cvs than the susceptible wheat (cv Anahuac) or durum wheat (cv Altaika) with exception of line PFC8026.

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Table 1. Soil analyses of the soil after the application of four lime levels

Lime levels (%)[1]	pH H_2O	Al^{2}	Ca^{2}	Mg^{2}	P^{3}	K^{3}	Organic Matter[4]
		------$cmol_c$/L--------			--- mg/L ----		--%---
0	4.4	2.33	1.5	0.9	10.4	154	3.3
25	4.7	0.80	2.8	2.2	12.1	163	3.3
50	5.1	0.19	4.5	3.4	9.1	114	3.5
100[5]	5.7	0.05	5.9	4.7	9.3	138	3.1

[1] Percentage of lime to increase soil pH in water to 6.0, according to SMP buffer solution method.
[2] Exchangeable with KCl 1 mol/L.
[3] Mehlich-I extractant.
[4] Wet combustion.
[5] 12.6 t lime/ha.

Table 2. Dry matter of plants and roots and plant height in unlimed soil as percentage of the 12.6 t/ha limed soil treatment

Genotypes	Shoot dry matter	Root dry matter	Plant height
	----------(%)----------		(cm)
Wheat			
BR35	41	53	88
Anahuac	4	11	42
Durum Wheat	1	2	10
Barley			
FM404	14	24	64
MN599	12	20	58
Volla/Dayton	13	23	64
Volla	11	10	55
PFC85104	8	17	39
PFC8026	3	5	37
Blocks	ns	ns	ns
Genotype (G)	**	**	**
Liming (L)	**	**	**
(G x L)	**	**	**
CV (%)	15	46	9

ns, not significant.
** significant at 1 % probability level.

Phenology for Optimal Barley Production in South-Eastern Australia.

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Introduction. Australian barley breeding programmes for improved malting quality rely heavily upon the use of parents from Japan, North America and Europe. These parents are generally unsuitable for Australian conditions, due to low and unstable yields and small grain size in this environment; grain size is one of the most important parameters in determining malting quality. Explanation for this poor performance is hindered by a lack of phenological information. The objectives of this study were to determine differences in response to temperature and photoperiod of important parental genotypes, adapted cultivars and promising advanced breeding lines using time of sowing experiments. These studies will allow the identification of parents most suitable for Australian breeding programmes, the development and employment of suitable agronomic practices (to ensure yield stability and uniform, large grain size) during selection and possibly the identification of genotypes suitable for niche-production areas within Australia. Additionally, this study will aim to increase the understanding of the importance of the three pre-anthesis phases in determining yield and grain size.

Materials and Methods. Fifteen cultivars from a range of geographic origins, representing the various sources of germplasm used by the breeding program, were studied during the first year of these investigations but for brevity only a small sub-set of these data from 6 cultivars is presented. This sub-set includes Arapiles and Schooner, which are the dominant malting cultivars in Victoria, Skiff, which is a high yielding, semi-dwarf, feed cultivar in southern Australia, and Harrington, Haruna Nijo and Chariot which are important representative malting cultivars from Canada, Japan and the UK respectively. Data presented here were collected from field and pot experiments conducted in 1995 at Horsham (36°40' S, 142°18' E). Field experiments were sown on 9 May, 2 June and 15 August. Phenological development and the time of awn emergence was recorded and an automatic weather-station recorded appropriate climatic data. The pot experiments sown on 19 July (sow time 1) and 4 Sept (sow time 2) used natural and an 18 hr daylength (natural daylight supplemented with 200 W incandescent bulbs) treatment to determine photoperiod response.

Results. The comparative photoperiod and temperature responses were determined from the pot experiment at Horsham (Table 1). Results from the pot experiment showed that Harrington and Chariot have a medium and Haruna Nijo a weak photoperiod response while the locally adapted cultivars Schooner and Arapiles have a medium to strong response. There was a weak response to temperature in both Harrington and Chariot, a medium response in Haruna Nijo and Schooner, while Arapiles and Skiff had a strong response.

The period from sowing to double ridge (DR) in day-degrees (°C days) decreased as time-of-sowing (TOS) was delayed in Haruna Nijo, but in the other five cultivars TOS 2 was equal to, or greater than, for TOS 1 and 3, although the differences were not always significant (Fig. 1a). There were small and sometimes significant differences between the cultivars, which were most marked in TOS 3; Haruna Nijo had the lowest values for all sowing times. The duration from DR to the attainment of maximum spikelet primordia number (MPN) was more strongly effected by TOS than the other phases of development, with the duration declining markedly with each successive TOS (Fig. 1b).

Table 1: Day-degrees from sowing to awn emergence (AE) at Horsham using two day lengths (Natural vs 18hr) and two sowing times (19 July, 4 Sept). Temperature responsiveness is indicated by the difference between time to AE between sowing times under 18 hr day length. Photoperiod responsiveness is indicated by the difference between time to AE at different day lengths for each time of sowing.

Sowing time	Day-degrees (S - AE)											
	Harrington		H. Nijo		Chariot		Arapiles		Schooner		Skiff	
	Nat	18hr	Nat	18hr	Nat	18hr	Nat	18hr	Nat	18hr	Nat	18hr
1	1137	968	1064	925	1169	979	1135	968	1084	904	1092	948
2	992	846	878	775	998	866	910	751	831	726	831	718

The rate of development in the Japanese variety Haruna Nijo was generally significantly faster than all other cultivars for all phases of development. During the period from MPN to awn emergence (AE) the response to TOS varied between cultivars with the duration increasing (Harrington) and decreasing (Haruna Nijo, Arapiles and Schooner) as TOS was delayed; with Chariot the period declined from TOS 1 to 2 but increased from TOS 2 to 3 (Fig. 1c).

Table 2: Days from sowing to awn emergence at Horsham field experiment for three times of sowing (9 May, 2 June and 15 August).

Sow time	Cultivars					
	Harrington	H. Nijo	Chariot	Arapiles	Schooner	Skiff
1	127.0	111.0	137.0	138.0	132.0	136.0
2	121.0	104.0	122.5	123.0	120.5	121.5
3	78.0	66.0	81.5	74.0	71.0	70.0
l.s.d. ($P< 0.05$) Sowing time x cultivar =1.5						

The days from sowing to AE declined as TOS was delayed in all six cultivars. Haruna Nijo had the lowest values at all TOS. There were significant differences between all cultivars for TOS 1 and 3. Three cultivars had similar values for TOS 3, although there were significant differences between some of cultivars (Table 2).

Discussion. The general rainfall pattern in south-eastern Australia is variable and the timing of adequate rainfall to allow sowing to start can range from mid April to late July. The developmental patterns of Arapiles, Schooner and Skiff, associated with relatively large responses to photoperiod and temperature, provide a degree of "phenological flexibility". For example, despite the first and second sowing times being 24 days apart, awn emergence occurred over a period of only 12 days for the cultivar Schooner; this contrasts with Harrington for which awn emergence was over a 17 day period.

Figures 1a - 1c: Comparative lengths of the three pre-anthesis developmental phases for 6 cultivars; Hgt=Harrington, H. Nijo=Haruna Nijo, Chrt=Chariot, Ara=Arapiles, Sch=Schooner, Skf=Skiff.

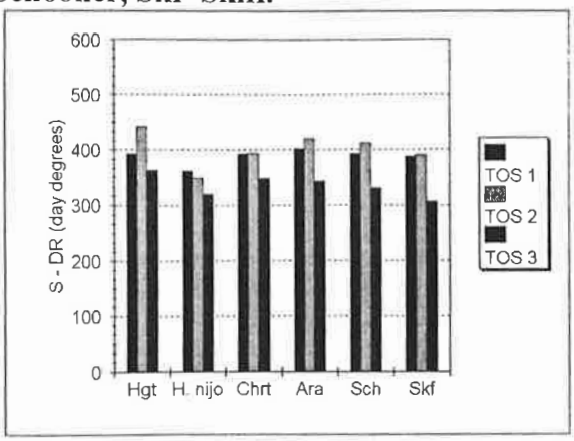

Figure 1a: Comparative developmental time from Sowing to DR

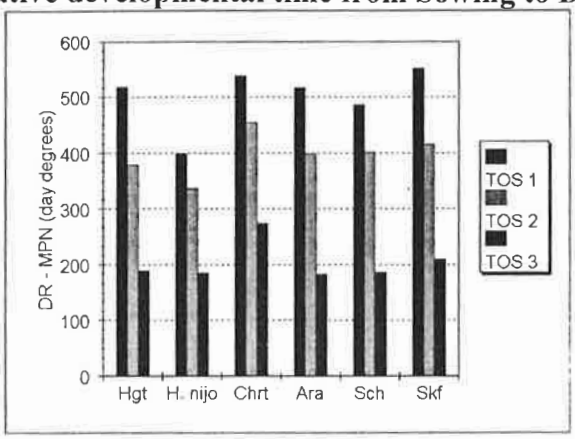

Figure 1b: Comparative developmental time from DR to MPN

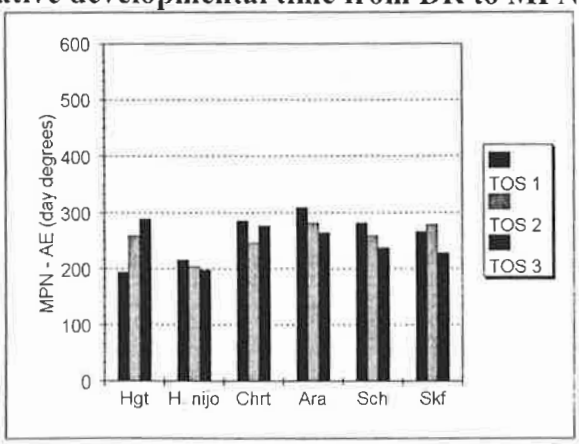

Figure 1c: Comparative developmental time from MPN to Awn Emergence

Conclusions. Cultivars adapted to the local environment in south-eastern Australia have medium to high responses to photoperiod and temperature, giving greater flexibility of sowing time. Results suggest the developmental phases from DR to AE for the Japanese cultivar are too short for high yields to be obtained in south-eastern Australia. The English cultivar Chariot has a developmental response similar to the commercially grown Australian cultivars. However, the relatively low photoperiod and temperature responses of Chariot reduced the phenological flexibility of this cultivar.

Acknowledgements. The project is funded through an Agriculture Victoria AFI grant.

PATH-COEFFICIENT ANALYSIS FOR GRAIN YIELD AND RELATED CHARACTERS UNDER SEMIARID CONDITIONS IN BARLEY.

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INTRODUCTION

Barley is an important agricultural commodity in Turkey where it is mostly grown under drought stress conditions. Therefore high and stabile yield capacity under low-rainfall environments is a major task of barley breeding programs. Although there is no increase in biological yield of barley bred in this century for normal yielding environments, an increased biological yield under stress conditions may improve yielding capacity. And a better partitioning of the biomass to grain yield (harvest index) should result better economic yield for barley. In order to test this idea, a two-year path-coefficient study was initiated to determine the contribution of biological yield, harvest index and other yield related characters to grain yield under semiarid conditions.

MATERIALS AND METHODS

Studies were conducted in Urkutlu, Burdur province, Turkey in 1993 and 1994. In the region, a Mediterranean type precipitation distribution prevails, which is totally 429 mm as long-term average. In the first year, a randomized complete blocks design was used with two replicates, sown in 24-25 March 1996 including 49 advanced lines in F5, selected for early heading. Each plot consisted of 1 row of 1 m long and 30 cm apart. Sixty seeds per rows were sown. In the second year, 81 entries were sown on 29th October grown in single plots of 4 m^2. At both season biological yield, grain yield, plant height, 1000 grains weight, grains per spike and days to heading were observed and then harvest index was calculated according to the formulae (grain yield/total biomass) x 100. Simple correlation coefficients among grain yield and related characters measured were calculated and then the path-coefficient technique was performed as suggested by Dewey and Lu (1).

RESULTS AND DISCUSSION

Phenotypic correlation coefficients between grain yield and related characters are shown in Table 1. It is clearly seen that grain yield was positively and significantly ($p < 0.01$) correlated with biological yield, harvest index and 1000 grains yield in both years, and with plant height in one year, 1994. It should be noted that correlation coefficients were higher in the first year than in the second year since the second was the better environment, permitting to the some lines to perform better, which gave rise to higher variances among the lines and the higher variances caused the higher covariances.

However, correlation coefficients measures the relationship of any two traits, so it may not give a real scheme of the traits association. Path-coefficient analysis offers a means of separating the correlation coefficient to direct and indirect effects, and thus gives us an

accurate idea about the most contributing character to grain yield so that estimation of the relative importance of each character will be possible (1-4). Regarding the results of path-coefficient analysis in Table 1, biological yield and harvest index had the two greatest positive and direct effects on grain yield in both years. Since direct effects of these components on grain yield are almost equal to their correlation coefficients with grain yield, selection for biological yield and harvest index should be effective and should improve yielding capacity under semi-dry conditions.

Plant height and harvest index had positive and indirect effects on grain yield via biological yield. Hence, plant height might be useful to select indirectly for high biological yield where data for biomass itself are unavailable. On the other hand, days to heading had the negative direct effects on grain yield and negative and quite high indirect effects, via biological yield, on grain yield, showing importance of early heading under semi-dry conditions in order to increase biological yield and also harvest index. Although the greatest increase in grain yield has been due to an increase in harvest index in cool season cereals, further improvements through harvest index may be limited. Hence, increasing biological yield should lead to increased grain yield in barley while maintaining harvest index. We found that early heading lines produced high biomass and quite high harvest index. While 1000 grain weight had positive and indirect contribution to grain yield via biological yield, the direct and indirect effects of number of grains on grain yield were inconsistent between the two years.

It was found that grain yield of barley was highly correlated with biological yield, harvest index, 1000 grain weight and plant height under drought stress conditions; and first two components had strong direct effect on grain yield, suggesting that the grain yield capacity under drought stress conditions may be predicted by these components.

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Table. Path-coefficient estimates for grain yield and related characters under semi-dry conditions in two years (1993, 1994).

Components	1993		1994	
	Path Coefficient	Percent	Path Coefficient	Percent
Grain yield vs plant height				
Direct effect	-0.029	13.8	-0.087	13.3
Indirect effect via biological yield	0.161	77.1	0.363	55.4
via harvest index	-0.004	1.7	0.130	19.9
via days to heading	-0.001	0.5	0.026	3.9
via grains/spike	0.010	5.0	0.005	0.9
via 1000 grain weight	0.004	1.9	0.044	6.7
TOTAL	0.142		0.480**	
Grain yield vs biological yield				
Direct effect	0.779	86.3	0.725	71.9
Indirect effect via plant height	-0.006	0.7	-0.044	4.3
via harvest index	0.103	11.4	0.205	20.3
via days to heading	0.001	0.1	-0.016	1.6
via grains/spike	0.004	0.5	-0.003	0.3
via 1000 grain weight	0.010	1.1	0.016	1.6
TOTAL	0.891**		0.916**	
Grain yield vs harvest index				
Direct effect	0.434	68.3	0.413	49.5
Indirect effect via plant height	0.000	0.0	-0.028	3.3
via biological yield	0.185	29.1	0.360	43.1
via days to heading	0.001	0.2	-0.006	0.7
via grains/spike	-0.003	0.4	0.004	0.5
via 1000 grain weight	0.013	2.0	0.024	2.9
TOTAL	0.630**		0.766**	
Grain yield vs days to heading				
Direct effect	-0.007	3.5	-0.100	31.1
Indirect effect via plant height	-0.005	2.5	0.022	7.0
via biological yield	-0.089	46.6	-0.119	37.1
via harvest index	-0.074	38.7	0.025	7.8
via grains/spike	0.013	6.9	0.020	6.3
via 1000 grain weight	-0.003	1.8	-0.034	10.7
TOTAL	-0.164		-0.185	
Grain yield vs grains/spike				
Direct effect	0.022	8.8	0.037	17.1
Indirect effect via plant height	-0.014	5.6	-0.012	5.7
via biological yield	0.154	62.5	-0.056	25.8
via harvest index	-0.050	20.3	0.044	20.3
via days to heading	-0.004	1.6	-0.054	24.9
via 1000 grain weight	0.003	1.1	-0.014	6.2
TOTAL	0.110		-0.055	
Grain yield vs 1000 grain weight				
Direct effect	0.041	11.1	0.066	12.8
Indirect effect via plant height	-0.003	0.8	-0.058	11.4
via biological yield	0.192	51.8	0.177	34.7
via harvest index	0.133	35.9	0.150	29.4
via days to heading	0.001	0.2	0.052	10.2
via grains/spike	0.002	0.4	-0.008	1.5
TOTAL	0.365**		0.379**	

*,**: Statistically significant at 0.05 and 0.01 significance level, respectively.

Light effects on the accumulation of a chloroplast cold-regulated protein. C. CROSATTI, M. GROSSI, F. RIZZA, E. GIORNI and L. CATTIVELLI, Experimental Institute for Cereal Research, Section of Fiorenzuola d'Arda, I-29017, Via S.Protaso, 302, Fiorenzuola d'Arda (PC), Italy

It is well known that light has many effects on the plant life, and the expression of many genes is altered by its presence or absence. We are interested in elucidate the relationship between light and the expression of a specific barley gene induced by low temperatures (*pt59* - Cattivelli and Bartels, 1990) which encodes for a cold-regulated protein of 14 kDa (COR14) localized in the stroma of the chloroplasts. In *H. vulgare* the COR14 antibody cross-reacts with two proteins with sligthly different relative molecular weight around the marker of 14.4 kDa refered to as COR14a and COR14b (high and low relative molecular weight, respectively). In a collection of *H. spontaneum* genotypes a clear polymorphism was found for the corresponding COR proteins. While some accessions showed the same COR pattern as cultivated barley, in 38 out of 61 accessions examined, the COR14 antibody cross-reacted with an additional cold-regulated protein with a relative molecular weight of about 24 kDa (COR24). The accumulation of COR24 was often associated with the absence of COR14b; the relationship between the COR14b/COR24 polymorphism and the adaptation of *H. spontaneum* to different environments is discussed. By studying the COR14 accumulation in cultivated barley we have found that the threshold induction-temperature of COR14a is associated with the loci controlling winterhardiness. The association was demonstrated by using either a set of 30 cultivars of different origin, or two sets of frost-tolerant and frost-sensitive F_1 doubled-haploid lines derived from the cross Dicktoo (winter type) X Morex (spring type). These results suggest that the threshold induction-temperature of COR14a can be a potential biochemical marker for identification of superior frost-resistant barley genotypes (Crosatti et al., in press).
The role of the light was tested by comparing plants grown and hardened under standard and non-standard photoperiods. While the transcription of the *pt59* gene and the translation of the corresponding mRNA occur only at low temperature, the light markedly stimulates gene expression and is needed for the protein's accumulation (Crosatti et al., 1995). Plants only needs to be exposed to light for a short time (i. e. 5 min) to induce *pt59*-corresponding mRNAs when the temperature is low. This fact indicates that the expression of *pt59* mRNA is mediated by a light-regulated factor and that even a 5-min light exposure is enough to induce this factor to such an amount as to normalize the gene's expression. A brief exposure of plants to light ensures normal *pt59* expression but is not enough to determine normal COR14 accumulation. This suggests that the presence of the light, or the chloroplast activity, influences, beside gene expression also the accumulation of the protein.

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The agricultural value of barley cultivar mixtures and of different species mixtures containing barley and oats in disease control.

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Introduction. The use of cultivar and species mixtures of cereals can provide an-ecologically sound and cheap method of disease restriction. (Wolfe 1985,Czembor,Gacek 1996). Disease control in this way can lead to an improvement both in yield and in yield stability compared with the performance of pure stands.(Gacek et al.1996a,b). In addition to restriction of diseases there is potential in mixed stands for reduction of the negative effects of other stresses on crop plants, such as abiotic stress and insect and weed infestation.(Czembor,Gacek 1996). Currently, based on the seed sales it is calculated that at least 6-8% of the total barley growing area in Poland is sown with cultivar mixtures. Even more popular than cultivar mixtures are species mixtures. Currently, about 1.3 Mio hectares of cereal species mixtures are grown. About 80% of these are barley oats mixtures, and the rest are three - species mixtures (barley + oats + wheat).

In addition to cereal species mixtures, cereal - legume mixtures are becoming increasingly important in Poland.

The mixture area increased rapidly during the last decades without support from science and breeding.

Over the past decade, a number of experiments with barley cultivar mixtures and cereal species mixtures have been investigated for their effect on disease development and on grain yield and its stability over environments.

Materials and Methods. In the experiments commercial cultivars of barley were studied in pure stands and in different mixture compositions. Experiments were performed at 3-4 sites, in four replicates. Experimental plots, usually of 10 m^2 size were interspaced with border plots of the same size in order to reduce inoculum interference.

In the experiments with cereal species mixtures, two-component mixtures (Barley + oats), and three-component mixtures (barley + oats + wheat) were studied to investigate their influence on disease development and on grain yield. Whereas, in the experiments with cereal - legume mixtures, different combinations of barley, wheat and oats with peas were studied, to investigate their effect on disease, weed and insect infestation and on final grain yield.

Results and Discussion. The investigations of genetically diverse mixed stands of cereals have confirmed their great effectiveness in restraining development of major foliar diseases of cereals.
the observed disease reductions in different cultivar and species mixtures, compared to their levels on the mixture components grown in pure stands, are presented in Table.

Table. Disease reductions in cereal mixtures

Mixture type	Barley powdery mildew relative to pure stands	Effects on non-target diseases and pests
Cultivar mixtures		
- Spring barley	max. 45 - 60%	Net blotch, Scald, Brown rust
- Winter barley	max. 30 - 50%	Scald
Species mixtures		
- cereals	max. 65 - 75%	Different cereal foliar diseases
- cereals + legumes	max. 70 - 75%	Different cereal foliar diseases, insects, weeds

Disease restrictions in mixed stands depended on the range of their genetical diversity and on the overall disease level in the growing period. Large - scale use of barley cultivar mixtures has confirmed the effectiveness of mixtures in restraining development of cereal foliar diseases. The reductions were greater and more stable in species mixtures than in the cultivar mixtures.
We expect that the species mixture benefits could be greater if the mixing was more controlled and scientifically supported.
Mixtures in most cases gave higher and more stable yields than their individual components grown in pure stands.(Wolfe 1985). In our experiments yield increases in the best mixtures were 6,2% (winter barley), 7,1% (spring barley) (Czembor,Gacek 1996, Gacek et al. 1996a,b).
It is worth to note, that yield increases of spring barley were around 9-10% on large-scale demonstration fields (ca. 1 ha).
Generally, mixtures gave more stable yields, than their components grown in pure stands. The coefficient of variance (CV%) was used as a

stability parametr in our experiments. The CV´s for winter barley cultivar mixtures (1,4 - 8,9%) were lower than those for their component cultivars grown in pure stands (4,1 - 11,7%).(Gacek et al.1996a). The use of cultivar and species mixtures is widely accepted in Poland as an inexpensive, simple and environmentally safe way for cereal disease control.

Current large-scale development of mixtures in Poland needs to be supported from science and breeding. Attempts have been made to breed and select components specifically for use in cultivar and/or species mixtures. The main mixture pre-breeding objectives are: (i) breeding of cultivars/lines with different disease resistance genes, (ii) production of lines with good "mixing abilities", (iii) development of earlier and shorter lines of wheat and oats, (iv) breeding lines,that when grown together, are complementary for uncontrollable environmental variables.

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Starch, Protein and Beta-glucan Accumulation During Grain Development in Oat.
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Introduction. The mixed linkage (1→3), (1→4)-β-D-glucan (beta-glucan) component of oat grain are of particular importance because of the hypocholesterolemic effects it imparts to humans when it is incorporated into the diet (Peterson 1992). One goal of this research program is to determine genetic and cultural approaches to increasing the beta-glucan concentration in oat. It was important to know the developmental sequence to the deposition of beta-glucans relative to other storage products in the developing oat kernel. In this study, two cultivars of oat were grown in the field and groats were analyzed for composition at progressive stages of development.

Materials and Methods. Oat (*Avena sativa* L.), cultivars Marion and Premier, two cultivars known to be high in beta-glucan, were grown in Fargo North Dakota, USA during the 1994 growing season. A randomized complete block experimental design with 3 replications was used. Anthesis was considered the time when at least half of the spikelets had shed pollen. At each harvest time (7, 14, 21, 28, and 35 days after anthesis), three panicles from each replicate were harvested and all of the primary spikelets were dehulled by hand. Groat weight and moistures were determined from groats pooled from the three panicles. Groat samples were lyophylized and ground to a fine powder. Starch was determined by a procedure used by Doehlert (1994). Protein was determined by combustion analysis with a Leco Nitrogen Analyzer[1] (Leco Corportion, St Joseph, MI). Beta-glucan was determined enzymicly, by the method of McCleary and Glennie-Holmes (1985).

Results. Both Marion and Premier oats exhibited a linear increase in dry weight from 7 to 28 days after anthesis, afterwhich no further significant dry matter accumulation occured. Groat fresh weights also increased from 7 to 28 days, but decreased substantially after 28 days (Fig. 1). Moisture content of groats decreased linearly from 7 to 35 days after anthesis. Dry matter accumulation in both genotypes ceased when percentage moisture went below 35%. Mature groat dry weight was greater in Marion than in Premier, and because the grain fill period was similar, the rate of dry matter accumulation per groat was greater in Marion than in Premier.

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[1]Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA or imply approval to the exclusion of other products that may also be suitable.

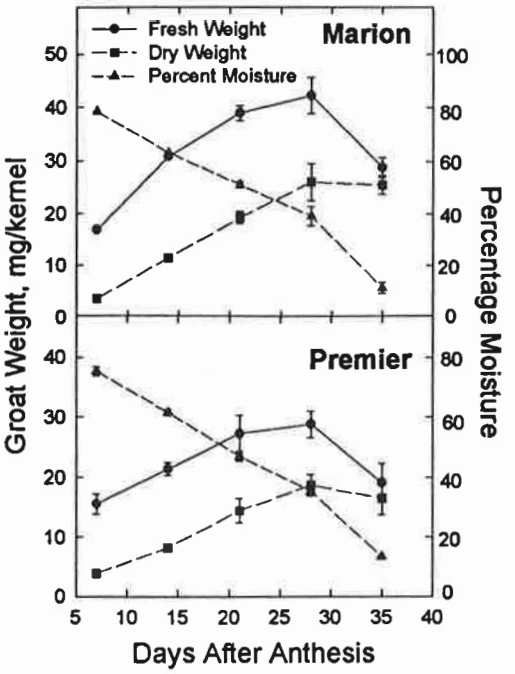

Figure 1. Accumulation of fresh and dry weight and moisture loss during development of Marion and Premier groats.

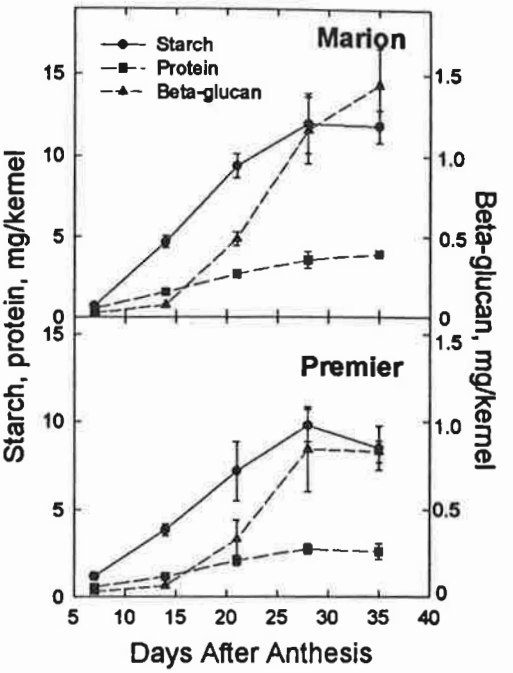

Figure 2. Accumulation of starch, protein,and beta-glucan during development of Marion and Premier groats.

Starch and protein content in developing groats of both Marion and Premier increased linearly from 7 to 28 days after anthesis (Fig. 2), but the pattern of beta-glucan accumulation was quite different. From 7 to 14 days, very little beta-glucan accumulated, but the rate of beta-glucan accumulation increased during the period from 21 to 28 days after antheses for both Marion and Premier oats. Much less beta-glucan accumulated in the final interval, 28 to 35 days after anthesis (Fig. 2).

Discussion. The results indicate a different pattern of accumulation of beta-glucan than that of starch and protein. Starch and protein contents appear to increase linearly in developing groats from 7 to 28 days (Fig. 2), corresponding well with the period of dry matter accumulation (Fig 1). Beta-glucan accumulation occurred primarily in the late stages of development. Åman *et al.* (1989) also reported that most of the oat kernel dry matter is deposited before the majority of beta-glucan accumulation occurs. Because beta-glucan accumulation occurs primarily late in development, it is likely that environmental stress that might lead to an early end to grain development would affect beta-glucan more than other storage components. A study by Brown *et al.* (1970) indicated a biphasic pattern to dry matter and protein accumulation in developing groats, where the most rapid dry matter accumulation occured in the first 15 days after anthesis, then slowed in the later half of the development. Beringer (1971), Peterson and Smith (1976), Peterson *et al.*

(1985), and Saigo *et al.* (1983) have also published reports examining the developmental process in oat groats.

The results presented here suggest that grain development in oat may follow a pattern similar to that in maize, as reported by Doehlert *et al.* (1994). The early stage of development, where very little dry matter accumulates, is refered to as the lag phase. This is followed by a linear grain-fill stage, where dry matter accumulates linearly with time. This is finally followed by a dry down period, leading to kernel maturity. In oat, it would appear that the lag phase of development ended at about 7 days after anthesis, and the linear grain-fill phase continued until about 28 days after anthesis. In maize (Doehlert *et al.* 1994), dry matter accumulation also ended when moisture in kernels dropped below 35%, as observed in oats (Fig. 1). Moisture content of grain may be the best indicater of developmental stage. Moisture contents of over 75% would indicate lag phase, moistures of 35 to 75 % would indicate linear grain-fill, and moistures of less than 35% would indicate the dry-down phase or kernel maturity.

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Near-isogenic analysis of uniculm and conventional-tillering barley lines. S.M. Dofing, Palmer Research Center, University of Alaska Fairbanks, 533 E. Fireweed, Palmer, Alaska 99645, USA.

Introduction. The uniculm phenotype has been proposed as a component of a small grain ideotype that maximizes crop productivity (Donald, 1968). Uniculm plants do not produce late-developing tillers that delay maturity and harvest date. This is especially important in short-season or northern areas of production, where inclement weather or early snowfall could result in complete crop loss. Additionally, the presence of green spikes at harvest may reduce grain quality or require higher grain drying costs. This study was conducted to compare the performance of uniculm and conventional-tillering near-isogenic barley lines that differed by the presence of the uc_2 (uniculm) gene.

Materials and Methods. The experimental material consisted of two pairs of near-isogenic barley lines differing in tillering type, caused by the presence of the uc_2 gene. The first pair of lines was 'Kindred' and Uniculm Kindred, and the second pair of lines was 'Morex' and Uniculm Morex. Uniculm Kindred was derived from thermal neutron radiation of Kindred, while Uniculm Morex was developed by E.A. Hockett at Montana State University from the cross Uniculm Kindred/Morex, followed by six backcrosses of uniculm plants to Morex as the recurrent parent.

The lines were evaluated for three years (1993-1995) at Palmer, Alaska. Thirty-two treatments were evaluated in a 2 x 2 x 4 x 2 factorial. Treatments consisted of 1) uniculm and conventional-tillering types, 2) Kindred and Morex genetic backgrounds, 3) seeding densities of 50, 140, 230, and 320 kernels/m^{-2}, and 4) row spacings of 15 and 30 cm. Traits measured included maturity date, spikes m^{-2}, kernels/spike, kernel weight, and grain yield.

Results and Discussion. Averaged across genetic backgrounds, seeding rates, and row spacings, uniculm lines matured 70 growing degree days (0 C base temperature) than conventional-tillering lines (Table 1). This represents an economically significant difference of about six days in our location, an attractive advantage where early harvest is essential. Unlike conventional-tillering lines, the pattern of maturation of uniculm plants within plots was extremely uniform, due to the absence of late-developing tillers.

Grain yield of both uniculm and conventional-tillering lines was higher in narrow row spacing. Optimum seeding rate of uniculm lines was expected to be higher than that of conventional-tillering lines because of their lack of tillers. These higher seeding rates result in greater within-row interplant competition; therefore, it was speculated that uniculm lines might benefit relatively more from narrow row spacing. However, the relative yield increases due to narrow row spacing were approximately the same for both tillering types.

Performance of the uniculm and conventional-tillering lines at each seeding rate, averaged across genetic backgrounds, is shown in Figure 1. At all seeding rates, conventional-tillering lines had more spikes m^{-2} and kernels/spike. The pattern of response for the two types of lines was similar across seeding rates. Uniculm lines, however, had higher kernel weights,

especially at lower seeding rates. Grain yield, the product of the three yield components, was higher for the conventional-tillering lines at all seeding rates. Maximum grain yield of uniculm lines was only 52% of that of conventional-tillering lines. The higher kernel weight of uniculm lines was not sufficient to compensate for their lower spikes m^{-2} and kernels/spike.

These results show that uniculm lines, free of late-developing tillers, mature earlier and more uniformly than their conventional-tillering counterparts. However their grain yield is unacceptably low, even at higher seeding rates. The two pairs of lines used were developed without selection, and it is possible that higher-yielding uniculm lines could have been developed by selecting for high grain yield or kernels/spike. However, large gains would be required to raise their yield to acceptable levels. It should be noted that, because of the economic benefits associated with early maturity in northern areas, growers would likely accept lower grain yields than those from existing cultivars, provided that the yield reduction was not too large.

Table 1. Performance of two pairs of uniculm vs. conventional-tillering near-isogenic lines grown at four seeding rates and two row spacings at Palmer, Alaska, during 1993-1995.

Parameters evaluated	GDD to maturity	Spikes per square meter	Kernels per spike	Kernel weight	Grain yield
		--------no--------		mg	Mg ha^{-1}
Tillering type					
Uniculm	1277	130	35.5	49.3	4.57
Conventional	1347	272	45.0	43.9	1.95
Tillering type (spacing)					
Uniculm (15 cm)	1278	130	36.0	50.0	2.09
Uniculm (30 cm)	1276	130	35.0	48.5	1.81
Conventional (15 cm)	1349	282	45.1	44.2	4.88
Conventional (30 cm)	1344	262	45.0	43.6	4.27
$LSD_{0.05}$	5	5	0.5	0.3	0.08

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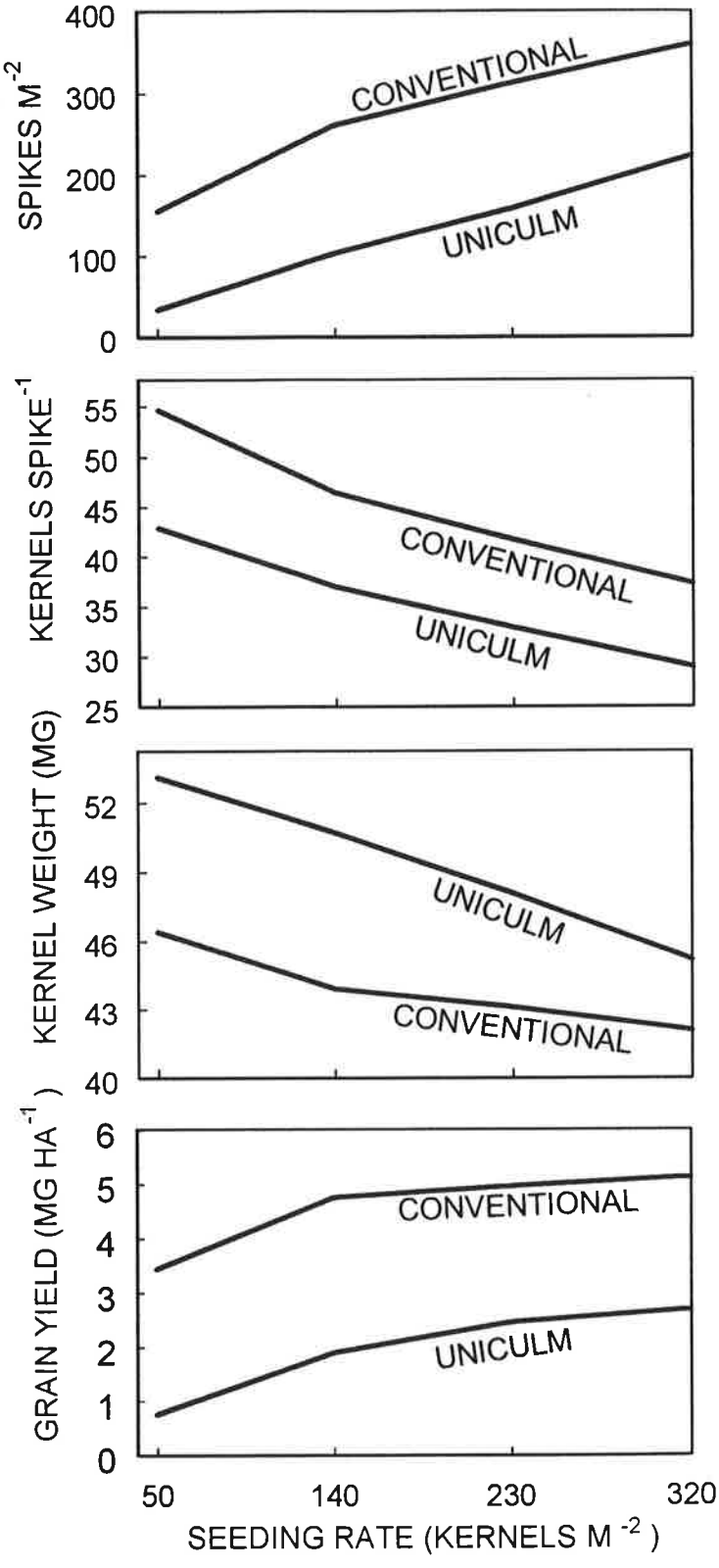

Figure 1. Performance of uniculm and conventional-tillering near-isogenic lines grown in Palmer, Alaska, between 1993-1995.

Comparison of Two and Six Row Barley Genotypes in Dry Areas in Syria

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Introduction

Several studies have been done to compare two and six row barley genotypes under good conditions.

In Australia it has been found that six rows have a higher grain yield, better lodging resistance, higher harvest index than two rows, but they were shorter and had lower 1000 kernel weight and hectoliter weight. (Portmann et al., 1991).

Jestin (1985) showed that six rows yielded more than two rows, but two rows had lower fertility which was compensated by higher tiller number, and larger 1000 kw. In a study done in France, Gaius (1991), showed that six rows winter barley yielded more, were taller, had longer awns, and more seed/spike/ m^2 in comparison with two rows, but two rows had higher 1000 kw, sheath number and rows in m^2.

For rapid growth, six rows are well adapted to short growing season (Kivi, 1977). This study shed some light on comparison between two rows and six rows barley genotypes in low rainfed barley areas in Syria.

Materials and Methods

This study has been carried out in Al Mogargi Research Station (Al Hasaka Province) north-east of Syria (annual rainfall of 261mm) in 1989-1990 (186 mm rainfall) and in 1990-1991 (190 mm rainfall), in an area characterized by low rainfall bad distribution, and frost.

72 barley genotypes were studied in the first season, and 178 (including the 72 tested the first year) in the second season: half of genotypes were two rows and half six rows. All the genotypes were spring type.

Each genotype was sown in six rows plots, 3m lenght, 25 cm inter rows spacing. Field observation were taken on grain yield (Kg/ha), plant height (cm), number of days to heading and maturity, and 1000 kernel weight (g).

Results and Discussion

Table 1. Grain yield, 1000 kernel weight, plant height, days to heading and maturity for two rows and six rows barley genotypes in Almogargi research station 1989 - 1990.

Row type	Grain yield	1000 kw	plant height	days to heading	days to maturity
two row	662	39.7	32	117	159
six row	585	35.6	33	120	160
Sign. of diff.	.05	.01	n.s.	.05	n.s.

Table 1 shows that two rows has higher in Grain yield,1000 K.W and 3 days earlier in heading than six rows.

Table 2. Grain yield, 1000 kernel weight, plant height days to heading and maturity for two rows and six rows barley genotypes (178 entries) in Almogargi research station 1990-1991.

Row Type	Grain yield	1000 K.w	plant height	days to heading	days to maturity
tow row	500	31.2	32.6	82	112
six row	320	24.8	28	84	113
Sign. of diff.	.01	.01	.01	.01	.05.

Table 2 shows that two rows was superior in terms of grain yield, and 1000/K W, and earlier in heading and maturity in comparison with six rows.

Fig I : Friquences distribution of plant hight shows that 48.4% of 2 RT genotypes were (tall to modereate tall) , to 75 % of 6 RT (1989-1990). In (1990 - 1991) 32.6% of 2 RT gehotypes were (tall to modureate tall), to 12.2 % of 6 RT.

Fig II Friquences of 1000 kernel weight shows that 92.4 % of 2 RT genotypes were (high to modereate high) 34-50/g , to 62.9% of 6 RT (1989-1990). In (1990-1991) 37.5% of 2 RT genotypes were (high to modereate high), to 25% of 6 RT .

Fig III Friquences of grain yield shows that 67% of 2 RT genetypes were (high to modereate high) 600-1000 g, to 49.9% of 6 RT (1989-1990). In (1990-1991) 69.9% of 2 RT were (high to wodereate high) , to 40.4 % of 6 RT .

So using 2 RT barley genotypes in breading program give a good shance to emprove; he yield, 1000 kernel weight, earley heading, and plant height more than 6 RT in dry ereas in Syria.

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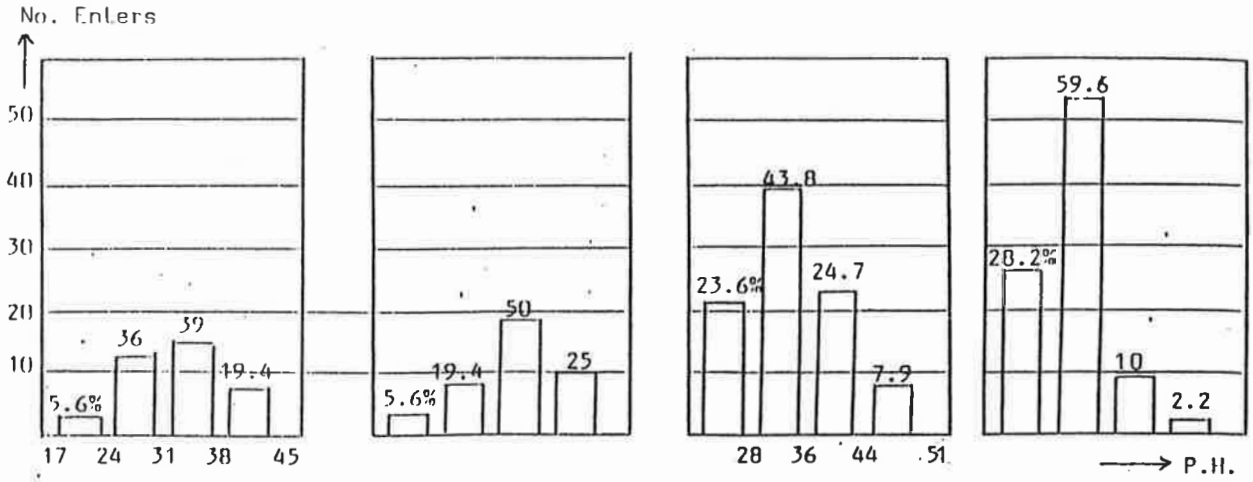
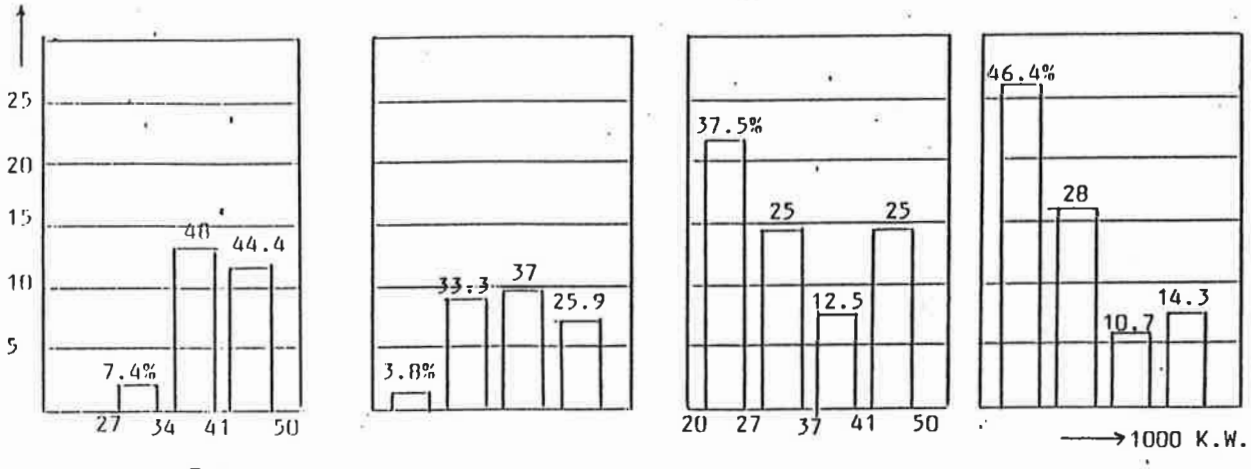


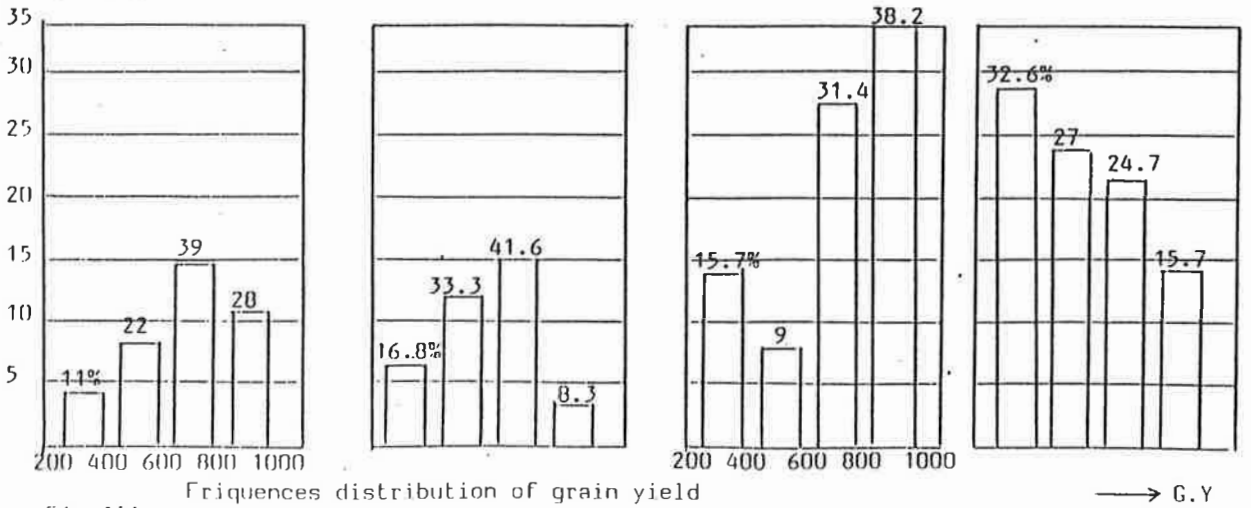
Friquences distribution of plant hight

Fig I :



Friquences distribution of 1000 kernel weight

Fig II:



Friquences distribution of grain yield

Fig III :

Oat Genotypes Evaluation to Aluminum Toxicity . E.L.FLOSS; A.R. DECHEN; Q.A. de C. CARMELO; F.A. MONTEIRO. University of Passo Fundo, C.P. 271. Passo Fundo, Rio Grande do Sul, Brazil. 99070-970.

Introduction. Acid soils are found extensively throught the south brazilian regions where oat are cultivated. Aluminum toxicity is a growth-limiting factor for crop production in these areas. The toxicity can be prevented by liming raise soil pH up to 5,5 to 6,0 or to grow cultivars that have greater tolerance to Al. However,the Al tolerance of brazilian oat cultivars and germplasm collection is not know.The main goal of this work, carried out at ESALQ/USP, Piracicaba, SP, was to study the responses of white (*Avena sativa* L.) and black oats (*A. strigosa* Schreb.) genotypes to levels of aluminum in nutrient solution. This study was undertaken to select tolerant genotypes, as well to study the possible causes of nutritional and physiological effects of the aluminum toxicity.

Materials and Methods. The oat genotypes were grown for 11 days in nutrient solutions (FURLANI & HANNA, 1990) in a growth chamber at 25^{0}C. It was determined the level of Al in nutrient solution for selection of oat genotypes, and 156 white oat genotypes and 14 black oat were screened for tolerance. The main parameter evaluated was the seminal root growth (SRG) whose values were correlated with other parameters of root growth and dry matter (DM) yield. Two white tolerant oat cultivars (UPF 82079 and UPF 86AL169-2b) and the most tolerant black oat cultivar (UPF 77434) were compared with two sensitive oat genotypes (UPF 7 and UPF 79239-1) during 21 days for evaluation in 0, 5,10, 15 and 20 mg Al/liter of nutrient solution.

Results and Discussion.The concentration threshold of Al in nutrient solution was 7,5 mg/liter. This level promoted a reduction of 50% in the SRG in the most sensitive genotypes. Initial seminal root lenght (ISRL) did not correlate significantly with SRG, relative seminal root growth (RSRG), lenght of seminal root (LSR) and relative length of seminal root (RLSR) and sometime the ISRL correlated with the LSR. SRG showed a highly significant correlation with RSRG, LSR and RLSR (Table 1). The SRG showed better correlation with DM relative yield for the whole plant - PDM (r = 39,18**) and shoots - SDM (r = 38,96**) and lower correlation with DM root yield - RDM(r = 30,73**) and relative root DM -RRDM (r = 35,15**). The best correlation were observed with SRG and the relative shoot DM - RSDM (r = 56,31**) and relative whole plant DM - RPDM (r = 59,3**).

Table 1 - Linear correlation coefficients for various traits in oat genotypes grouth on Al levels in nutrient solution

	SRG	RSRG	LSR	RLSR	ISRL
SRG	-	64,6**	94,1**	65,7**	ns
RSRG	-	-	67,0**	98,5**	ns
LSR	-	-	-	63,2**	30,3**
RLSR	-	-	-	-	ns

** significantly at the level of 1%.

At the 7,5 mg Al/liter, the white oat genotypes UPF82079 (1563CRcpx/C7512/SRcpx), UPF86Al198-5-4b (QR336=X2051-6/X1913-3/3/DC9/Coker 74C17/OTEE), UPF3 (Coronado/X1779-2), UFRGS 6 , UPF86Al068 (C5-2, 1563 CR cpx/T312/Srcpx), UFRGS 1 (DAL/CDA 292), UFRGS 4 (DAL/CDA 292), UPF 15 (QR306=COKER82-33//IL3376/OA338), UPF86120 (COKER 85A85), UPF84297 (X2051/X2670//X2300/

X2682/3/CORON/BCLA), UPF84125 (X2795/X2682/2/C62-26/3/C62-26), UPF87070 (OT224/W78181), UPF84321 (CORON/BCLA//PA7804), UPF87128 (X2795-2/X2682-3/COKER 62-26 2), CTC 1 (BCLA/COKER234//RLE83), UPF79302 (X2681-1/(74C17/X2888-2), CTC84412-3 and UPF79159 734470-2/CI8360), showed tolerance (SRG) and responsiveness (RSRG). The genotypes UPF82079 (1563CRcpx/C7512/SRcpx), UPF77394-1 (Auto Tetraploid Sais), UFRGS 6 (Unknown), UPF86006 (79 Barrow Seln.), UPF81360 (MN1107/DAL), UPF86160 (COKER 62-26/EEA10//X2503/X2299), UPF86112 (STEELE), UPF81359 (MN320/DIAMANTE) and UPF2 (X2505-4), showed tolerance by the relative tolerance index (RAlTI) at the level of 15 mg Al/liter.

The black oat genotypes UPF77434 (CD3820), UPF84AP01 (Unknown), UPF77066 (PI244471), UPF77436 (CD7847(CI9035)), Argentina Black Oat , UPF77352 (K2588/URSS), and UPF85AP01 (Unknown), were tolerant/responsive at the concentration of 30 mg Al/liter.

The seminal lenght (SRL) of the black oat genotypes were higher than de most tolerant white oat (UPF 82079 and UPF 86 AL169-2b) only at the level 15 and 20 mg Al / liter (Table 2). However,the black oat UPF 77434 seminal root length was superior than the sensitive white oat genotypes (UPF 7 and UPF 79239-1) at all levels of Al.

Table 2 - Effects of levels of aluminum in nutrient solution on the seminal root lenght oat genotypes

Genotypes	Levels of Aluminum (mg/l)				
	0	5	10	15	20
	cm				
UPF 77434	40,4 aA	30,0 aB	26,8 aB	26,2 aB	25,3 aB
UPF 82079	34,6 aA	30,0 aA	21,3 aB	16,8 bB	14,0 bB
UPF 86Al169-2b	42,2 aA	27,6 abB	19,0 ac	12,0 bcCD	8,6 bd
UPF 7	26,0 bA	19,5 bcA	9,9 bB	6,3 cB	6,1 bB
UPF 79239-1	39,2 aA	16,3 cB	9,1 bBC	7,7 cC	7,8 bC
Average	20,9				
C.V. Genotypes (%)	10,2				
C.V. Levels of Al (%)	16,7				

Means followed by the same letter are not significantly different by Tukey's multiple range test ($P<0,05$).

On the other hand, the black oat produced higher plant dry matter than sensitive genotypes at all levels of Al but not differe from the tolerant genotypes only at 5 mg Al/liter (Table 3).

Table 3: Effect of aluminum in nutrient solution on the plant dry matter of oat genotypes

Genotypes	Levels of Aluminum (mg/l) 0	5	10	15	20
	g/ vaso				
UPF 77434	3,1 c C	4,2 a AB	4,9 a BC	3,7 a BC	3,8 a BC
UPF 82079	4,6 ab C	3,5 a B	3,0 b B	1,4 b C	1,1 bc C
UPF 86Al169-2b	5,2 a A	4,2 a B	2,8 b C	1,5 b C	1,4 b D
UPF 79239-1	5,0 a A	2,1 b B	1,2 c C	0,6 c C	0,6 bc C
UPF 7	3,96 bc A	2,1 b B	1,3 c BC	0,9 bc CD	0,5 c D
Average 2,7					
C.V. Genotypes(%) 4,1					
C.V. Leves of Al (%) 12,7					

Means followed by the same letter are not significantly different by Tukey's multiple range test (P<0,05).

In this trial was observed a better correlation of the seminal root lenght (SRL) with shoot dry motter(SDM) and plant dry mather (PDM) than with root dry matter (RDM) (Table 4).

Table 4 - Linear correlation coefficients (r) for various traits in oat genotypes growing in different Al levels in nutrient solution

	RLSR	RDM	RRDM	SDM	RSDM	PDM	RPDM
LSR	0.91**	0.83**	0.71**	0.87**	0.79**	0.87**	0.79**
RLSR	-	0.80**	0.73**	0.84**	0.77**	0.85**	0.77**
RDM	-	-	0.85**	0.90**	0.85**	0.93**	0.87**
RRDM	-	-	-	0.83**	0.94**	0.85**	0.96**
SDM	-	-	-	-	0.94**	1.00**	0.91**
RSDM	-	-	-	-	-	0.91**	1.00**
PDM	-	-	-	-	-	-	0.91**

** significantly at level of 1% .

It was concluded that the white oat showed a high variability and were less tolerant than the black oat. The white oat genotypes UPF82079 and UFRGS6 showed the best tolerance and responsiveness considering the Al levels used and might be used for breeding purposes.

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POSSIBLE REASONS FOR PHENOTYPIC CORRELATIONS IN VARIETIES AND HYBRIDS OF SPRING BARLEY DIFFERING IN DEVELOPMENTAL RATES.

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Introduction. The problem of variability of quantitative traits is the background of quantitative genetics. Parameters of variation are the main ones for estimating the genetic processes in early generations following the F_1.

With early maturity being one of the basic agricultural problems of cereal crops, the pattern of variability and correlations between different quantitative traits of a barley plant and their dependence on genetic differences concerning developmental rates would be of a practical and theoretical interest. Therefore, the main aim of our investigation was to estimate correlation patterns for several quantitative traits describing the barley plant taking into consideration genetic differences among varieties of spring barley which differ in developmental rates. The second task was to determine the biological reasons stipulating different correlation patterns.

Materials and Methods. Eight varieties of spring barley were planted during 3 seasons. We used contrasting groups: early and late maturing specimens of spring barley. Early maturing varieties were Sampos and Ranny 1 (2-row), Pirrka and Hankija 60250 (6-row); late maturing varieties were Robin and Linga (2-row), Glenn and Dissa (6-row). Every year complete structural analysis of yield resulted in 18 traits being measured in each plant. These traits correspond to general structural analysis described by many authors. Longevity of the main ontogenetic phases was also measured. Eight F_2 hybrid families containing the early maturing varieties mentioned above as the maternal component of crosses were also planted during two seasons. At the same time additional samples of contrasting varieties (Sampos, Hankija, Robin, Dissa) were treated with gibberellin, heteroauxin and kinetin solutions (100 mg/l) before heading and the same structural analysis was conducted. The degree of correlation was generally estimated for varieties and hybrids by means of multidimensional scaling. Also, factor analysis was performed on the same samples to reveal the main factors working on the distribution of plants in hybrid populations. The results of hormonal treatment were estimated as the effects on the main and side shoots of a barley plant.

Results and Discussion. Early maturing barley varieties exhibited a higher degree of instability of the traits which describe the barley plant in general. Moreover, early varieties (especially 2-row ones) showed higher variation of kernel weight per plant if compared to other traits describing the plant as a whole (i.e., plant height, general vegetative weight, number of shoots per plant, number of productive shoots per plant). It may be suggested that the yield of late varieties is more stable than the yield of early ones. Multidimensional scaling revealed that correlation pattern was higher in late varieties (Table 1) - fractal dimension of trait space comprised about 2.4 for late varieties and 2.8-2.9 for early ones. F_2 hybrids also varied in this parameter and also more axes in multidimensional scaling were found for earlier hybrids if compared to late ones (Table 1).

Table 1. Multidimensional scaling of 18 agronomic traits in varieties and hybrids of spring barley.

Varieties, hybrids	Fractal dimension of trait space
Sampos	2.8
Robin	2.3
Hankija	2.9
Dissa	3.5
F_2 Sampos x Robin	4.6
F_2 Robin x Sampos	2.4

The main agronomic traits are more strongly correlated in late cultivars. Factor analysis showed that among 5 hypothetical factors the traits describing the main shoot corresponded to the first factor. Thus the main reason of different correlation patterns is weaker correlations between traits describing a plant in general exhibited by early varieties and hybrids.

Analysing effects of plant hormones we divided all effects into ones influencing the main shoot vs. other shoots on the plant. It has been shown that all quantitative traits were less affected by the hormones in early varieties than in late ones. Moreover, ranging all the effects in comparison with the weakest one we obtained a matrix of effects, ranging from 1 to 5. Each phytohormone produced an effect both on the main shoot and other shoots of the barley plant. Therefore, the figures could be interpreted as a temporal row describing the temporal distribution of processes controlling growth and development of the main shoot and the other shoots. According to the matrix, the points corresponding to the processes of main shoot growth were organized as a compact group on a temporal vector for early varieties and a dispersed group with effects on main and side shoots following each other for late cultivars. It can be suggested with a simple pattern of interactions between the main stem and other shoots of the plant - at first, growth processes are allowed in the main stem and than the system triggers growth of the other shoots. This triggering procedure may occur several times during ontogenesis. In late cultivars the system is triggered constantly during short time intervals. Thus, the variability of main quantitative traits is higher for early cultivars. This also leads to increasing number of axes in multidimensional scaling. Moreover, the character of interactions between the main shoot and other shoots of a plant could be regarded as a heritable trait which can be estimated both on varieties and hybrids.

Barley mixtures to increase stability of production under stress conditions. S. GRANDO and S. CECCARELLI, Germplasm Program, International Center for Agricultural Research in Dry Areas (ICARDA), P.O. Box 5466, Aleppo, Syria.

Introduction. Landraces are still widely used in subsistence agriculture where maximizing stability of performance is more important than maximizing yield *per se*. There is evidence that landraces are mixtures of different genotypes (Ceccarelli *et al.*, 1995). Pure line selection within Syrian barley landraces has produced lines with superior performances for the dry areas of Syria, but pure lines may not be the most suitable type of cultivars in adverse and variable conditions. It is reasonable to speculate that heterogeneity within landraces is a buffer mechanism against environmental fluctuations and that it may enhance stability, even at levels of heterogeneity lower than in the original population. The use of mixtures rather as opposed to homogeneous varieties has been suggested by several authors as a mean to promote stability. Improved stability and decreased disease severity are common features of mixtures relative to their component in monoculture. A recent review (Lenne and Smithson, 1995) has shown that although their yield advantage is generally small, mixtures are viable strategy for sustainable productivity in subsistence agriculture. The purpose of this research was to evaluate the role of different level of heterogeneity in stabilizing barley yield in stress environments in West Asia. As a model we used the two barley landraces, Arabi Abiad (white seeded) and Arabi Aswad (black seeded) which constitute the bulk of barley production in Syria. The first is common in slightly better environments (between 250 and 400 mm rainfall) and the second in harsher environments (less than 250 mm rainfall).

Materials and Methods. We conducted a trial for five cropping seasons (from 1990/91 to 1994/95) in three to six locations per year (total of 26 year-location combinations). Two groups of mixtures were included in the trial, one black seeded and one white seeded. For each group the mixtures were constituted with lines extracted from the same population. We started with 72 lines extracted from a black seeded population and 75 from a white seeded population. The lines went through three consecutive cycles of selection for grain yield under stress conditions, promoting at each cycle the best lines to the following year testing. With the lines we constitute mixtures at different level of heterogeneity. The black seeded group had mixtures of 72, 34, 17, and 5 components, the white seeded had mixtures with 75, 34, 15, and 5 components. In the mixtures with the highest levels of heterogeneity, all lines available for each population were included. The mixtures at second highest level (34 lines, in both groups) were constituted with the lines selected for one cycle. In the mixtures with 17 and 15 components the lines selected for two cycles were used, while the mixtures with the lowest number of components contained the lines selected for three cycles. The experiment included 10 pure lines (the best 5 black seeded and the best 5 white seeded), and six checks.

Results and discussion. Mean yields ranged from 36 to 4385 kg/ha. Black seeded

material tends to have lower average grain yield, lower response, and higher frequency of positive intercept than white seeded material. In both groups the mixtures with five components had an advantage over the more complex mixtures. In the black seeded group (Table 1), the mixture of five lines did not have a clear advantage over the components. In particular SLB 5-96 had a high average yield (1880 kg/ha), combined with a relatively good response (b=0.97) and a positive intercept (a=91.5).
In the white seeded group (Table 2) the mixture of five components had an advantage over the single lines, combining a high average grain yield (1966 kg/ha), with a good response (b=1.04) and positive intercept (a=48.1). The only line (SLB 9-98) with a positive intercept, had a very low average grain yield (1588 kg/ha) and low response (b=0.81).
The results indicate the possibility that the two Syrian barley landraces possess different buffering mechanisms. The advantage of both five component mixtures over the more heterogenous mixtures would suggest that the level of heterogeneity of landraces may not be needed to maintain yield stability.

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Table 1. Grain yield (kg/ha), regression coefficient (b) and intercept (a) of four black seeded mixtures, five lines, and three checks.

Material	Grain yield Mean ±s.e.	b	a
MIXB 72	1747 ±232	0.89	102.9
MIXB 34	1773 ±249	0.97	-8.9
MIXB 17	1786 ±253	0.98	-12.1
MIXB 5	1841 ±245	0.95	92.5
SLB 5-96	1880 ±254	0.97	91.5
SLB 5-07	1868 ±261	1.00	24.1
SLB 5-86	1696 ±221	0.85	135.7
SLB 5-31	1946 ±274	1.06	-9.2
SLB 5-30	1723 ±227	0.87	118.6
Arabi Aswad	1636 ±220	0.85	66.3
Tadmor	1696 ±231	0.88	67.4
Zanbaka	1678 ±224	0.86	89.5

Table 2. Grain yield (kg/ha), regression coefficient (b) and intercept (a) of four white seeded mixtures, five lines, and three checks.

Material	Grain yield Mean ±s.e.	b	a
MIXW 75	1941 ±	1.12	-117.5
MIXW 34	1910 ±	1.13	-167.5
MIXW 15	1869 ±	1.07	-109.2
MIXW 5	1966 ±	1.04	48.1
SLB 9-63	1978 ±	1.12	-85.8
SLB 9-71	1985 ±	1.13	-104.7
SLB 9-76	2053 ±	1.21	-180.1
SLB 9-09	2021 ±	1.11	-26.4
SLB 9-98	1588 ±	0.81	102.7
Arabi Abiad	1887 ±	1.12	-176.3
Arta	2087 ±	1.17	-66.1
Harmal	1931 ±	1.09	-82.0

Involvement of the fatty acid metabolism in barley cold acclimation. M. GROSSI, C. CROSATTI, C. MURELLI, P. BALDI, F. RIZZA and L. CATTIVELLI, Experimental Institute for Cereal Research, Section of Fiorenzuola d'Arda, I-29017, Via S.Protaso, 302, Fiorenzuola d'Arda (PC), Italy

Activation of new genes whose expression is regulated by low temperatures as well as variation in fatty acid content and composition has been associated with cold-acclimation in barley and in other cereals. In the present work we have cloned a barley gene homologous to Acyl CoA-oxidase which expression is regulated by low temperatures. Because this enzyme is involved in the degradation of the fatty acids we have analysed the variations of the fatty acid content during cold acclimation.
The cDNA clone *cdr29* was isolated by differential screening, the nucleotide and the deduced amino acid sequences were compared with those reported in the data banks.
A comparison with the sequences in the data bank showed both at the nucleotide and at the amino acid level a region of high homology with known sequences coding for peroxisomal acyl-Coenzyme A oxidase of rat, human, *Caenorhabditis elegans* and *Candida* (Grossi et al., 1995). A multiple alignment was performed between a internal fragment of CDR29 and the corresponding sequence of all other acyl-Coenzyme A oxidases. The results showed the existence of a highly conserved domain through all the sequences.
The low temperature induction of *cdr29* was compared with the variations in the free fatty acid content during hardening. During the 21-day cold acclimation period, in the frost-resistant cultivar Onice the total amount of fatty acid decreased significantly from ca 16 mg(g dry weight)$^{-1}$ of the control to ca 1 mg(g dry weight)$^{-1}$ after 21 days. No appreciable variations occurred in the frost-sensitive cultivar, where the free fatty acid content at days 0 and 21 were similar (Murelli et al., 1995).

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Effects of environments on awn length in barley varieties having short awned lateral spikelets (Bozu barley). J. HAYASHI [1] and S. YASUDA [2], [1] Institute for Agricultural and Biological Sciences, Okayama University, [2] Okayama Women's College, 1089−31, Itsukaichi, Kurashiki 710, Japan.

Introduction. Barley cultivars characterized by awnless or shorter−awned lateral spikelets are known to be found only within the Oriental barley region(Vavilov 1926, Takahashi 1949), and they are collectively called from old times "Bozu" barley in Japan. In this experiment, Bozu barley cvs with shorter−awned lateral spikelets were used as the materials. Takahashi and Hayashi(1982) carried out genetic analysis using many crosses among awnless and shorter−awned lateral spikelets cvs, and proposed new gene symbol, v^{lr} for I_2 (Shakudo 1948) and *lr* (Leonard 1942) on the chromosome 2.

Materials and methods. Experiment 1: A total of 20 cvs consisting of 17 Bozu cvs and 3 ordinal six−rowed cvs were sown in the field in mid−November, mid−February and mid−April at Kurashiki. Sprouted seeds of the materials were treated at 3 °C for 20 days in the mid−February sowing and for 40 days in the mid−April sowing, respectively. At ripening stage, spike length and central and lateral awn lengths were measured on main stem of each plant. Experiment 2: Two Bozu cvs, Hayakiso 2 and Honagashiro were sown in pots in end−November and mid−January, respectively, and grown outdoors. At different growth stages, two pots each of these two cvs were kept at 25 ° or 15 °C in a greenhouse for 5 days, and then moved again to the open field. This high−temperature treatments were made every 5 days until early April. Different stages of spike primordia differentiation were discriminated by random sampling from each treatment. Experiment 3: Hayakiso 2 and Honagashiro, and ordinal six−rowed long awn cv, Marumi 16 were grown in pots with the same manner as those in Experiment 2, and at spikelet differentiation stage, they were treated at 20 °C for from 1 day to 5 days.

Results and discussion. In the Experiment 1, when the Bozu type cvs were sown outdoors in November, February and April, development of the lateral awns differed with the cvs and also sowing times. Many of the Bozu cvs sown in February and

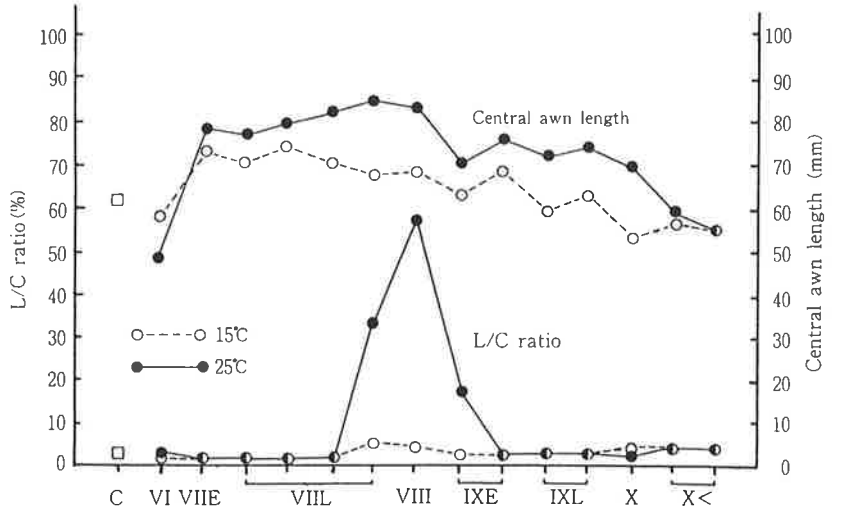

Fig. 1. Changes in central awn length and L/C ratio of normal Bozu cultiva, Hayakiso 2, which was treated by high temperature for 5 days at different stages of spike primordia differentiation. C:control, V:bract initiation, VI:spikelet primordia initiation, VIIE:early double ridge, VIIL:late double ridge, VIII:late stage of spikelet differentiation, IXE: early stage of flower differentiation, IXL:late stage of flower differentiation , X:flower primordia development.

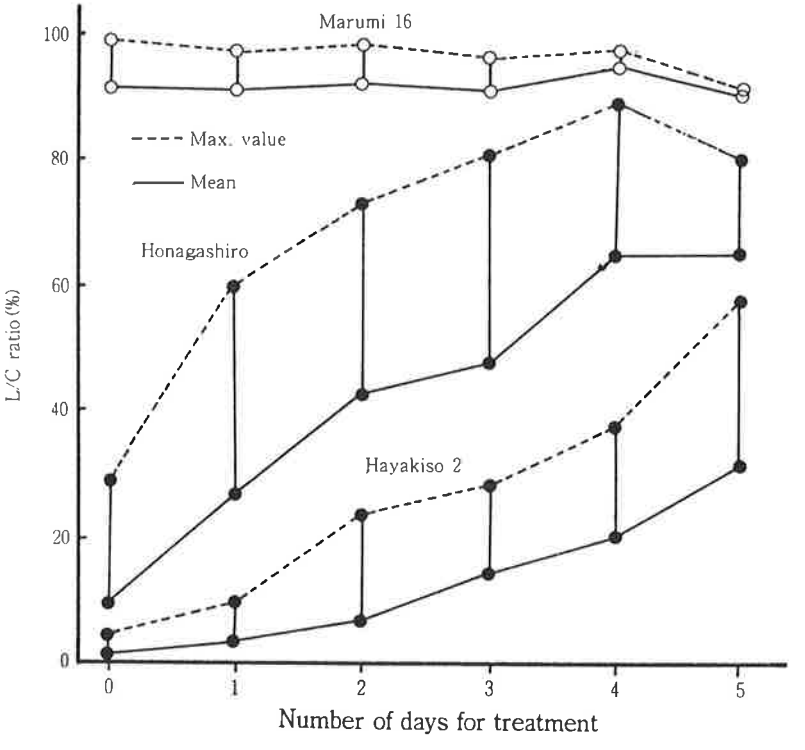

Fig. 2. Changes in L/C ratio of two Bozu cvs (Honagashiro and Hayakiso 2)and one ordinary six-rowed cv (Marumi 16) treated at 20 o C during different days.

April developed unusually longer latral awns as compared with those of the ordinal November sowing, and the trend of their awns elongation became more conspicuous with the advance in sowing date from February to April. Accordingly, the L/C ratio, represented by the length of lateral awn against central awn, became higher. However, when sown in February and April, some "Bozu" cvs whose central awns were ordinarily short or medium long, exhibited marked elongation of both central awns as well as lateral awns, so that they appeared to be ordinary normal long awned six-rowed form. This may be considered as follows: An expressivity of the gene or genes for long-awn involved in the Bozu cvs tested here was inhibited by the v^{lr} gene involved together in them when grown under ordinary sowing time of mid-November. But when such Bozu cvs were sown in February or later time, the inhibitory effect of v^{lr} gene on awn elongation was reduced with the rise of air-temperature. From these results, the marked elongation of lateral awns, and in some cases central awns as well, by the spring sowing led us to assume that the major causal agent might be the rise in temperature during the period of spike formation. The most sensitive spike formation stage to high temperature for awn elongation was verified by subjecting the Bozu barley plants to a rather high temperature from 15 ° to 25 ° C at the time of spikelet differentiation which corresponded to stages VII and VIII in Fig. 1. Furthermore, the effective length of time of high temperature treatment was ascertained to be rather short, mostly for five days, if the treatment was made arround the late stage of spikelet differentiation (Stage VIII, Fig.2). This fact clearly indicates how and when the gene for reduced lateral awn, such as v^{lr} and others, are hindered to exert its action by environmental condition, in this case, a somewhat higher temperature. As to biochemical mechanism, it is not clear at present, however.

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TYPE OF ACCUMULATION AND DISTRIBUTION OF THE IONS OF NICKEL AND CADMIUM BY SPRING OAT. **M.V. Ivanov, A.V. Grandjean, C.G. Starodvorski, Belogorka, Institutskaya 1 Street, C3HUUCX, Leningrad Region 188231, Russia.**

The environment has become contaminated with toxic heavy metals due to human activity. Heavy metals arise from different sources and tend to accumulate in soils. Crops can take up heavy metals from the soils; if heavy metal concentrations in the edible parts reach certain levels, agricultural product safety related to human consumption is endangered

By breeding, one can produce new varieties of crops with lower levels of uptake and accumulation of heavy metals; or crops which accumulate the toxic species in the non-edible parts. This method seems to have more potential than, for instance, decreasing heavy metal uptake by addition of some chemical reagent or organic matter to bind heavy metals. It is clear that any addition to the soil leads to contamination of the environment.

For this reason we initially carried out investigations in order to reveal the varietal differences and natural phenomenon of uptake and distribution of nickel and cadmium by spring oat. The stressors are the water solutions of $NiCl_2$ and $CdCl_2$ with concentration 2.5 mM. Under otherwise equal conditions the experiments also included the variants with NPK-fertilizers with concentration of each nutrient 1.2 mM. The analysis of Ni and Cd were made by Perkin-Elmer 370 atomic-absorption spectrophotometer with graphite furnace HGA-76. The conditions of atomization were standard. The different organs of the plant (roots, leaves, stems, husk and seeds) were analyzed. The plant samples were air-dried and prepared for analysis by dry digestion at 450°C. The vegetation experiments were made in three repetitions; the results of analysis were from duplicate analyses.

The highest accumulation of nickel occurred with K-fertility (Ni+K); and the maximum concentration - 56.8 mg kg Ni (0.03 mg/kg Ni in blank experiment) was present in the seeds. We believe that the K - ions affect water exchange of plants and result in uptake of the highest quantity of dissociated ions of Ni. These processs are blocking the protein-transport complex to provide the highest accumulation of nickel in the seed endosperm.

Table 1. Nickel uptake by oat cultivar Dedal. (Ni - mg/kg of air-dried matter)

Variant	Root	Leaf	Stem	Husk	Seed
Ni	15.5	10.1	11.1	8.6	35.9
Ni + N	17.9	17.3	6.1	20.5	39.7
Ni + P	19.3	19.3	6.1	8.4	18.7
Ni + K	46.8	46.8	26.6	52.9	56.8
Ni + NPK	32.5	32.5	22.7	41.3	39.7

The results of studies showed that P - fertility stimulates the process of Ni-uptake, but on the other hand the availability of the knot partitions with the high level of sugars block entry of Ni into the seeds. This also permits uptake of water containing the dissociated Ni - ions by the leaves because of the large amount of phosphorus needed for photosynthesis.

In the experiments with cadmium to take place another processes. The highest concentration of Cd was present in the N treatment (Cd + N) in all plant parts.

Table 2. Cadmium uptake by oat cultivar Dedal. (Cd - mg/kg air-dried matter).

Variant	Root	Leaf	Stem	Husk	Seed
Cd	48.0	28.0	36.8	36.2	10.5
Cd + N	155.7	37.4	115.2	64.7	26.1
Cd + P	36.7	11.6	19.9	18.5	9.1
Cd + K	28.3	10.4	10.6	11.4	10.4
CD + NPK	25.4	14.8	17.4	17.2	7.7

The high level of nitrogen intensifies the processes of protein synthesis and growth of the entire plant. Active uptake of water with dissolved Cd - ions occurred and Cd is distributed to all overground organs of the plants. The highest concentration of cadmium accumulated in the roots.

In all fertilizer treatments the types of uptake and distribution of heavy metals in various plant organs depends on the duration of the vegetative stage. The greater the vegetative stage the lower the differences in locations of heavy metals among organs of the plants.

As the results of this work it will be noted that:

1. Nickel - ions are concentrated in the seeds. The accumulation depends on the level of nutrition - the less nutrition the higher the Ni concentration. Phosphorus decreased the accumulation of nickel in the seeds.

2. The accumulation and distribution of cadmium is different from that of nickel and depends largely on the concentration of N in the environment, Cd, as distinguished from Ni, is only slightly concentrated in the seeds.

3. The ability of plants to immobilize the different heavy metals is strongly dependent on the concentration of sugars and the type of distribution of proteins in plant organs.

Evaluation of barley germplasm collection to nitrogen fixing ability by Bacteria.
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Introduction. To evlauate and examine the available germplasm of barley which collected by the Dept. of Agronomy, Assiut Univ., a greenhouse experiment was carried out using clay loamy soil. The soil was amended with maize stalks as an organic manure. Maize stalks were mixed with soil at a rate of 0.2% (2 tons/feddan).

Then evaluation of the local isolates of non-symbiotic nitrogen fixing bacteria was carried out. Fifty strains for either *Azotobacter* or *Azospirillum* for barley germplasm screening were purified and five efficient strains selected for use as inocula. These isolates from fields cultivated with barley at Assiut & Minia provinces.

Grains of the tested accessions for inoculated treatments were soaked before planting in a suspension of *Azotobacter* and *Azospirillum* strains containing about 10^7 cell m^{-1} for 30 min. Uninoculated grains were planted as control.

Samples of vegetative parts and roots were obtained after 45-50 days to determine plant height, number of leaves per plant, fresh and dry weight/plant. Counts of either *Azotobacter* or *Azospirillum* were determined in the rhizosphere zone of the tested germplasm.

Grain inoculation tended to stimulate the growth of the tested accessions presented as an increase in plant height, number of leaves per plant, fresh and dry weights of the barley plants.

Regarding the total counts of *Azotobacter* and *Azospirillum* in the rhizosphere zone of the tested accessions, the results indicated that inoculation considerably affected the population counts of these microorganisms in the rhizosphere zone.

In the winter seasons of 1990, 1991 and 1992 about 379 accessions of barley were grown under inoculation conditions to study their response to inoculation with asymbiotic nitrogen fixers. In the first season (1990), 161 accessions were tested and 10 out of them were selected, while in 1991 and 1992 seasons, 92 and 126 accessions, respectively, were tested and 10 also from each season were selected as high in plant growth and counts of both *Azotobacter* and *Azospirillum*.

Field experiments were conducted in the Experimental Farm, Fac. of Agric., Univ. of El-Minia, during the winter season of 1993. Three treatments of fertilizer i.e. 100%, 50% and 25% of the normal field rate of inorganic fertilizer in the presence of inoculation were used to evaluate the selected barley accessions which responded at the grenhouse tests.

The results showed that the highest and significant increase in plant height was observed for accession No. 31 followed by accession 2628 then 1065. Significant increase in plant weight was recorded for all accessions except 1065 and 2628. The highest increase was noted for accession 1944 followed by 1465 then 497. On the other hand, grain yield was affected by inoculation. Significant increase was noted in all accessions except 479. The highest increase was recorded for accession No. 1944 followed by 2207 then 31. It could be concluded that inoculation with N-fixing microorganisms resulted in an increase in plant growth and reduced the requirements of inorganic fertilizer to half the amount needed with non-inoculation.

Materials & Methods.

In the first 6 months we collect and survey then evaluate the local species of non-symbiotic nitrogen fixing bacteria, from Assiut & Minia Provinces out of 50 strains for either *Azotobacter* or *Azospirillum* from cereal fields 5 efficient local strains were selected, for use as inocula, which isolated from rhizosphere zones of wheat and barley. According to the method of Dobereiner (1978) using N-free semi-solid malate medium. Grains of barley of inoculated treatments were soaked before planting in the suspension of *Azotobacter* and *Azospirillum* strains (containing about 10^7 cell m^{-1}) for 30 min. Uninoculated grains were used as control. Three replicates for each accession were used for each treatment in the greenhouse pots Exp. Samples of vegetative parts and roots were obtained after 50 days to determine plant height, number of leaves and dry weight per plant, as well as counts of both *Azotobacter* and *Azospirillum* in the rhizosphere of the tested accessions. Samples from rhizosphere soil of each treatment were taken after 50 days after sowing for determination of nerobic non-symbiotic nitrogen fixing bacteria (*Azotobacter* and *Azospirillum*). The visible numbers of *Azotobacter* and *Azospirillum* were determined by dilution frequency method using Cochran's table (Cochran, 1950) for estimating the MPN of these microorganisms. The numbers were calculated/gram oven-dried soil. Semi solid malate medium (Dobereiner *et al.*, 1976) and modified Ashby's medium (Abdel-Malek and Ishac, 1968) were used for estimating numbers of *Azospirillum* and *Azotobacter*, respectively. Nine selected accessions out of 379 barley, which evaluated under greenhouse experiments and showed significant response to inoculation with asymbiotic N_2-fixers, were tested under field conditions to study the effect of inoculation as well as N-fertilizer on growth and yield of the selected accessions. Combinations of nitrogen fertilizer & inoculation were used in three replications as mentioned previously.

Results & Discussion. Purification of isolates of *Azospirillum* as well as *Azotobacter* were carried out according to Ishac *et al.* (1986). Nitrogenase activities of either *Azospirillum* or *Azotobacter* isolates were determined in the Dept. of Botany, Fac. of Science, Assiut University, according to the methods of Schollhorn and Durris (1967). Table (1) show nitrogenase activity for purification isolates of both *Azosprillum* and *Azotobacter*. The season of 1990, 161 accessions of barley were tested under greenhouse conditions to study their response to inoculation. Data presented in Table (2) showed a slight increase in plant height fresh and dry weight of plants was observed as a result of grains inoculation. Out of the 161 germplasms which were tested, 11 were selected as the best. The results showed that *Azospirilla* counts exceeded those of *Azotobacter*. *Azosprilla* population in biofertilized treatments were in the range of 1.63-158.22 x 10^5 while the corresponding figures for *Azotobacter* were 0.91-116.61x10^4. The increase in bacteria counts mainly due to grains inoculation.

N-ase activity was carried out for the selected accessions. The results showed that high values were recorded for accessions 2625 followed by 2762 then 2628 at 60 days, age.

The data recorded were in line with those obtained by Hgazi *et al.* (1979). They reported that inoculation of maize plants with *Azospirilla* as well as *Azotobacter* resulted in traditional increase in their densities at early stages of growth and maximum. N-ase activities were observed during flowering and grain filling.

In winter season of 1991, 92 more accessions were tested for their response to inoculation. Out of the 92 accessions tested 15 lines were selected. Data of microbiological determination showed the same trend of plant growth with some exceptions. Out of the 92 accessions examined, 10 which gave high counts of N-fixer were selected. N-ase activity was carried out for these selected accessions from the results, line No. 497 gave high N-activity followed by No. 1065 then 31. The results showed that inoculation with asymbiotic N-fixers affected the growth and yield of barley plants. The effect differfed according to the genetic make up used as well as the treatment of fertilizer.

In the control treatment (received 100% N but uninoculated) produced tallest plants increased dry weight and grain yield for accessions, 31, 1944 and 2725, respectively.

In the inoculated treatments, significant increase in plant height was recorded for all accessions except in the case of germplasms 497 and 2628, the increase was insignificant. The highest and significant increase was observed for line 31 followed by 2628 then 1065, Table (4).

In the light of the forementioned results, it could be concluded that grain inoculation with *Azotobacter* and *Azospirillum* increased the densities of the asymbiotic N-fixers (*Azotobacter* and *Azospirillum*). Similar results were obtained by other investigators (Saleh *et al.*, 1986).

The effect of inoculation with asymbiotic nitrogen fixers on plant growth and crop yield was emphasized by many investigators (Rovira, 1963 and Brown, 1974). They reported that inoculation of different crops with asymbiotic N-fixers significantly increased plant growth dry weight & grain yield. The beneficial effect of these organisms on plant development could be attributed not only to the N-fixation but also to the production of growth promoting substances (Harper and Lynch, 1979; Tien *et al.*, 1979 and Renders and Vlassak, 1982).

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Varietal differences for nitrogen use efficiency in winter barley (*Hordeum vulgare* L).

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Introduction. During the last thirty years grain yield of winter barley increased of about 120 $kg.ha^{-1}.year^{-1}$ in France to reach an average of 6 $t.ha^{-1}$ in the early nineties. Plant breeders have released varieties adapted to new agricultural practices characterised by a higher level of inputs. Chemical control of weeds, diseases and lodging and nitrogen fertilisers have been widely used. In recent years, high input management was however contested. The first reason was the overproduction of the most common crops including winter barley. A reform of the European Common Agricultural Policy was initiated in 1993 to decrease production through lower selling prices and compulsory fallow. The second reason was that ecological risks are more and more taken into account. Nitrogen is one of the main inputs in France and is responsible for an important part of phreatic water pollution. Plant breeders can contribute to agriculture evolution in creating new varieties with a better nitrogen use efficiency. Some studies have been carried out in the field on winter wheat (*Triticum aestivum* L) or Durum Wheat (*Triticum turgidum* L var *durum* Desf) in order to assess varietal differences for nitrogen use efficiency (e.g. Dubois and Fossati 1981, Dhugga and Waines 1989). It seems that no such studies have been devoted to winter barley and so it is not known whether a valuable genetic variability exists which could be used in plant breeding.

Material and methods. Field studies were conducted in 1994 and 1995 at Estrées-Mons (Somme, northern France) on a deep loam. The experimental design was a randomised, complete block with 4 replications as a factorial combination of two nitrogen treatments (0 and 100 kg.ha-1 in 1994 and 110 kg.ha-1 in 1995 as ammonium nitrate) and 24 winter barley varieties (12 six-rowed and 12 two-rowed). Both years soil nitrogen between 0 and 1.2 m was measured at about 60 kg.ha-1 in February. Except for one breeding line from INRA station of Montpellier, all the others were registered cultivars bred between 1977 and 1993. Experiments were sown early in October and plant density at the beginning of stem elongation averaged 250 $plants.m^{-2}$. Chemical treatments were applied in order to achieve a total control of weeds and diseases and to limit the risk of lodging. A single treatment was represented by two adjacent plots each consisting of six 5-m rows 0.2 m apart. Grain yield, yield components, total above-ground dry matter and nitrogen grain content were measured at maturity.

Results and discussion. In barley a large part of the variability may be explained by the existence of two groups of cultivars, 2-rows and 6-rows. Table 1 presents results for both groups and for the two N levels averaged over the two years. At high N level, corresponding to common agricultural practices in the region, 6-rows outyielded 2-rows by 5%. The difference was even higher at low N level with 12%. As a whole, 6-rows seem then to be more resistant to N deficiency then 2-rows. The decrease in number of ears per m^2 from high to low N level was higher for six-rows, but there was an increase of number of grains per ear for 6-rows. The decrease in number of grains per m^2 was then equivalent for both groups (28 and 26 % for 2- and 6-rows respectively). On average, 1000-grain weight of 6-rows increased of 2.5 g between the high and the low N level. This increase was much lower for 2-rows with only 1.0 g.

	Low N level		High N level	
	2-rows	6-rows	2-rows	6-rows
Grain yield ($g.m^{-2}$)	563b	637a	763b	801a
Number of ears (m^{-2})	629a	394b	852a	589b
Number of grains per ear	21.7b	44.4a	22.1b	40.5a
Number of grains (m^{-2})	13420b	17480a	18580b	23550a
1000-grain weight (g)	42.2a	37.0b	41.2a	34.5b
Total aerial dry matter ($g.m^{-2}$)	1184b	1239a	1658a	1680a
Harvest index (%)	47.6b	51.5a	47.9b	46.1a
Anthesis date (days from May 1st)	20.0a	17.3b	20.2a	18.0b
Grain N concentration (%)	1.60a	1.56b	1.80a	1.76b
Straw N concentration (%)	0.48a	0.48a	0.63a	0.62a
Grain N yield ($g.m^{-2}$)	9.0b	9.9a	13.8a	14.1a
Total N ($g.m^{-2}$)	12.0b	12.8a	19.3a	19.6a
N harvest index (%)	75.2b	77.8a	71.4b	72.9a
N utilisation efficiency	47.6b	50.2a	40.0b	41.9a

Table 1: characteritics of 2-row and 6-row winter barley cultivars at two nitrogen levels averaged over two years. Within a given nitrogen level means of 2-rows and 6-rows followed by the same letter are not significantly different at the 5% level.

At the high N level, 2- and 6-rows had equivalent straw N concentration, grain N yield and total N. Grain N concentration is higher for 2-rows. These results are in agreement with a previous study carried out in the same place with different varieties (Le Gouis 1992). At the low N level, 2-rows and 6-rows straw N concentration were still equivalent and grain N concentration was still higher for 2-rows. Grain N yield and total N were however higher for 6-rows. These results showed that at high nitrogen level, the higher grain yield of 6-rows was due to a better N utilisation efficiency (grain yield on total nitrogen). At low nitrogen level, it was due both to a better N utilisation efficiency and to a better N uptake efficiency (provided that N supply is the same, N uptake efficiency corresponds to total N).

Within each group, an analysis of variance (data not shown) showed that a large genetic variability existed for all characteristics measured. Genotype X environment interactions

were generally high, especially the genotype X year interaction. Genotype X N level interactions were on the whole not significant for 2-rows and significant for 6-rows. This result means that on average 2-row cultivars had the same behaviour at the two N levels and so that the level of resistance to a N deficiency is equivalent for all cultivars. On the contrary, for 6-rows it was possible to identify cultivars which seemed, at least on one year, to have a better level of resistance to N deficiency. For example, a cultivar had in 1995 a grain yield at low N level equivalent to 95 % of its grain yield at high N level.

These experiments showed that a genetic variability exists within barley for resistance against N deficiency. As a whole, winter six-rows seemed to be more resistant than two-rows as far as grain yield is concerned.

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DURATION OF THE VEGETATIVE PERIOD OF AVENA WILD SPECIES AND THEIR RESPONSE TO VERNALIZATION AND PHOTOPERIODISM.

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Introduction. Wild forms of plants are of great interest to plant breeders as sources of valuable agricultural traits. For instance, many wild oat species possess such useful properties as resistance to unfavourable environmental factors and diseases, high protein and oil content in kernel. Large number of spikelets in the panicle, etc. (Marshall H.G. et al., 1992). Centres of origin and diversity of wild relatives of oat are situated below 45' N. Lat., namely in Mediterranean regions, in the countries on the coasts of the Black and Caspian Seas and the countries of Central Asia. Vegetative growth of many forms of wild oat species occurs during the winter months. Part of this area is at high elevations (2,000 m) in the mountains. Therefore, these types have specific responses to environmental factors, such as photoperiod and temperature. These response have strong interactions with the duration of separate phases of plant ontogenesis, especially duration of the early periods of plant development (germination-tillering-heading). It is very important to take this into account when using definite wild forms as the initial material in breeding (Rodionova N.A. et al., 1994). Influence of daylength and effect of lower temperatures in the early phase of plant development on oat varieties and species have been reported by many researchers (Razumov, V.I., 1961; Sampson, D.R., Burrows, V.D., 1972; Sorrells, M.E., Simmons, S.R., 1992).

Materials and Methods. The present research was dedicated to two problems. The first one was obtaining of seeds during their reproduction in the environment of St. Petersburg (60' N. Lat.), for some oat accessions grown in the field started heading too late and hence could not yield grain of good quality or did not enter the reproductive phase at all. The second was searching for daylength neutral types of oat and identification of true winter forms for subsequent utilization in breeding.

Studying wild species in the field showed that many of the forms did not head at all or had a prolonged heading phase, which enhanced sterility and decreased quality of seeds. The following materials were used in this research: diploid species - A. bruhnsiana Gruner. (1 accession), A. ventricosa Bul. (1 accession), A. clauda Dur. (11 accessions), A. pilosa M.B. (12 accessions), A. wiestii Steud. (18 accessions), A. hirtula Lag. (7 accessions), A. longiglumis Dur. (9 accessions), A. atlantica Baum et Fedak (1 accession), A. prostrata Ladiz. (1 accession); tetraploid spp. A. barbata Pott. (73 accessions), A. vavilovana Mordv. (43 accessions), A. magna Murphy et Ter. (11 accessions), A. murphyi La-diz. (2 accessions), A. macrostachya Bal. (1 accession); hexaploid spp. A sterilis L. (200 accessions), A. fatua L. (26 accessions); all in all, 421 accessions of 18 wild species of oat.

In field conditions 59 late-ripening samples were identified in all the species under study. From 1991 to 1995 they were studied in the framework of a plant vegetation experiment at Pushkin Laboratories of VIR. A special site was arranged for photoperiod experiments with the duration of photoperiod artificially shortened to 12 hours (SD). A longer day (LD) typical for Leningrad Province (60' N. Lat.) was used for control. Average daylength during the period from germination to heading was 18 hours with monthly deviations of 18, 19 and 17 hours in late May,

June and July, respectively. Seeds were vernalized for 40 days at +2°C. Duration of the period for germination to heading was taken as the main criterion for evaluation of the response of the species to duration of daylength and vernalization. The difference between the time of heading during SD and LD determined the degree time of photoperiod susceptibility of each sample.

Results and Discussion. Results of the vegetation experiment showed that the majority of the samples in almost all species, if sown with non-vernalized seeds, were sufficiently late ripening, and the duration of the germination/heading period with the natural LD fluctuated from 80 to 110 days. In the LD experiment weak responses to vernalization (less than 20 days of delay in heading) were manifested by the forms of the following species: A. bruhnsiana, A. clauda, A. wiestii, A. longiglumis A. canariensis, A. atlantica A. vaviloviana, A. magna and A. fatua; strong responses (more than 20 days of delay) were shown by A. pilosa , A. prostrata, A. barbata, A. murphyi and A. sterilis. Several samples were unable to proceed to reproductive development without vernalization. These forms were A. clauda from Azerbaijan, A. barbata from Italy and Portugal, A. sterilis from Georgia, Ukraine (Crimea), Russia (Krasnodar Region), Morocco and Lebanon. In the latest forms of tetraploid A. vaviloviana from Ethiopia vernalization under the LD conditions helped to accelerate heading only for 1-19 days. Samples of hexaploid A. fatua showed the weakest response to vernalization before planting. Two relatively late samples from Tadjikistan and Zerbaijan manifested acceleration in heading only for 3-8 days.

Studying the development of individual plants of wild species of genus Avena L. in the process of ontogenesis in LD and SD conditions showed that after 40-day-long vernalization weak photoperiodic sensitivity was typical for diploid spp. A. longiglumis and A. hirtula; tetraploid A. vaviloviana and A. magna; and hexaploid A. fatua. Strong photoperiodic sensitivity was shown by the samples of diploid spp. A. clauda, A. pilosa and A. bruhnsiana; tetraploid A. barbata and A. murphyi; their heading phase was delayed by 30-70 days as compared with the LD version.

Analysis of responses displayed by the samples of different species helped to identify several forms of diploid A. clauda from Turkey, A. longiglumis from Israel, A. hirtula from Corsica and Greece, tetraploid A. barbata from Turkey and hexaploid A. sterilis from Tunisia and Morocco, which demonstrated week susceptibility to the change of daylength.

It became possible to identify early samples of spp. A. hirtula from Italy, A. wiestii (several samples) from Azerbaijan, Turkey and Italy, and A. fatua from Tadjikistan, which headed on the 33d to 40th day after 40 days of vernalization in the LD experiment. A part of them (A. wiestii from Azerbaijan, A. vaviloviana from Ethiopia and A. fatua from Tadjikistan) also displayed the weakest sensitivity to the duration of daylight. The latest ripening sample, A. sterilis from Ukraine (Crimea), was characterized by strong photoperiodic sensitivity; it failed to become reproductive in the SD experiment (12 hours of daylength).

Thus, such complex study of wild Avena L. spp. in the field and during vegetation demonstrated wide polymorphism of responses to photoperiodism and vernalization. Obviously it is possible to find true winter forms among spp. A. clauda, A. barbata and A. sterilis, since several forms of these species, being late in the field, showed strong responses to the effect of vernalization in vegetation experiments. Such samples were colleced either at high altitudes in mountains, or in

witnter months. Such species as A. vaviloviana and A. fatua may be regarded as true spring species. The forms identified in the vegetation experiment manifested weak responses to vernalization. This lack of response probably pertained to the fact that these species are regarded as weeds in spring plantings. Worth mentioning as chiefly spring by types are such species as A. wiestii, A. canariensis and A. magna. From the vast divesity of the two latter species it was possible to identify only 2-3 late samples showing medium response to vernalization. Generally, these species always headed in the field and produced normal grain yields.

Daylength neutral types may be identified among spp. A. hirtula, A. vaviloviana and A. fatua. Late-ripening samples with strong response to the duration of daylength were chosen from diploid A. clauda, tetraploid A. murphyi and hexaploid A. sterilis.

This study has shown that the response of wild species of genus Avena L. to vernalization is determined to some extent by the geographic distribution of each form, but the response to daylength depended primarily on the species.

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Barley seed vitality in relation to heat-susceptibility and -resistance.

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Introduction.

Quantification of seed vigour in malting barley is based on determination of the germination curve of a range of heat-treated sub-samples with water content 12%, treated at 68°C in 0-4½ hours.
The calculation is based on the method of Aastrup et al. (1989). The loss of vigour is assumed to follow a normal distribution curve at constant storage conditions. By mathematical transformation, using a probit scale, the distribution can thus be described by a straight line. The point where the straight line hits the y-axis is then taken as a quantitative measure for the initial seed vigour.
Following Aastrup et al. the vigour potential status at any time can be calculated using the models and equations, and knowing the storage conditions.
If two barley samples have the same content of water and equal storage conditions, the germination curves should be the same.
We will in the following investigate if this model is adequate in real life.

Materials and Methods.

Plant material. Field experiment I Barley samples with natural differences in vigour.
A total of 22 different samples of the varieties Alexis, Ariel, Blenheim, Etna and Meltan. The samples were received from "Skånska Lantmännen", Malmö. The barley samples were harvested in 1993, and grown on two locations in Denmark and on one location in Sweeden in 1994.

Field experiment II The varieties Alexis, Carula, Etna and Lysimax were used. The samples were harvested in 1993 and grown on two locations in Denmark and one location in Sweeden in 1994. The 4 samples all contain 12 % water. Ageing (loss of vigour) is simulated by a heat-treatment of the barley lots in water-tight plastic bags in a water bath at 58°C in 0, 10, 30, 40 and 50 hours.

Methods. Germination Index (GI) To determinate the germination index 4 x 100 kernels are germinated in 50 ml 0.75 % H_2O_2 for three days. Percent germinated kernels (n) is calculated after 24, 48 and 72 hours.

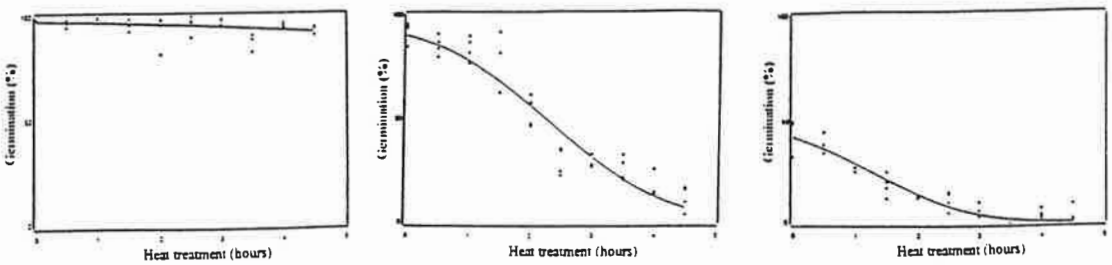
$$GI = \frac{10 \times (n_{24} + n_{48} + n_{72})}{n_{24} + (n_{48} \times 2) + (n_{72} \times 3)}$$

n = percent germinated kernels after 24, 48 and 72 hours of germination.

Vigour Potential (VP) The vigour potential (defined by Aastrup et al., 1989) is determinated by heat-treating 10 sub-samples of every barley lot (12% water) in a water bath at 68°C ("storage temperature") in 0-4½ hours ("storage time"). After the heat-treatment 4 x 100 kernels of every sub-sample are germinated in 90 mm petri dishes with two layers of filterpaper and 5 ml H_2O for 8 days. The germination results of the 10 sub-samples will end up with a "germination curve" for each barley sample. After transforming the germination data to probit the VP can be determinated as explained in the introduction.

Results and Discussion.

We found a large variation in the form of the germination curves for all samples, although the water content, storage temperature and time were equal (figure 1).



a) Blenheim b) Meltan c) Alexis

Figure 1. The germination curves for samples of a) Blenheim, b) Meltan and c) Alexis. The three samples were heat-treated at 68°C for 0-4½ hours. The water content for the three samples are 12.0 %.

In contrast to the assumption by Aastrup et al. (1989) we have found, by using the multivariate analysis PCA (Principal Component Analysis), that the barley samples can be classified into three types of germination curves. One type for the samples showing heat-sensitivity and one for the samples showing heat-resistance. The third group consists of samples with low initial germination percent. The samples in this group are also quite heat-resistant and it can be discussed if there thus are only two types of germination curves (figure 2).

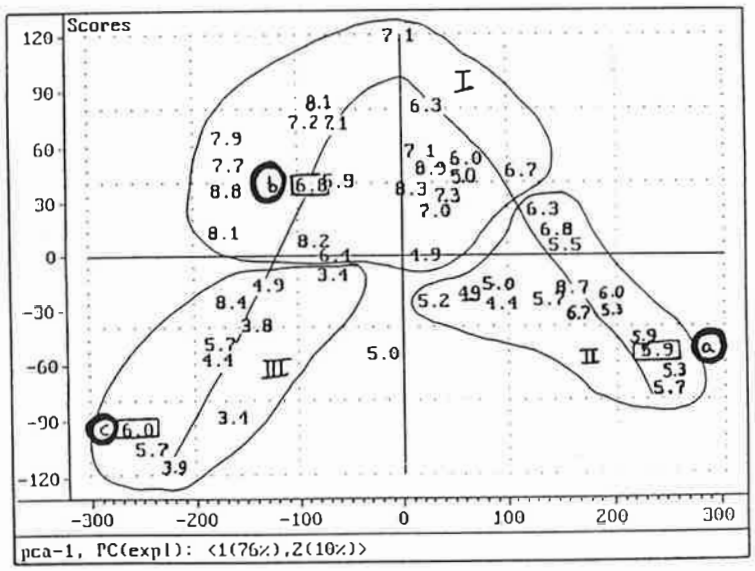


Figure 2. Score plot for the germination data determinated after heat-treatment of the barley samples. The numbers indicate the germination index (GI) for every barley sample. The letters a,b and c refer to the germination curves in figure 1, and show that the three samples are found in the extremity of the parabola. From the values of the GI the data can be divided into three groups which are significantly different.

When comparing the germination curves with the Germination Indices (GI), we noted that heat-sensitive samples had a higher GI than heat-resistant samples. The barley samples which could resist the stressfactor of heat had a slower initial germination than the barley samples which could not resist the heat-treatment (figure 2).

We have found significant difference between the three groups.

$LSD_{0.95}$ (I) = 7,26 $LSD_{0.95}$ (II) = 5,88 $LSD_{0.95}$ (III) = 4,96

By studying the litterature most of the experiments have been made using only one variety (Goodspeed, 1911, Robertson, 1939, Roberts, 1960, Roberts & Abdalla, 1968, Ellis & Roberts, 1980, Ellis & Roberts, 1981). Therefore we found it interesting to examine several varieties. Furthermore to our knowledge there have only been vigour-experiments on artificially aged barley, and never on naturally aged barley as we did in this experiment. If we only look at the results from the artificially aged samples, it seems like the Aastrup-model is useful. However, if we look at the results from the naturally aged barley lots, they show that some of the samples follow this model and some do not.

We have seen differences in heat-sensitivity and -resistance between varieties but also between seed lots of the same variety. In a later study we examined 17 different samples of the variety Alexis, harvested in 1994, and grown on different locations all over Europe (in cooperation with Dr. J. Molina-Cano, University of Lleida, IRTA, 25006 Lleida, Spain). We heat-treated these samples, and found major differences in their heat-sensitivity. We do not yet know the reasons for this. It seems that there is no clear connection between heat-resistance and the geographical environment.

Conclusion.

We did not find the vigour model of Aastrup et al. (1989) useful in practice, because the assumptions of normal distribution and linearity of the effect of heat-ageing were not valid for all lines in our material. By PCA analysis which has been verified by classical statistics we identified both heat-sensitive barley samples which follow the above model by Aastrup et al. as well as heat-resistant types which defeat the model. We will continue to study the issue of heat-resistance and-sensitivity in relation to vitality, malting quality and yield (t/ha).

Acknowledgement.

We wish to thank the SL-foundation for financing this study.

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Stem breakage and its relationship to oat plant characteristics. C.M. MUNDSTOCK and A.B. DA ROCHA, Plantas de Lavoura Department, University of Rio Grande do Sul, P.O. Box 776, Porto Alegre, 91501-970, RS, Brazil.

Introduction. Stem breakage is a particular type of lodging, and has been recognized to severely reduce small grains yields in most parts of the world. In Brazil, oat varieties show high stem breakage levels and this problem is responsible for up to 60% yield decrease (FEDERIZZI et al., 1994). Susceptibility to stem breakage has been attributed to inherent genotypes characteristics. In oat plants, culm length, culm thickness to internode length ratio and number of vascular bundles have been associated with stem breakage. Culm length, which comprises the lever of the lodging-inducing torque, has been positively correlated with stem breakage. Number of vascular bundles and culm thickness: internode length ratio was also related to lodging resistance (PINTHUS, 1973). However, stem breakage is associated to the processes that occur during senescence in oats (GRAFIUS et al., 1955). Senescing plants show high amount of lignin in the straw while water content slowly decreases. Both aspects affect the modulus of stem elasticity, and may determine stem breakage. The purpose of this work was to characterize the stem of different oat genotypes and verify the relationship between some plant characteristics and stem breakage.

Material and Methods. A field experiment was carried out at University of Rio Grande do Sul Experimental Station (Southern Brazil) with six oat varieties sowed at two dates (June 13, and July 14, 1994). Oat genotypes were: UFRGS 10, UFRGS 14, UFRGS 15, and UFRGS 901717 (all from the University of Rio Grande do Sul Oat Breeding Program) and also UPF-7 and UPF 890167-18 (from University of Passo Fundo Oat Program). Thirty plants/plot were sampled by booting stage for culm thickness: internode length ratio determination. Another sampling was done two weeks after anthesis for vascular bundles analysis. Both were observed in the upper three internodes of the plants. Lignin content (VAN SOEST & MOORE, 1965), culm water content and culm length were recorded in plants sampled by maturity. Stem breakage was determined at the same time.

Results and Discussion. The percentage of broken culms increased with plant maturity (Table 1) in both sowing dates. This was also observed by Grafius et al. (1955) who proposed the use of a lodging resistance factor in green plants as a measure of stem breakage resistance in senescent oats. Besides that, there were varietal differences which turned a genotype more resistent to stem breakage than the others, as seen in UPF 890167-18 and UFRGS 901717. UPF 890167-18 showed the highest values in all the plant characteristics studied in this experiment (Table 2). Culm thickness: internode length ratio did not vary among genotypes, but the culm length did. The plant characteristics that were correlated with stem breakage were number of vascular bundles, culm thickness: internode length ratio, water content and culm length. Lignin content was not associated with stem breakage. The culm water content showed, on the other hand, the most marked relationship to stem breakage, demonstrating that when stem tissues are wet, with great elasticity, there are less chances for it to break, as also seen by PATTERSON et al. (1957). As the stem tissues die in the late maturity stage, lignin builds up and water content decreases. These modifications in the components of the culm change its elasticity and the chances of stem breakage increase. This pattern can be affected by the other stem structural characteristics.

The associations between number of vascular bundles (positively), culm length (negatively) and the ratio of culm thickness: internode length (positively) and stem breakage resistance are poor. The two last characteristics may affect the modulus of the lodging-inducing torque. This work indicates that plants with of short culms or high culm thickness: internode length ratio are not necessarily resistant to stem breakage. The interaction betweem these characteristics with maturity stage is more important than a single plant characteristic.

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TABLE 1. Percentage of broken culms in six oat genotypes, at two sowing dates and different maturity stage

Genotypes	Sowing dates			
	June 13		July 14	
Sampling dates	Nov, 01	Nov, 11	Nov, 01	Nov, 11
UFRGS 10	A 77 ab*	A 87 a	B 13 a	A 75 a
UFRGS 14	A 95 a	A 96 a	B 1 b	A 40 b
UFRGS 15	A 50 bc	A 68 ab	A 0 b	A 1 c
UFRGS 901717	B 29 cd	A 51 bc	A 0 b	A 0 c
UPF -7	A 90 a	A 96 a	B 0 b	A 58 ab
UPF 890167-18	B 6 d	A 35 c	A 0 b	A 0 c

* At each sowing dates, capital letter = line comparison, and minute letter = column comparison. Tukey test at 5% of probability.

TABLE 2. Oat culm characteristics in six oat genotypes

Genotypes	Number of vascular bundles (upper three internodes)	Culm thickness/internode length (X 100) (upper three internodes)	Lignin content (% in dry matter)	Water content (%)	Culm length (cm)
UFRGS 10	26 b*	1,2 a	10,9 a	24,9 c	113 b
UFRGS 14	27 b	1,5 a	10,0 a	24,3 c	101 c
UFRGS 15	30 a	1,2 a	10,2 a	30,8 b	84 d
UFRGS 901717	23 c	1,0 a	8,7 b	33,9 ab	101 c
UPF-7	29 a	1,2 a	9,8 ab	23,3 c	107 bc
UPF 890167-18	29 a	1,2 a	10,9 a	37,8 a	133 a

* Tukey 5% of probability

TABLE 3. Correlation coefficients between culm characteristics and stem-breakage

Characteristics	Stem-breakage
Number of vascular bundles	-0,56*
Culm thickness: internode length ratio	-0,45*
Lignin content	$0,42^{NS}$
Water content	-0,77**
Culm length	0,50*

* $P < 0,05$

Tiller yield contribution under different plant spacings. C.M. MUNDSTOCK and A. GALLI. Plantas de Lavoura Department, University of Rio Grande do Sul. P.O. Box 776, Porto Alegre, RS, 91501-970, Brazil.

Introduction. Inter plant competition determines the amount of tiller production and survival. This morphological adaptation is determined by the supply of light, water, and nutrients per plant. Oat plants are quite sensible to the availability of environmental factors and show it by altering the size of the plant. This can be seen in panicle size and the presence or absence of tillers. As tillers might contribute to grain yield, it would be interesting to have a larger contribution of tillers in addition to the main stem. Current varieties used in Brazil have a low tiller production under current cultivation methods. The objective of this work is to study the plasticity of yield components under two environmental conditions.

Material and Methods. A field experiment was conducted with UFRGS-15 oat cultivar (spring type, short stature) using six different plant densities (50,150, 250, 350, 450, and 550 seeds/m^2) Row spacing was constant (20 cm). Dates of seeding were June 10 and July 8, 1994. Five plants/plot were selected and individual tillers were identified. At maturity, main stem and tillers panicles were counted and harvested separatelly and determined the number of grains.

Results and Discussion. Tillers were present in great number at early plant development specially at low densities, decreasing progressively at later stages. Tiller survival ocurred only on densities lower than 350 seeds-m^2 in both dates of seeding. Tillers from first (T1), second (T2), and third (T3) leaves were the most common in low densities. The more basal tillers (T1 and T2) survived better than T3 and were more productive. Tiller contribution to final yield was important at 50 seeds/m^2 density and made over 65% of grain production (Table 1). As densities increased, number of tillers decreased and this was the strongest effect of the plant competition in both seeding dates. Grains/panicle in both the main stem and tillers also decreased as expected (Table 1) but not as drastically as tiller number. The contribution of tillers to final yield was reduced as a resultant of both less fertile tillers and less grains/panicle. Nevertheless, the first one exerted the strongest effect. This indicates that the process of tiller survival is crucial to increase grain yield. A better equilibrium in the yield components is necessary to increase the number of grains/area.

TABLE 1. Main stem and tillers characteristics

Densities seeds/m^2	Date of planting			
	June, 10		July, 8	
	Main stem	Tillers	Main stem	Tillers
	% of grain yield			
50	36 c	64 a	34 d	66 a
150	69 b	31 b	55 c	45 b
250	97 a	3 c	80 b	20 c
350	96 a	4 c	80 b	20 c
450	100 a	0 c	100 a	0 d
550	100 a	0 c	100 a	0 d
	Grains/panicle			
50	87	53	55	55
150	64	35	47	47
250	51	15	39	39
350	36	33	31	31
450	34	-	33	-
550	22	-	22	-
	Mean tillers/plant			
50		3,25		4,05
150		0,80		1,60
250		0,20		0,55
350		0,05		0,40
450		0		0
550		0		0

$P < 0.05$ - Tukey

Genotypic correlation coefficients of some agronomic characters among Nordic barleys. M. Nurminiemi and O.A. Rognli, Dept of Biotechnological Sciences, Agric. Univ. of Norway, P.O. Box 5040, N-1432 Ås, Norway.

Introduction. Simultaneous selection for all characters by index selection requires information about: economical values, heritabilities, and genotypic and phenotypic correlations of the characters. Correlations have both genetic causes: pleiotropy and linkage, and environmental causes. Correlation resulting from pleitropy is a net effect of all the segregating genes that affect the characters, and therefore it does not necessarily cause a detectable correlation. Linkage causes transient correlations, especially in populations derived from crosses between widely different inbred lines. The environment is a cause of correlation when two characters are influenced in the same manner by changes in environmental conditions. Pleitropy is a common feature of major genes, but little is known about how it affects the genetics of quantitative characters. It is also important to know how selection in one character will cause simultaneous changes in other characters (Falconer 1989).

Materials and Methods. Most of the entries were inbred lines or pure line varieties. Some of the entries were heterogeneous populations of nearly homozygous individuals, like bulks, multilines, or landraces. The 220 lines were divided into 3 subsets by earliness and row type. Subset EARLY consisted of 37 early maturing 6r-feed barleys. Subset 6R included all 71 6r-barleys, while subset 2R comprised 149 2r-barleys. Early maturing barleys were tested at all sites in 1988 and 1989, while subsets 6R and 2R were tested at the 4 southernmost locations from 1987 to 1989. The experimental sites were: 1) Højbakkegård (55°40'N 12°18'E) near Copenhagen, Denmark, 2) Korpa (63°46'N 20°15'W) in southern Iceland, 3) Röbäcksdalen (63°51'N 20°16'E) near Umeå in northern Sweden, 4) Svalöv (55°57'N 13°8'E) in southern Sweden, 5) Viikki (60°15'N 25°3'E) in Helsinki, Finland, and 6) Ås (59°40'N 10°48'E) in South-East Norway (Nurminiemi et al. 1996). The characters recorded were: days from sowing to heading HD, days from sowing to ripening YR, average lodging score, LODG, plant height, HEIG (cm), resistance to cereal powdery mildew, MILD (*Erysiphe graminis* DC. f.sp. *hordei* Em. Marchal), 15 % moisture corrected grain yield, YIEL (kg/ha), straw yield, STRW (g/m^2), harvest index, HI, kernel weight TSW (g), and test weight HLW (kg/hl). Raw protein content of grains, PROT (%) was measured from the early maturing lines only.

PROC GLM and IML of SAS/STAT (SAS 1987) were used to obtain estimates of different variance components. Phenotypic variances were estimated as: $\sigma^2_p = \sigma^2_g + \sigma^2_{gl}/_L + \sigma^2_{gy}/_Y + \sigma^2_{gly}/_{LY} + \sigma^2_e/_{rLY}$, where L, Y and r represent the no. of locations, years and replications, respectively. Heritabilities were estimated as: $h^2 = \sigma^2_g/\sigma^2_p$ and its error as: $S.E.(h^2) = (S.E.\ \sigma^2_g)/\sigma^2_p$. The variances of variance components were estimated as proposed by Wricke and Weber (1986). The genotypic correlation between two characters X and Y is: $r_g = COV_{xy}/S_xS_y$, where COV_{xy} is the genotypic covariance of the two characters, and S_x and S_y are the genotypic standard deviations from the matrix of genotypic mean squares. Error correlations, r_e, were estimated from the matrix of error mean squares. Singh (1991) proposed a method for testing of significance of r_g when the heritabilities, h^2_x and h^2_y, r_g, and r_e of characters X and Y are known. $r_g/\sqrt{AV(r_g)}$ is distributed as a standard normal variate, where asymptotic variance (AV) of $r_g = 1/q(1-r^2_g)^2 + 1/bq\ (1-r^2_g)(k^2_x+k^2_y - 2r_er_gk_xk_y) + 1/bf\ \{(1+r^2_e+ r^2_er^2_g)k^2_xk^2_y - 2r_gr_ek_xk_y(k^2_x+k^2_y) + (1/2)r^2_g(k^4_x+k^4_y)\}$, where b= no. of blocks, q= no. of genotypes -1, f= q(b-1), $k^2_x = (1/h^2_x)-1$, $k^2_y = (1/h^2_y)-1$.

Results and Discussion. Generally, genotypic correlation coefficients among reproductive characters, i.e. grain yield, kernel weight, test weight, and harvest index, and among days to heading and days to ripening were most often positive and significant. This was also the case with plant height, lodging, and straw yield (Table 1). Correlation between plant height and straw yield is partly caused by interdependency, because taller plants give higher straw yield, but effects of linked genes and/or pleiotropy can also be present (Powell et al. 1990, Wettstein-Knowles 1992, Hayes et al. 1993). Lodging reduced reproductive characters, which was frequently demonstrated by negative correlations between these characters. There was no correlation between lodging and straw yield among 6r-lines. Lodging affects the two upper culm internodes, and since these internodes comprise about 2/3 of the total culm length, straw yield is reduced (Pinthus 1973).

Grain yield was negatively associated with protein content, $r_g = -0.85 \pm 0.07$. Large genotypic differences in protein content and widely varying growing conditions partly explain the high correlation. The protein in grains originates primarily from nitrogen accumulated in the foliage prior to heading. Therefore, the absolute amount of protein in the kernels is hardly affected by lodging, while carbohydrate assimilation is interfered (Pinthus 1973). This was demonstrated by the positive correlation between lodging and protein content, and negative between lodging and grain yield.

The average of the reproductive characters and incidence of powdery mildew disease increased from north to south, and caused positive correlations between these characters in subset EARLY. A positive relation between lodging and powdery mildew attack was expected (Fejer & Fedak 1978), but Korpa and Röbäcksdalen (moderate lodging and no disease) vs. Viikki and Ås (little lodging and moderate disease incidence) contributed to a non-significant correlation (data not shown). When subsets EARLY and 6R are compared, the correlations between powdery mildew vs. reproductive characters or rate of development changed from positive to negative. The negative effect of lodging on kernel weight was most pronounced ($r_g = -0.52 \pm 0.12$) in subset 6R. Kernel weight was negatively and significantly associated with days to heading only in subset EARLY. The grain must be filled rapidly under northern cool temperatures, while at Højbakkegård a long vegetative growth period causes lodging and reduced grain weight. Kernel weight of 6r-barleys is sensitive to environmental stress because daylength and temperature affect fertility of lateral spikelets (Guitard 1960).

2r-lines had a better lodging and powdery mildew resistance, and higher kernel weight than 6r-lines. Subsequently, correlations including these characters showed the largest differences between subsets 2R and 6R. Greater genotypic variance in powdery mildew resistance of 2r-lines and larger number of genotypes tested contributed to higher correlations with grain yield, days to heading and ripening than in subset 6R. Genotypic variance of lodging among 2r-lines was smaller than among 6r-lines, and correlations between lodging and reproductive characters were smaller in subset 2R. Plant height had higher correlation with grain yield and days to heading in subset 2R than in 6R.

Phenotypic correlations between the year of release and agronomic characters for 69 2r- and 26 6r-varieties revealed that reduced plant height, increased harvest index, and improved lodging resistance have been equally important for both row types, while powdery mildew resistance has increased significantly among 2r-varieties. QTL's (quantitative trait loci) and major genes controlling lodging have been detected on all chromosomes (Wettstein-Knowles 1992, Barua et al. 1993, Hayes et al. 1993). Therefore, linkage between genes affecting lodging and row type cannot alone explain the relatively better lodging resistance of 2r-barleys. Genes affecting row type, plant height, developmental rate, and photoperiod response are located on chromosome 2 (Powell et al. 1990, Hayes et al. 1993, Laurie et al. 1994). The *I-i* locus on

chromosome 4 controls the fertility in the lateral spikelets and epistatically the row type (Powell et al. 1990). Hackett et al. (1992) found QTL for plant height and days to heading on chromosome 4. Genes affecting developmental rate have been reported on all chromosomes (Kjær et al. 1991, Wettstein-Knowles 1992, Barua et al. 1993, Hayes et al. 1993). Thus, both linkage and pleiotropy are probably involved in the genetic control of row type, developmental rate and photoperiod-sensitivity.

Table 1. Genotypic correlation coefficients of different traits among subsets.

Subset	Trait	Hd	Lodg	Tsw	Hlw	Mild	Heig	Yr	Hi	Strw	Prot
Early+	Yiel	0.47	-0.70	0.20	0.58	0.45	-0.22	0.35	0.72	0.05	-0.85
	Hd		-0.24	-0.35	0.02	-0.02	-0.08	0.45	0.22	0.41	-0.28*
	Lodg			-0.19	-0.43	-0.28	0.56	-0.18	-0.49	-0.05	0.38
	Tsw				0.43	0.31	0.39	0.19	-0.26	0.20	0.30
	Hlw					0.42	-0.07	0.40	0.51	-0.15	0.13
	Mild						-0.14	0.10	0.37	-0.06	-0.11
	Heig							-0.12	-0.71	0.54	0.67
	Yr								0.08	0.02	-0.04
	Hi									-0.76	-0.84
	Strw										0.28
6R++	Yiel	0.60	-0.68	0.46	0.69	-0.26	-0.16	0.61	0.68	0.33*	
	Hd		-0.35	0.12	0.21	-0.34	-0.12	0.80	0.09	0.59	
	Lodg			-0.52	-0.50	-0.04	0.47	-0.37	-0.72	0.00	
	Tsw				0.61	-0.13	0.42	0.31	0.22	0.48	
	Hlw					-0.20	0.27	0.46	0.41	0.39	
	Mild						-0.12	-0.41	0.22ns	-0.54	
	Heig							-0.07	-0.64	0.74	
	Yr								0.21	0.45	
	Hi									-0.51	
2R+++	Yiel	0.55	-0.21	0.13	0.59	-0.76	-0.44	0.70	0.63	0.28	
	Hd		-0.17	-0.16	0.26	-0.49	-0.35	0.73	0.10	0.44	
	Lodg			0.03	-0.14	0.04	0.62	-0.15	-0.58	0.47	
	Tsw				0.09	-0.13	0.22	0.00	-0.09	0.26	
	Hlw					-0.38	-0.07	0.63	0.23	0.33	
	Mild						0.24	-0.65	-0.19	-0.30	
	Heig							-0.16	-0.83	0.55	
	Yr								0.10	0.62	
	Hi									-0.58	

+) H_0: $r_g=0$ is rejected at $p<0.05$=* when $|r_g|>0.28$, at $p<0.01$ when $|r_g|>0.38$ and at $p<0.001$ when $|r_g|>0.51$, ns=$p>0.05$

++) H_0: $r_g=0$ is rejected at $p<0.05$=* when $|r_g|>0.20$, at $p<0.01$ when $|r_g|>0.30$, and at $p<0.001$ when $|r_g|>0.40$, ns=$p>0.05$

+++) H_0: $r_g=0$ is rejected at $p<0.05$=* when $|r_g|>0.15$, at $p<0.01$ when $|r_g|>0.21$, and at $p<0.001$ when $|r_g|>0.29$

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VARIABILITY, HERITABILITY AND CORRELATION IN COLD AND YIELD CHARACTERS IN WINTER / FACULTATIVE BARLEY.

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INTRODUCTION. Barley is an important second crop in *Türkiye* with 3.525.000 ha planted area and, 7.500.000 ton production. With 2130 kg/ha yield is nearly equal to World barley yield (*State Statistics Inst.* 1995). The barley production area is largely restricted to *Central Anatolia* and *Southeastern Anatolia.*

Like other crops; barley yield is also results of mutual effects of morphological, physiological, and genotypic characteristics and environmental factors. Hence, to increase heritability of the yield it should be given emphasis not only to yield potential, but also to the other characteristics which determine the yield potential.

The purposes of the study were; to determine the available genetic variability and association among yield and yield components in some barley varieties released for *Central Anatolia and Transitional Zones in Türkiye*.

The research carried out by Amin, et al. (1992) on durum wheat, the genotypic coefficient of variation was found highest for grain yield (31.38), followed by harvest index (30.85), and grain numbers per spike (27.69). Heritability estimates in broad sense showed high value for days to anthesis (89.19%), grain yield (84.93%), and harvest index (80.00%) and moderate value for thousand kernel weight (72.58%). This study indicated that there was a significant positive correlation of grain numbers per spike with grain yield (+ 0.447*). Heritability estimates in winter facultative barley lines were highest for days to heading (82.6%), growth habit (66.2%) and maturity (65.0%) and lowest for grain yield (29.7%). Moderate heritability was shown by plant height (51.2%). There was a positive correlation between plant height and grain yield, but negative correlations between days to heading and maturity with grain yield (Cai et al. 1993). The study revealed that genotypic variance was very small compared to the environmental variance. This resulted in low heritability estimates for grain yield in winter wheat (068%-25%) (Yakar, 1984). As a result of the study conducted in Central Anatolia high elevation in Türkiye, significant negative correlation was determined among the yield with cold damage and winter damage (Ottekin et al. 1995).

MATERIALS AND METHODS. This experiment was conducted at eight locations; Haymana, Kırıkkale, Bala, Altınova, Ulaş, Koçaş, Ankara and Yozgat representing cold region in Türkiye's high elevation during 1990-1991, and 1991-1992 planting season, with eight winter facultative cultivars of barley (Hordeum Vulgare). Cultivar Tokak 157/37 was the oldest one, others were Cumhuriyet-50, Anadolu-86, Obruk-86; two rowed barley cultivars, six rowed barley cultivar Yıldırım, two rowed barley cultivars as recently registered Bülbül-89, Tarm-92, Yesevi-93. The experiment was laid out in randomized block design with three replications. The heritability and correlations of various agronomic characters were investigated. Genotypes data on spike numbers per square meter, grain numbers per spike, thousand kernel weight, grain yield, protein content, cold tolerance value were analyzed (Yürür et al. 1981).

Analysis of variance was calculated according to the methods suggested by Comstock and Moll (1963) and genotype x environment interaction variance was determined. Heritability estimates in the broad sense were defined as the proportion of

genotypic variance that was computed from the variance components according to the expectation of the mean squares, to the total variance. To determine the effects of genotype x environment, variance component method was used.

The parameters were defined as below;
y = The number of year
p = The number of locations
g = The number of genotype
r = The number of replications
$(X)_{ikjr}$ = Phenotypic value for (i). genotype, (k). year, (j). environment, (r). replication was formulated as fallows:

$$X_{ikjr} = m \pm g \pm yk \pm pj \pm (gp)_{ij} \pm (yp)_{kj} \pm (gyp)_{ikj} \pm (e)_{ikj}$$

RESULT AND DISCUSSION. Analysis of variance was used to determine if significant differences among the genotypes regarding to cold tolerance, yield, quality and yield components such as spike numbers per square meter, grain numbers per spike, thousand kernel weight, grain yield, plant height, and protein content. It was found that there was a significant differences among the cultivars at the 1% level for the traits indicating presence of genetic variability.

The genotypic co-efficient of variation was highest in grain numbers per spike (Table 1). Spike numbers per square meter and protein content showed the lowest genotypic co-efficient of variation. Other characteristics and grain yield exhibited moderate value of genotypic co-efficient of variation. If any character shows comparatively high genetic co-efficient of variation may response favorably to selection. Otherwise phenotype can not reflected genotype (Demir, 1975). The phenotypic co-efficient of variation indicated that the highest value for grain numbers per spike and the lowest value for protein content. All characters showed that small differences in genotypic and phenotypic co-efficient of variation, indicating less environmental influencing on them.

Heritability estimates in broad sense (Table 1) showed high value for cold tolerance, plant height, spike height, grain numbers per spike indicating that these characters were controlled by genetic factors. Heritabilities were moderate for grain yield, thousand kernel weight, protein content and low spike numbers per square meter. According to Demir (1975), heritability estimates provide the basis for selection on phenotypic performance, however if high heritability is due to additive gene effect, the genetic gain would be high.

Table 1. The phenotypic and genotypic co-efficient of variation for all characters together with their heritability estimates.

Characters	*Mean*	*Co-efficient of Variation*		*Heritability*
		Genotypic	*Phenotypic*	*(%)*
Cold Damage	5.49	12.20	12.87	0.90
Plant Height (cm)	74.95	9.30	9.80	0.90
Spikes / m^2	838.38	2.06	3.39	0.38
Spike Height (cm)	6.02	9.09	9.96	0.83
Grains / Spike	22.22	29.70	32.79	0.82
Grain Yield (kg/ha)	3285.60	14.41	16.52	0.76
1000-Grain Weight (g)	44.30	10.59	11.98	0.78
Protein Content (%)	12.24	0.81	1.02	0.64

In correlation study, the relations between cold damage and grain yield were investigated. Although, correlation value showed differences based on the cultivars, in general, grain yield was significantly and negatively correlated with cold. Same conclusion is drawn by Ottekin et al (1995). This relationship indicated that cold damage was one of the important limited factors for barley grain yield in the high elevation of Türkiye. On the other hand, there was a significant positive and negative correlation at different level between yield and yield components, yield and quality components. Especially, significant positive correlations were determined among yield with spike numbers per square meter ($r = + 0.781^*$) and plant height ($r = + 0.631^*$). The same result was obtained by Cai et al. (1993) related to the plant height.

Based on this study it can be concluded that significant genetic variability was determined between cultivars for the characters studied. Improving spike numbers per square meter and plant height characters could result in high grain yield in the high elevation of Türkiye.

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Effect of semi-dwarf mutants on salt tolerance in barley. H. PAKNIYAT, E. BAIRD, W.T.B. THOMAS, P.D.S. CALIGARI, W. POWELL and B.P. FORSTER, Department of Cell and Molecular Genetics, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland, UK.

Introduction. Salt tolerance in the Triticeae is correlated with shoot sodium accumulation (Greenway & Munns, 1980). Recent experiments by ourselves have shown that the *GPert* mutation has a positive effect on salt tolerance in barley (Forster *et al.*, 1994). The *GPert* mutation is located on barley chromosome 7 (5H) and is allelic to *Ari-e* (Franckowiak, 1991). We therefore decided to test other mutants at the *Ari-e* locus and compare their response to salt stress to that of other semi-dwarf mutations; *sdw* (syn. *denso*) and *ert-k*^{32}, located on chromosomes 3 (3H) and 6 (6H) respectively (Barua *et al.*, 1993; Gustafsson *et al.*, 1971 respectively). We also tested the response of these mutants and their parental lines to exogenous gibberellic acid.

Methods. Seeds of mutant lines and their parents were grown in hydroculture with the addition of 25 (control) and 175 (stress) mol m^{-3} NaCl as described in Forster *et al.* (1994). The experiment was carried out in a glasshouse at 16–24°C with supplementary lighting providing a 16 h photoperiod. After 4 weeks in test salt concentrations shoots were harvested, washed, dried and ground of a fine powder. Each sample (10 mg) was vortexed in 1 ml of deionised water and allowed to stand for 1 h. Sample Na^+ was measured using a sodium ion electrode (Lazar, ISM-146).

For the gibberellic acid sensitivity test, one week old seedlings were transferred to hydroculture tanks with or without the addition of 50 ppm GA_3. Shoot lengths were measured after 10 days.

Results.

Mutant	Control	Treatment
ari-e.1	10.19**	19.46**
ari-e.119	10.65*	19.22**
ari-e.156	10.58*	18.65**
ari-e.228	10.40**	18.53**
G. Promise	10.73**	19.47**
Diamant	10.31	21.24
Alf	10.56	21.86
Pallas	10.85	20.84

Parent	Control	Treatment
Bonus	11.21	21.51
Foma	11.55	21.21
Foma	11.55	21.21
Foma	11.55	21.21
Maythorpe	11.74	22.35
Valticky	10.47	21.38
Bomi	10.68	22.39
Bonus	11.21	21.51

Table 1. Mean shoot Na^+ content (mg of sodium/g dried shoot) of the mutant and parental lines in control (25 mol m^{-3} NaCl) and salt stress (175 mol m^{-3} NaCl), s.e.d. = 0.497. ** and * indicate where mutant lines have highly significant ($p<0.01$) and significant ($p<0.05$) different responses to their respective parental lines.

Mutant	$+GA_3/-GA_3$	Parent	$+GA_3/-GA_3$
ari–e.1	1.09	Bonus	1.39
ari–e.119	1.09	Foma	1.35
ari–e.156	1.09	Foma	1.35
ari–e.228	1.09	Foma	1.35
GPert (G. Promise)	1.08	Maythorpe	1.48
ert–k^{32} (Pallas)	1.33	Bonus	1.39
sdw (Diamant)	1.45	Valticky	1.52
sdw (Alf)	1.22	Bomi	1.27

Table 2. Ratio of shoot length in GA_3 over shoot length without GA_3 for the mutant and parental lines.

Discussion. In these experiments the *ari–e* mutants performed in a similar manner to *GPert* in both salt and gibberellic acid treatments. The *ari–e* and *GPert* lines were relatively more salt tolerant (accumulated less Na^+) and relatively insensitive to GA_3 (ratio close to unity) when compared with their parental lines and with other mutant dwarfing genes (*ert–k*^{32} and *sdw*). The results support the findings of Franckowiak (1991) that *GPert* and *ari–e* are allelic; we therefore suggest the *GPert* symbol is changed to *ari–e*.GP. The effect on salt tolerance was not found to be a general feature of semi–dwarfism, but specific to the *GPert* and *ari–e* genotypes. The *GPert* and *ari–e* mutants are characterised by semi–dwarf stature and short awns. The *ert–k*^{32} and *sdw* are also classed as semi–dwarf, but have a different growth habit and do not have short awns. The genes are also distinct in being located on separate chromosomes and probably have different modes of action: insensitivity to gibberellic acid may account for semi–dwarf stature in *GPert* and *ari–e* lines. The data suggest that *ari–e* mutants (including *GPert*) are better adapted to salt stress than other semi–dwarf genotypes. The isogenic relationship between Golden Promise and Maythorpe has been confirmed by randomly amplified polymorphic DNAs (RAPDs), no difference in band pattern could be found using over 300 10–mer primers, and also by AFLP analyses. In the latter case a band shift has been detected which may be associated with the mutation causing the change in growth habit, this is being investigated further.

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Barley and other cereals root development in a Brazilian acid soil. G. PERUZZO; G. ARIAS. EMBRAPA-National Wheat Research Center (CNPT). P. O. Box 569, 99001-970 Passo Fundo, RS, Brazil.

Introduction. In acid soils crops with low Al tolerance concentrate their roots in the upper soil layer. Cereal species have different tolerance levels and barley (*Hordeum vulgare*) is more sensitive than wheat (*Triticum aestivum*), sand oats (*Avena strigosa*) and rye (*Secale cereale*). The objective of this experiment was to determine the effect of barley root development in soil layers as deep as 60 cm.

Material and methods. Over the years of 1992 and 1993 the development of roots in soil up to a 60 cm depth was studied under field conditions. The length of roots in the soil layers was measured with a 5 cm reticular grid (Böhm, 1979). Each 5 cm soil layer was sampled for soil Al analysis. In 1992 the root system of two Brazilian barley genotypes, cv. BR2 and line PFC85104, sand oats, and the Brazilian wheat PF87453 were studied in a limed acid soil. After this first evaluation, the soil was replaced in the trenches and mixed with 400 g/m2 fine milled lime, in each 20 cm layer. In 1993, these trenches were cultivated with two Brazilian barley genotypes, cvs. MN599 and PFC85104, the Brazilian wheat cv. BR23, sand oats and the Brazilian rye cv. BR1. At heading the trenches were reopened to describe root development, at one side of the trench in soil limed from 0 to 20 cm and at the opposite side in soil limed from 0 to 60 cm.

Results and discussion. In 1992 (Table 1), a year of normal rainfall, more than 93 % of the barley roots concentrated in the upper 0-20 cm layer and were not deeper than 25 cm. In the deeper layers, Al was higher than 2.3 $cmol_c/L$ soil. Sand oats had 79.1 % and wheat 76.9 % of their roots in the first 20 cm, and the rest developed as deep as 60 cm and beyond (Table 1). In 1993 (Table 2), it was very dry before heading and the roots of barley genotypes developed deeper than in 1992, reaching 45 cm in the soil limed up to 20 cm. Barley cv. MN599 had 82.8 % and PFC85104 83 % of their roots in the first 20 cm layer (Tables 2 and 3). Rye had 61.1 %, sand oats 66.2 %, and wheat 72 % of their roots in the first 20 cm and the rest reached 60 cm and deeper (Table 2). In the trenches limed up to 60 cm depth, the percentage of roots in the 0-20 cm layer was 61.7 % for MN599 and 58.9 % for PFC85104 (Table 3). With deep liming the root development of barley was similar to the other cereals. Grain yield increase due to liming up to 60 cm were 63 % for cv. MN599 and 89 % for line PFC85104. MN599 had 49 % more heads per m^2 and 28 % less spike sterility in the plot limed to 60 cm, as compared to the 0-20 cm limed layer, which presented spike sterility of 54 % (Table 4).

Conclusions. Exchangeable Al up to 2.3 $cmol_c/L$ was a barrier for root development in the wet year, while in the dry year roots developed deeper in soil layers containing up to 3.0 $cmol_c/L$ soil. In a dry year superficial root development of barley caused by the presence of Al in soil layers below 20 cm could strongly reduce barley yield potential.

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Table 1. Al ($cmol_c$/L soil) and percentage of roots of barley cv. BR2 and line PFC85104, sand oats and wheat PF87453, developed in each layer of soil, in 1992

Soil depth (cm)	BR2		PFC85104		Sand oats		Wheat PF87453	
	Al	Roots (%)	Al	Roots (%)	Al	Roots (%)	Al	Roots (%)
0- 5	0.0	45.7	0.0	46.0	0.0	40.4	0.0	34.4
5-10	0.0	27.7	0.0	27.3	0.0	20.5	0.0	17.5
10-15	0.0	14.9	0.0	19.0	0.0	12.4	0.0	14.5
15-20	0.0	10.5	0.0	7.1	0.0	5.8	0.0	10.5
20-25	2.9	1.2	3.8	0.6	2.2	2.6	1.0	3.8
25-30	2.9	0.0	3.8	0.0	2.2	3.5	1.0	2.9
30-35	3.2	0.0	3.9	0.0	2.3	2.5	2.9	3.2
35-40	3.2	0.0	3.9	0.0	2.3	3.1	2.9	3.0
40-45	3.9	0.0	3.7	0.0	3.5	3.1	3.7	3.9
45-50	3.9	0.0	3.7	0.0	3.5	2.3	3.7	2.4
50-55	4.1	0.0	3.6	0.0	3.7	2.2	3.7	2.8
55-60	4.1	0.0	3.6	0.0	3.7	1.6	3.7	1.1

Table 2. Al ($cmol_c$/L) and percentage of roots of barley line PFC8104, rye cv. BR1, sand oats and wheat cv. BR23, developed in each layer of soil in 1993

Soil depth (cm)	PFC85104		Rye BR1		Sand oats		Wheat BR23	
	Al	Roots (%)	Al	Roots (%)	Al	Roots (%)	Al	Roots (%)
0- 5	0.0	43.5	0.0	32.0	0.2	33.4	0.0	42.5
5-10	0.0	19.2	0.0	13.7	0.2	15.8	0.0	14.3
10-15	0.0	12.0	0.0	8.3	0.2	9.2	0.0	8.5
15-20	0.0	8.3	0.0	7.1	0.2	7.8	0.0	6.7
20-25	0.7	5.6	0.0	8.9	1.4	5.6	1.6	5.4
25-30	0.7	3.8	0.0	7.0	1.4	5.0	1.6	4.3
30-35	2.0	3.3	2.2	5.1	2.9	4.0	2.8	3.6
35-40	2.0	2.2	2.2	3.9	2.9	3.4	2.8	3.3
40-45	2.7	1.1	2.8	4.2	2.9	3.7	3.2	3.3
45-50	2.7	1.0	2.8	4.0	2.9	4.0	3.2	3.4
50-55	2.9	0.0	3.1	3.4	3.0	4.8	3.2	2.7
55-60	2.9	0.0	3.1	2.4	3.0	3.3	3.2	2.0

Table 3 Al ($cmol_c/L$) and percentage of roots of barley cv. MN599 and line PFC85104, developed in each layer of soil limed at 60 cm depth in 1993.

Soil depth (cm)	MN599				PFC85104			
	20 cm		60 cm		20 cm		60 cm	
	Al	Roots (%)	Al	Roots (%)	Al	Roots (%)	Al	Roots (%)
0- 5 cm	0.0	40.2	0.0	16.5	0.0	43.5	0.0	15.2
5-10 cm	0.0	19.4	0.0	17.4	0.0	19.2	0.0	18.6
10-15 cm	0.0	12.9	0.0	15.3	0.0	12.0	0.0	14.6
15-20 cm	0.0	10.3	0.0	12.5	0.0	8.3	0.0	10.5
20-25 cm	2.3	6.4	0.0	7.7	0.7	5.6	0.0	8.4
25-30 cm	2.3	3.8	0.0	5.7	0.7	3.8	0.0	7.6
30-35 cm	2.7	3.5	0.0	6.2	2.0	3.3	0.0	6.4
35-40 cm	2.7	2.4	0.0	5.1	2.0	2.2	0.0	5.0
40-45 cm	3.0	1.1	0.0	5.1	2.7	1.1	0.0	4.1
45-50 cm	3.0	0.0	0.0	3.8	2.7	1.0	0.0	3.1
50-55 cm	3.3	0.0	0.0	3.0	2.9	0.0	0.0	3.9
55-60 cm	3.3	0.0	0.0	1.7	2.9	0.0	0.0	2.6

Table 4. Yield components of barley cv. MN599 and line PFC8104 limed (L.) up to 20 cm and up to 60 cm depth, percentage in relation to the L 0-20, in 1993

Soil layer limed cm	heads/m^2		kernel/head		1000 kernel weight		Spike sterility	Yield	
	(%)		(%)		g	(%)	(%)	kg/ha	(%)
MN599									
0-20	364	(100)	9.7	(100)	41.4	(100)	54.2	1550	(100)
0-60	543	(149)	15.6	(161)	30.4	(73)	26.2	2520	(163)
PFC85104									
0-20	324	(100)	17.8	(100)	22.9	(100)	12.1	525*	(100)
0-60	384	(119)	26.2	(147)	28.2	(123)	4.2	990*	(189)

* Yield loss due to *Stagnospora nodorum* incidence.

Germination and yield performance of 110-year-old oat and barley seeds, the VIENNA SAMPLE of 1877. Verification of effective ultra-dry storage at ambient temperature

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Summary

After 110 years of hermetic sealed storage at temperatures between 10 and 15°C and at a moisture content of 3.12 % the extremely heterogeneous oat sample of yellow and white kernel types showed 81 % germination. This demonstrates that ultra-dry, long-term seed storage under ambient temperature conditions can successfully be achieved with the intention of cutting down risks and costs in germplasm conservation.

The results of the field tests of the progenies of this land race from the former Austro-Hungarian cereal districts showed that its yield performance was about 52 % lower compared with present oat varieties in Austria and Germany. The reason for this was found in the lower grain numbers/spike, the very low thousand kernel weights and the high susceptibility for lodging together with a poor resistance to crown rust (*Puccinia coronata* Corda).

Introduction and results

In 1877 F. HABERLANDT, the first professor of Agronomy at the 1872 founded University of Agronomy in Vienna („BODENKULTUR"), having proven the advantage of low moisture hermetic sealed seed storage, had prepared glass vials for long-term storage experiments with wheat, barley, rye and oat seed, respectively. Shortly afterwards he died and the samples fell into oblivion. They were stored in a cellar about 15°C and rediscovered in 1967. The vial with wheat was opened and from 400 seeds 14 germinated (RUCKENBAUER: *Die Bodenkultur* **22**, 372-386, 1971). In 1987 the other three vials were opened, the analysis of the oat sample yielded the following results:

Total mass	27.2 g
Pure seeds	90.9 %
Other seeds	7.8 %
Inert matter	1.3 %
Oat seeds	1235, TKM 19.8 g
Germination	81 %
Barley seeds	54
Germination	91 %

Fig. 1. The refilled glass vial of the Vienna Sample of 1877 of oats

With decreasing moisture content the influence of temperature eventually becomes unimportant for longevity, and below a critical value even moisture no longer shows a significant effect on longevity (ELLIS et al.: *Am. Bot.* **65**, 493-504, 1990)

Hence, proposals were put forward for exploring ultra-dry storage under ambient temperatures as border-line case with view to cutting down costs in germplasm conservation (ELLIS et al.: *Plant Var. Seeds* **6**, 75-81, 1993). Having practically preserved the initial high germination percentages (97.5) after 110 years of storage at ambient temperature, the Vienna Sample of 1877 presents experimental proof that this can successfully be achieved!

Whereas the various progeny-tests of the oat sample showed the expected low yielding performance compared with the oat varieties at present (more than 52 % lower yields), a surprisingly high genetic variability was detected within 44 germinating barley grains, which were found also in the oat sample (SCHULZE et al.: *Plant Var. Seeds* **7**, 193-197,

1993). The hordeins extracted from this seed and separated by SDS-PAGE and PAGE pH 3.1 could be grouped into 26 different biotypes (Fig. 2). Protein patterns in the upper part of the gel were marked by the letter H (high molecular) and a number, patterns in the lower part of the gel by the letter L (low molecular) and a number. Thus each analysed seed could be described by a combination of four different protein patterns (C, B, H, L) representing its electrophoretic type.

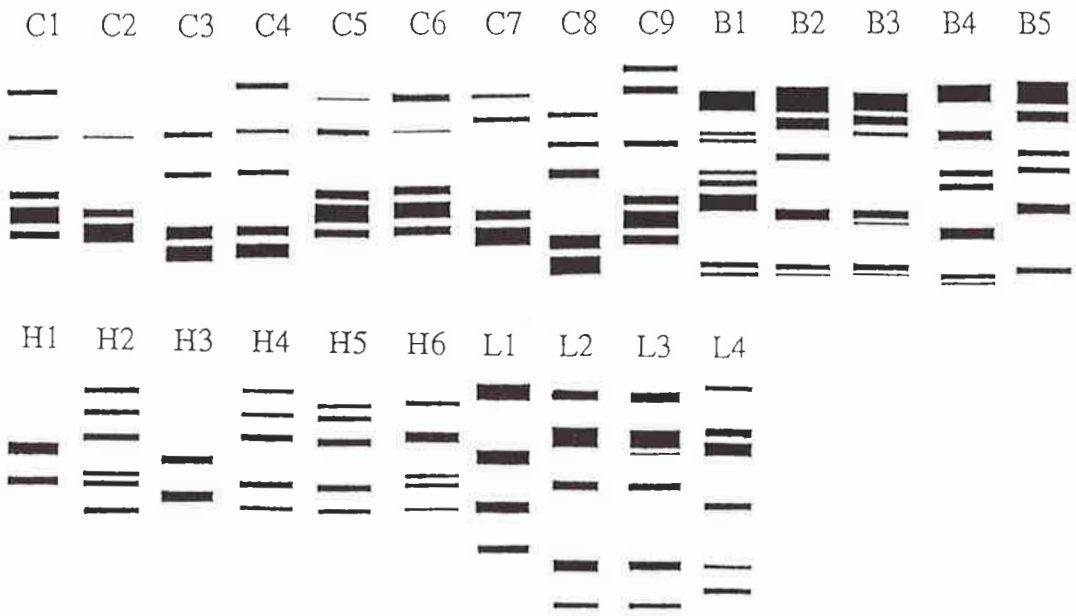

Fig. 2. Electrophoretic subunit patterns of hordeins of the 43 offspring of the Vienna Sample of barley of 1877 separated by SDS-PAGE (C- and B-patterns) and acid PAGE (H- and L-patterns)

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Performance of oat and barley in Eucalyptus based agroforestry system on salt affected soils. H.K. SINGH, C.B. SINGH and BANWARI LAL, Agro-Energy centre, Division of Agronomy. Indian Agricultural Research Institute, New Delhi-110012 INDIA.

Introduction. The introduction of fast growing exotic tree species like *Eucalyptus* in agroforestry systems seems to be a most compatible to tie over the present food, fuel, fodder, oil and energy crises. In India, about 10.4 M ha area is affected due to salinity and alkalinity (Singh, 1992). Although, *Eucalyptus* tree on arable land has been a subject of criticisms without experimental evidences. The experimental results have proved that *Eucalyptus* is one of the least water consuming sp.(as 1.4 mm/g) for dry matter production in comparison to other tree sp.(Chaturvedi, 1983). This have been miss conception regarding intercropping of arable crops under *Eucalyptus* plantation such as severe attack of insects, pests and disease, reduction of total quantum of solar radiation, execessive use of water and nutrients and finally reduction of interspace. The experimental results on various aspects have thrown convincing evidences that comparable intercepts under *Eucalyptus* plantation gave sufficient higher biomass (grain, fodder, fuel/wood) for multipurpose uses in a sustainable manner for longer period. It suits well in traditional Indian farming systems. Also because of specific site situation of Indo-Genetic main zone where moisture fluctuation limit successful farming programme, compatible combination of *Eucalyptus* high density tree plantation and intercrop with various grain and forage species caters the needs of over population of human and livestock of the tract. Keeping the above facts in mind, a more specific programme to explore the potentialization of intercrops like oats and barley, sorghum and maize under various plant populations of *Eucalyptus* have been undertaken to satisfy the quarries stated above.

Materials and methods. The experiment was conducted in five year old plantation at the research farm of Indian Agricultural Research institute, New Delhi-110012 situated at 28.4^{0}N latitude and 77.10^{0}E longitude at altitude of 228.16 m above mean sea level. It has semi-arid and sub-tropical climate with hot summer and cold winter. Soil of the experimental field was loam in texture (40% sand 39% silt and 21% clay), medium in organic carbon, P_2O_5 and N and low in potassium. The crops, oats (PO-3) and barley (D2-454) were included in the test for fresh and dry biomass production. The intercrops were sown in 2nd week of November and harvested in the 3rd week of March in both the years.

Results and Discussion. The fresh and dry biomass yield of oats and barley were recorded higher significantly when intercropped with 3958 trees/ha as compared to other inntercropping combinations. But, these yields were about 20 per cent less in comparison to the yields produced by pure crops (without trees). However, the fresh and dry biomass yield were drastically reduced due to high density plantation i.e. 17083 trees/ha (65% fresh and dry biomass). The reduction in yields were observed in linear manner and detail results are presented in Table-1. Barley outyielded oats by 4.6% during 1989-90 and 5.52% during 1990-91 in terms of fresh and dry biomass grown as sole and intercrops.

Maximum solar radiation was intercepted by oats and barley crops at 50 DAS as compared to 75 DAS in both the years. Interception of solar radiation was drastically reduced due to high tree densities and reached only up to 30% in 17083 trees/ha. However, the reduction in solar radiation upto 46% in 3958 trees/ha, the biomass yield of oats and barley remain same in comparison to sole crops (Table-1). Thus, the data indicated that 5000 trees/ha of *Eucalyptus* can be planted for intercropping of forage sp. under these conditions.

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Table -1. Biomass accumulation by oat and barley as intercrops in Eucalyptus densities.

Eucalyptus trees/ha	Biomass yield (t/ha)			Solar radiation ($MJ/m^2/day$)			
	Oats and Barley		Eucalyptus	1989-90		1990-91	
	1989-90	1990-91	1991	50 DAS	75 DAS	50 DAS	75 DAS
Tree densities							
Open	39.09 (6.95)	38.57 (6.71)	-	7.50	6.18	8.26	6.66
17083	14.05 (2.49)	13.94 (2.43)	188.95	2.27	1.79	2.48	1.88
10138	17.82 (3.16)	17.62 (3.04)	187.76	2.56	2.02	2.80	2.17
9652	20.16 (3.58)	19.36 (3.40)	164.37	2.63	2.06	2.84	2.25
7842	22.45 (3.46)	21.24 (3.73)	163.46	2.70	2.16	2.99	2.38
7222	24.11 (4.30)	23.13 (4.06)	161.16	2.82	2.22	3.13	2.44
6944	26.84 (4.49)	26.01 (4.58)	160.13	2.91	2.35	3.28	2.57
5347	28.68 (5.12)	27.64 (4.85)	140.61	3.14	2.60	3.61	2.81
4789	28.62 (5.20)	27.77 (4.89)	130.20	3.13	2.59	3.59	2.88
3958	31.87 (5.68)	30.15 (5.31)	125.39	3.46	2.84	4.04	3.07
CD at 5%	1.75 (0.24)	1.59 (0.35)	12.05	0.34	0.46	0.67	0.48
Intercrops							
Oats	24.80 (4.11)	23.57 (3.98)	-	3.31	2.69	3.70	2.91
Barley	25.94 (4.82)	24.87 (4.67)	-	3.31	2.67	3.67	2.91
Cd at 5%	0.78 (0.10)	0.62 (0.62)	-	NS	NS	NS	NS

NS - Non significant

Figures in parenthesis are dry biomass yield.

Effect of seeds rates and phosphorus on growth, yield and nutrient uptake of oat varieties. H.K. SINGH, M.V. VENUGOPALAN, BANWARI LAL and C.B. Singh, Agro-energy cntre Division of Agronomy. Indian Agricultural Research Institute New Delhi-110012 INDIA.

Introduction. Oats (*Avena sativa* L.) is most important and ideal winter cereal fodder crop grown on about 1.0 lakh ha in India (Hazra, 1995). It occupies a very significant place in intensive fodder cropping systems for modern dairy needs of India (Mehra, 1978). The production potential of oat depends on moisture and nutrient management to a great extent (Gill *et al*, 1976). The detail studies on population densities (seed rate) and Phosphorus requirement which directly influences the total biomass production has not been taken in detail studies in sub-tropical India. Whenever, new varieties evaluated their growth performance is a first need to be tested on field level for grain and green fodder production.

Materials and methods. A field experiment was conducted during winter season at Research Farm of Indian Agricultural Research Institute, New Delhi-110012, India to study the seed rate, Phosphorus levels of oat varieties. The soil was neutral in reaction (pH 7.3), deficit in nitrogen (0.06%N), Medium in Phosphorus (10.46 Kg/ha) and potash (186 kg/ha). The experiment was laid out in randomized block design. Sowing was done manually by Kera method (behind plough) at a uniform row to row distance of 25 cm. The seed was sown on 70 and 100 kg/ha as per treatment in rows, N was applied in the form of urea in three splits (50% basal + 25% at tillering + 25% at pre-bloom stage). Graded doses of phosphorus at the rate of 0, 40 and 80 kg P_2O_5/ ha in the form of single super phosphate were applied in the randomly allotted plots as basal application. A uniform basal dose of potassium at the rate of 30 kg K_2O/ha in the form of murate of potash was also applied. The varieties used in the test (PO-1, PO-2 and Kent) sown in 3rd week of November and harvested in last week on March. Five irrigations were given during crop season. The entire produce was harvested in one cut taken at milk stage.

Results and Discussion. The data on the green fodder production are presented in Table-1. The variety PO-1 and PO-2 were at par with respect to rate of green fodder production and significantly superior to Kent. Application of 40 and 80 kg P_2O_5/ha produced significantly more green fodder over control. Varying seed rates were toatally fail to produce any significant difference in rate of green fodder and dry matter yield/ha (Table-1).

The highest N uptake and protein yield was recorded by variety PO-2 which is at par with PO-1. Application of 40 kg P_2O_5/ha resulted in a significant increase in the nitrogen uptake over control. But a further increase in phosphorus level i.e. 80 kg P_2O_5/ha failed to increase crude protein, N and P uptakes. The Phosphorus application results are in close conformity with the results obtained on various research centres in India. i.e. Kanke (Bihar) Pantnagar (U.P.), Palampur (H.P.), Jabalpur (M.P.) and Hissar (Haryana), Anonymons (1983).

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Table -1. Green biomass, dry matter, protein production, N and P uptake of oat varieties as influenced by seed rate and phosphorus levels.

Treatments	Yield (q/ha)			Uptake (kg/ha)	
	Green fodder	Dry matter	Crude protein	N	P
Varieties					
P0-1	366.0	92.33	9.56	152.89	25.20
P0-2	375.0	97.31	9.87	157.78	29.66
Kent	337.0	86.31	8.33	133.27	25.33
CD at 5%	19.3	4.86	0.52	8.48	3.27
Seed rate (kg/ha)					
70	357.0	92.69	9.27	148.19	26.60
100	362.0	91.20	9.24	147.78	26.90
CD at 5%	NS	NS	NS	NS	NS
P_2O_5 (kg/ha)					
0	317.0	78.64	7.91	126.61	17.14
40	375.0	98.06	9.93	157.72	31.12
80	386.0	99.14	9.92	158.61	31.98
CD at 5%	19.3	4.84	0.52	8.48	3.27

NS - Non significant

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NS - Non significant

Barley Breeding for Winter Survival in Continental Mediterranean Environments. M. TAHIR[1], S. CECCARELLI[1], H. TOSUN[2], A. OTTEKIN[2], and V. SHEVTSOV[3], [1]ICARDA, P.O.Box 5466, Aleppo, Syria, and [2]Central Field Crops Research Center, Ankara, Turkey, [3]Agricultural Research Insititute, Krasnodar, Russia.

Introduction: Considerable damage to barley is caused by severe cold at various plant development phases in the continental mediterranean environments of Central and West Asia which is planted in conserved moisture. In major parts of these areas the long, severe winters, where the temperature can often plunge below -25°C, are followed by short springs and hot dry summers. The fluctuations in temperature are enormous. The barley crop has to pass through severe and variable cold during its vegetative phase and then complete its reproductive phase quickly to avoid terminal drought and high temperature. The suitable cultivars therefore must possess winter/cold survival traits and earliness in maturity. In view of the complexicity in cold tolerance and winter survival a three way approach has been adopted to develop suitable barley germplasm:

I. Identify lines which have deep crown node.
II. Slow primordia development during winter and rapid growth during spring.
III. Screening of the breeding material in the target environments under field and controlled conditions.

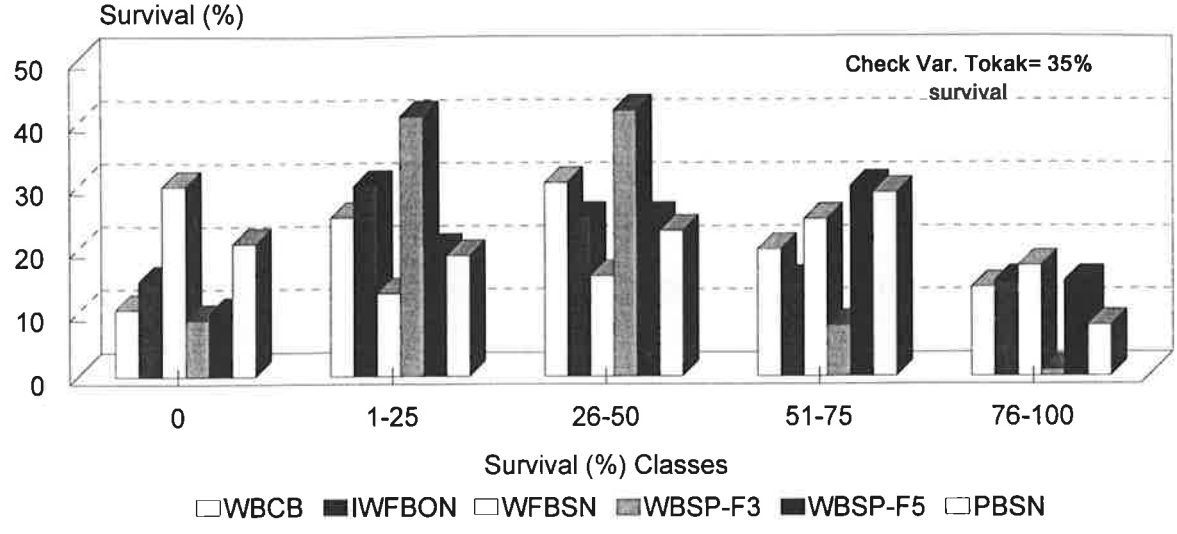
The results of studies carried out to develop suitable barley germplasm for winter survival are briefly reported.

Materials and Methods: Three types of barley material, i.e., parental lines (180 accessions), 850 F3 segregating populations and 835 genetically advanced lines were evaluated at Haymana Research Farm of the Central Field Crop Research Center, Ankara-Turkey. The material was planted in augmented design with systematic checks. The advanced material was then tested under controlled environments in freezing chamber (Warners and Johnson, 1972) at -11°C. Thereafter, 250 selected cold tolerant lines were tested for their primordia development (Inamura, *et al.*, 1955) during winter and early spring and crown node depth, because both these traits contribute to increased winter survival and drought tolerance. The data on crown node depth, tillers/plant, primordia development Stage (PDS) after 90 days of germination, culm length (cm), days to maturity and cold survival (%) were recorded.

Results and Discussions: In the first cycle of selection for cold tolerance under field conditions the percentage of lines with survival of over 75% was low in all the nurseries. From the data in Fig. 1, it is clear that there is enormous amount of genetic variability for cold tolerance in the tested material and the lines/varieties can be classified into different groups on the basis of their tolerance. Repeated

field testing is required to develop cold tolerant germplasm to overcome the problem of year to year fluctuation in temperature. It is also obvious that Ankara is a good location for evaluation of winter barley material for cold tolerance. A crown freezing test of the field selected cold tolerant lines/varieties was carried out under controlled environments at -11°C.

Fig. 1: Winter survival(%) in barley at Ankara



A great majority of the lines were found to be cold tolerant. This test is helpful in discarding those lines which escaped cold under field conditions. There is considerable genetic diversity for cold tolerance in the tested material (Fowler et al., 1977, Tahir *et al.*, 1991), however, adaptability of these cold tolerant lines in the continental mediterranean environments is essential. Therefore these lines were evaluated for other growth characteristics mentioned above. The data on the top 10 lines/varieties are summarized in Table 1. From these data it is obvious that it is possible toidentify winter type barley lines with deep crown node and at the same time have high tillering capacity which is in conformity with our earlier studies on these traits (Tahir and Shevtsov, 1994). Some of these lines, i.e. Roho/Mazurka//ICB103020; ZDM-938, Lignee131/5/cq... and ICB 101302 were found to have slow primordia development during winter but were much earlier in maturity. Therefore, this kind of germplasm is highly adapted to the continental mediterranean environments where the barley cultivars can suffer due to severe cold in winter and then because of terminal drought.

Therefore, to overcome the complex problem of cold and terminal drought, a multifacet approach (Tahir, *et al.*, 1991) based on field, and growth chamber evaluation in combination with desirable physiological/phenological attributes can be very useful in developing cold and drought tolerant germplasm for the continental mediterranean environments.

Table 1: Charcteristics of cold tolerant barley lines/varieties

Variety/Line	CND (cm)	Till./P	PDS	CL (mm)	DM	Cold Surv. %
Tokak	3.9	6.0	V	7	180	60
Roho/Mazu//ICB 103020	5.0	5.8	VII	7	173	90
ZDM - 93.8	5.1	4.6	V	7	175	80
Radical	4.4	4.0	VII	5	195	90
Plaisant	5.5	5.0	VI	4	194	90
Narcis	6.0	6.0	V	6	190	100
Dictoo	6.0	3.0	V	4	192	95
NE 89747	6.8	2.6	VI	4	182	100
Lignee 131/5/cq/...	5.8	4.4	V	7	174	80
Yugodar	5.7	3.8	V	6	185	100
ICB 101302	7.1	3.4	VI	6	177	90

PDS: Primordia Development stage after 90 days of germination.
CL: Culm Length; after 90 days of germination. DM: Days to Maturity. CND: Crown Node Depth.

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CANADIAN SPRING BARLEYS IN NORTH-WEST RUSSIA. I. Terentjeva, O. Kovaljeva, N.I. Vavilov Inst. of Plant Industry, B. Morskaya str.44, St. Petersburg, 19000 Russia.

Introduction. For producing new cultivars of barley which will satisfy current requirements of agriculture serious attention is paid to developing a germplasm collection. Canadian barley cultivars in the VIR collection consist of 290 samples, accessed between 1905 and 1995.

The samples are represented by varieties and selections mainly var. pallidum Ser., rikotense R.Reg., parallelum Koern., and single forms of medicum Koern., nigrinudum Vav., nigrum Lenk., nudum L. and coeleste L.

A study of the performance of Canadian barley cultivars in north-western Russia had been carried out to reveal the main source of valuable genetic characteristics.

Materials and Methods. In north-western Russia during the last 10 years 160 Canadian barley cultivars have been tested. Estimates of fungus disease resistance and insect resistance (frit fly - Occinella pusilla Mg) were made and agrobiological value was recorded according to VIR methodical instructions (1983). The collection was sowed in Pushkin.

For studying resistance to loose smut in the field artificially inoculated vacuum method was used (Krivchenko, 1983).

Benzilamide method had been applied for powdery mildew inoculation in the laboratory conditions. In the field powdery mildew resistance has been tested on provocative fone. The resulting infection was rated relative to the infection occurring on the susceptible control variety Pirkka.

Results. The Canadian barley cultivars in the VIR collection included early and medium maturing types and most were susceptible to lodging.

Some cultivars ripened 3-5 days earlier than the early ripening standard Potra. Such cultivars as Peguis, Perth, Klondike, Laurier, Leger, Buck, GP37, GP57, GP65, Otali, Jackson had a vegetative period of 75-80 days. The cultivars Buck and GP57 possessed a positive combination of early ripening and high yield.

It is interesting to emphasize that variety Buck v. coleste was one of the highest yielding naked forms.

Cultivar Rodeo was high yielding among the medium maturity forms. Cultivar Harrington had high yield and good malting quality.

Meteorological conditions on north-western Russia favored lodging. Cultivars Bruce, Heartland 1, Tupper, Samson, Ellice were resistant to lodging.

Some fungus diseases are intrinsic for this region.

Powdery mildew, loose smut and leaf rust were the most harmful diseases of barley. All cultivars from Canada were heavily infected with powdery mildew, only cultivar Ellice had an intermediate reaction type.

Cultivar Heartland 1, Pillsburg, Trent, Peguis, Kinkora, Leduc, Leger and selection lines BVN 66-2, BVP 54.5 were resistant to loose smut. Cultivar Heartland 1 possessed complex resistance to 3 types of smut.

In 1989 and 1995 we observed high infection levels of dwarf and stem rusts. Only single samples of resistance to these pathogens were evident. Among Canadian cultivars only the cultivar Morrison had resistance. Severe damage from the frit fly in north-western Russia occurs in the tillering stage leading to a large harvesting loss.

Canadian cultivars in north-western Russia normally do not yield higher than our standards Belogorskiy and Krenichniy. However, in the extreme meteorological conditions of 1995 such cultivars as Symko, Morrison, Duke and Manley yielded higher than our standards.

Table 1. Agronomic data for some Canadian cultivars in north-western Russia, 1995.

Cultivar	Yield (kg/m^2)	Vegetative period (days)	Resistance to[a] Pow. ml	Dw. rust	St. rust
Duke	0.48	95	1	1	1
Manley	0.50	98	1	1	1
Symko	0.32	93	1	1	1
Morrison	0.42	93	1	9	9
Potra	0.38	80	1	1	1
Belogorskiy	0.30	90	3	3	3
Krenichniy	0.30	92	5	3	3

[a]1-susceptible; 9 - resistant.

Which genes could be targets for drought tolerance improvement in barley? D. THIS, G. ARNAU, B. TEULAT, S. LEWICKI, C. BOREL*, T. SIMONNEAU*, C. BORRIES, I. SOUYRIS, P. MONNEVEUX, ENSAM-INRA, Genetics and Plant Breeding Department (*:LEPSE), 2 place P. Viala, 34060 Montpellier cedex, France.

Introduction. Drought limits crop yields in many environments. Cereals and particularly barley have developed a large range of tolerance strategies that could be used to improve yield stability in cultivated varieties. Genetic engineering via transformation (if one or a few genes are involved) or marker assisted introgression could help us to develop rapidly more tolerant genotypes. But drought tolerance appears to be a complex character, and then, if we want to obtain an interesting tolerance for the breeder, which genes do we have to introgress?
In order to analyse the real impact on tolerance of genes involved in the stress reaction, we have developed a comparison between nine barley lines from different origins, considered as "tolerant" and "susceptible" based on their yield stability behaviour. This short paper and the corresponding poster are an attempt to summarise the different research programs concerned with drought tolerance in barley hold in Montpellier.

Material and methods. The nine analysed lines are six row or two row lines from different origins: Albacète (Spain, 6 rows), Tichedrett (Algeria, 6 rows), LM 2887 (French advanced line, 6 rows), Alpha (France, 2 rows), Tadmor (ICARDA, Syria, 2 rows), Plaisant (France, 6 rows), Express (France, 6 rows), Mogador (France, 2 rows), and ER/APM (Syria, 2 rows).
All of those lines were evaluated for agronomic (yield parameters), morphological (plant architecture), physiological (water status and photosynthesis parameters), biochemical (xylem ABA and protein characterisation) or gene expression (molecular hybridisation after northern blotting) changes during drought stress. An F8 segregating population issued from a cross between Tadmor and ER/APM was used for genetical QTL research. Except for the two first types of characters, all measurements where done in controlled conditions as described by Lewicki et al. (submitted).

Evaluation of tolerance mechanisms. *Yield stability* evaluated in multilocal experiments is considered as our "tolerance" criterion. Field data allow us to consider in a first approach LM 2887, Albacète, Tichedrett, Alpha and Tadmor as "tolerant" whereas Plaisant, Express, Mogador and ER/APM are considered as "susceptible".
Morphological and physiological parameters were evaluated in growth chamber assays. Plants were grown in pots with the same substrate and stress was applied at the four leaves stage by withholding water.
Soil water content was followed during all experiments. The kinetics of soil drying is different for the nine lines analysed. This difference in soil drying could be explained by a different plant architecture and a different growth response during stress. No specific ideotype of tolerant lines could be identified. Leaf area and root development play an important role, but are difficult to measure in a large

experiment. However, soil water content is an important parameter to follow in order to compare stress response.

Stomatal conductance (gs) was followed for all of the nine lines in different greenhouse experiments. A difference was found, among the lines, between maximal stomatal conductance (gsmax) measured on well watered plants. Generally a higher gsmax was found in susceptible lines. The difference observed between the two Syrian lines was associated with differences in stomatal density, higher for ER/APM than for Tadmor, but the opposite result is found on the French lines. An important decrease was found for gs during stress, but when gs was related to the maximal stomatal conductance no difference was observed among the different lines. Therefore stomatal closure during stress cannot be considered as a good target for tolerance improvement in our case.

Water status was assessed during the stress imposition. Tolerant lines generally maintained a higher relative water content during water deficit than susceptible ones. As calculated by Morgan's methodology (1983), Albacète, Alpha, LM2887 and Tadmor (all tolerant lines) presented a high osmotic adjustment capacity compare to Plaisant, Express and ER/APM (susceptible) (Teulat et al., submitted). Osmotic adjustment capacity, considered as a good way to avoid drought stress by maintaining turgor, could then be a good target for tolerance improvement. But an exception was given by Tichedrett, considered as tolerant but presenting a low osmotic adjustment capacity.

Photosynthesis maintenance during stress is another way to sustain yield in limited conditions. At the more severe stress Albacète, Tichedrett and Alpha (tolerant) presented a significant higher CO_2 assimilation rate than Plaisant, Express and Mogador (Arnau et al., in press). A good photosynthesis maintenance is the only trait among those measured that could explain Tichedrett tolerance, whereas both osmotic adjustment and photosynthesis capacity interact for the other lines.

Biochemical characterisation. *Xylem ABA* , considered as a general stress signal, was measured in sap, extracted from leaves and roots systems, for LM2887, Express, Plaisant, Tadmor and ER/APM during water deficit together with stomatal response. When soil water content decreased an important increase was observed in xylem ABA content in both organs (Borel et al., submitted). Tadmor presented a slightly higher root ABA level and a lower leaf ABA level. Differences in drought induced ABA accumulation and in stomatal conductance sensitivity to ABA were evidenced among lines. LM 2887 and ER/APM accumulated more ABA at midday when the soil dries than the three other lines, but their stomatal sensitivity to ABA was lower. However these differences among lines were not consistent with their contrasted drought susceptibilities.

Leaf proteins and RNA were extracted at different levels of stress in another experiment (Arnau et al., submitted). *In vitro* translation and mono- or bidimensionnal PAGE separation of proteins allowed us to detect several proteins specifically expressed or regulated during stress, but few are specific for tolerant lines. However two polypeptides with a molecular weight of around 30kD and a protein of 22 kDa, specifically abundant in tolerant lines under severe stress, could be good candidates for further studies.

Candidate genes evaluation for drought tolerance. Different molecular probes corresponding to genes involved in the physiological mechanisms described previously, or with a putative role in drought stress response were collected from different laboratories, and evaluated for their expression during stress in tolerant and susceptible plants.
When related to the water stress intensity no difference was observed in three late embryogenesis abundant (lea) gene expression pattern between tolerant and susceptible lines. No difference was either detected in a saccharose synthase gene expression between the lines. Accumulation kinetics of RNA corresponding to chloroplastic proteins involved in photosynthesis were also studied. No modification was observed in all cases for a chlorophyll binding protein (CAB) gene expression during stress. A slight decrease was observed in both cases for atpB (acting in the pSI photosystem) and psaB (B-subunit of the chloroplastic ATPase) gene expression. On the other hand, susceptible varieties showed a high decrease in Ribulose 1.5 diphosphate carboxylase RNA accumulation during stress whereas it remained constant in tolerant lines (Arnau et al., submitted). Except for this gene, no difference in the transcriptionnal regulation of the candidate genes analysed could account for tolerance variability. Translational or posttranslational regulation could be rather involved.

Relative localisation of QTL for drought tolerance criteria and known function genes in barley chromosomes. A genetical approach has been initiated in order to localise key chromosomal regions involved in some tolerance parameters. The segregating population derived from the cross between Tadmor and ER/APM is being used to identify molecular markers (mainly RFLP markers from a comparative cereal study) linked to QTLs (Quantitative Trait Loci) related to drought tolerance, and especially QTLs related to osmotic adjustment capacity. Some other parameters' variation, such as leaf colour, number of tiller, fresh and dry aerial matter are also studied.
At the same time, known function genes polymorphism obtained via RFLP and PCR is being used to localise those genes on the same map. This will provide information on the direct or indirect role of those genes in the tolerance mechanism. First results will be presented in the poster session.

Conclusion. Both osmotic adjustment and photosynthesis maintenance appear to be major mechanisms involved in drought tolerance in barley, and the corresponding genes could be gene targets for tolerance improvement. Since many functional genes are already identified, the evaluation of their variability and regulation will help us to precise their role in tolerance. The identification of new tolerance markers implies a carefull comparison of different lines with different behaviours to avoid confusion between stress and tolerance markers, and precise the level of regulation involved. Beside this, the identification of molecular markers linked to the chromosomal regions involved in tolerance variation will provide tools for a rapid screen of genetic resources and for a marker assisted introgression of the desired tolerance into good cultivated lines.

Assessing relationship between yield and carbon isotope discrimination for barley across a wide range of rainfed Mediterranean environments

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INTRODUCTION. The Mediterranean region is characterized by the presence of important seasonal and spatial fluctuations in barley yields, causing large G×E interactions and thus hindering the process of screening and testing breeding material suitable for a particular area. Carbon isotope discrimination (Δ) is a physiological criteria susceptible to be incorporated into comercial breeding programmes aimed to improve tolerance to drought. As an integrative indicator of the crop water-use efficiency (WUE) (Farquhar and Richards, 1984), it has deserved growing attention in the last years. However, it remains unclear whether to favour selection for high or low Δ in rainfed environments (Hall et al. 1994), as well as their ability to assess G×E under complex interaction patterns (Romagosa and Araus, 1991).

The additive main effects and multiplicative interaction (AMMI) model is a powerful statistical tool for analysing G×E interaction (Zobel et al. 1988). The objective of this study was to examine G×E in a large set of rainfed environments, based on the AMMI model, for a further assessment of the genetic relationship present between Δ and grain yield.

MATERIALS AND METHODS. Five two-rowed and five six-rowed barley cultivars were grown in 22 environments in the 1992, 1993 and 1994 growing seasons in Northern Spain. Randomized complete block designs with four replications were used. Grain yields ranged from 1.1 t/ha to 6.5 t/ha. A simplified climatic characterization for each environment was obtained by the total rainfall to reference evapotranspiration (as calculated by the Hargreaves method) ratio (R/ETo).

G×E interaction was partitioned according to a predictive AMMI model (Gauch and Zobel, 1988). The optimum number of interaction principal component axes to be retained in the model (IPCA1 and IPCA2) was determined by the lowest value for the root mean square predictive difference after 40 validations runs. Based on the AMMI2 predictive model, estimates for all genotype by environment combinations were generated and used for input into cluster analysis (Crossa et al., 1990) and for determination of genetic correlated responses with Δ.

Ground mature grains and peduncles at heading were analyzed separately for stable ^{13}C isotope in a mass spectrometer, and corrected for the carbon isotope composition of air (-7.85‰), thus obtaining carbon isotope discrimination (Δ_{kernel} and $\Delta_{peduncle}$) values (‰).

RESULTS AND DISCUSSION. Significant differences between environments and genotypes were obtained for grain yield. As expected, environmental yield variation was much higher than that displayed among genotypes (scarcely 200 kg range). According to the predictive assessment of G×E, AMMI2 model captured 71% of the interaction sum of squares (SS) and accounted for 96.3% of the model SS; the remaining 3.7% of the model SS was identified as noise.

G×E was represented by means of a biplot (Fig. 1) showing IPCA1 against IPCA2. Environment clustering displayed three major groups, indicated in Fig. 1, which explained 74% of the environments and G×E SS. Cluster I contained six highly productive

environments with positive IPCA1 score; Cluster II was integrated by five environments of intermediate yield and moderately average negative IPCA1 score; finally, Cluster III was composed by eight poor and three medium-yielding environments with moderate to strong negative IPCA1 scores. The climatic characterizacion of clusters (Table 1) revealed that IPCA1 was related to the R/ETo ratio during the vegetative and reproductive phases of development. IPCA2 scores discriminated between Cluster II and Cluster III although, apart from differences in average productivity, IPCA2 values were not easily associated to any particular environmental variable. Genotype clustering displayed also three groups which accounted for 76% of the genotypes and G×E SS: negative IPCA1 scores were usually associated with old cultivars, well adapted to dry areas, whereas modern cultivars exhibited positive IPCA1 values.

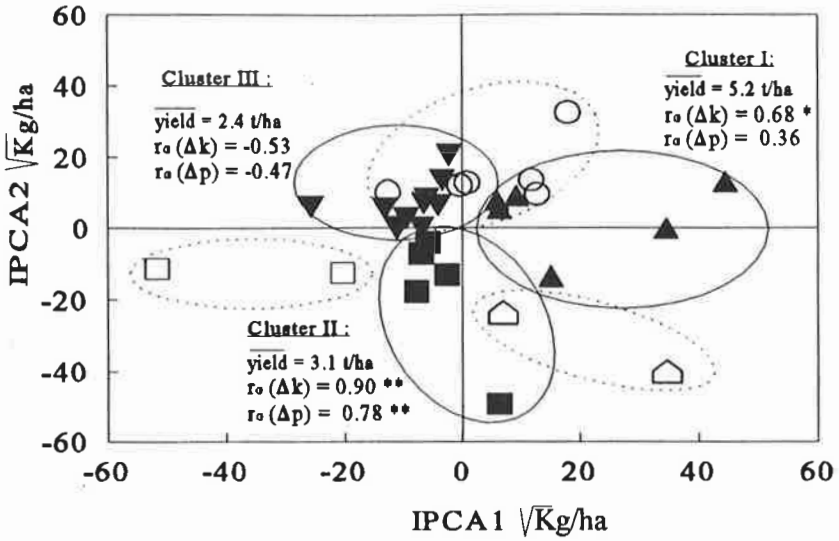

FIGURE 1. Biplot of the AMMI2 model for yield showing IPCA1, IPCA2 and cluster grouping of genotypes (dashed lines) and environments (solid lines). Genotypes-environments expressed by different open-filled symbols according to their cluster classification. Average yield for each cluster of environments, as well as genetic correlations (r_G) between yield and carbon isotope discrimination values of kernels (Δ_k) and peduncles (Δ_p), are indicated.

TABLE 1. Ratios of average rainfall (mm) and reference evapotranspiration (mm), and their standard deviations, (R/ETo±SD), during the vegetative and reproductive phases of development according to the cluster grouping of 22 environments.

	Cluster I	Cluster II	Cluster III
$(R/ETo)_{tillering}$	0.94±0.20	0.67±0.24	0.71±0.25
$(R/ETo)_{jointing+grain\ filling}$	0.59±0.19	0.33±0.14	0.32±0.12

There were highly significant differences among genotypes and environments for Δ_{kernel} and $\Delta_{peduncle}$ values. When comparing individual Δ of kernels and peduncles, those obtained from mature grains always achieved higher values. This could be due to the fact

that the carbon in the peduncles came from photosynthesis during the winter drought, and that grain filling benefited from rain during April and May. Genetic correlations between yield estimates and Δ were obtained separately for each cluster of environments in order to minimize G×E of yield within clusters. The correlation coefficients calculated for Δ_{kernel} were always higher, in absolute values, than those found for $\Delta_{peduncle}$ (Fig. 1), indicating that a better assessment of yield variation among genotypes could be obtained by using Δ values from mature grains.

Cluster I, the highest yielding subset, displayed moderate positive correlations between Δ values and yield (Fig. 1); Cluster II, grouping medium-yield trials, exhibited large positive correlations with Δ; finally, Cluster III, mostly composed by poor yielding trials, showed clearly negative relationships with Δ values. Given such different responses, the genetic implications of using Δ in breeding programs for a variable target environment are not straightforward.

It seems that a positive genetic relationship between yield and Δ is expected in favourable rainfed environments as found in Cluster I. In such cases, the usefulness of Δ in breeding programmes should depend on how well widely-adapted cultivars respond to suboptimal developmental conditions. In agreement with CIMMYT's approach, first screening stages could succesfully incorporate this trait. Nevertheless, the harsh growing conditions that usually characterized environments from Cluster III, showing a negative, although non-significant, correlation with Δ, seemed to favour those genotypes with higher WUE and address the question of whether to select for high or low Δ for these conditions. Besides, the behaviour of Cluster II regarding Δ is somehow disturbing: on average, grain yield of this subset slightly exceeded that of Cluster III, but probably the causes behind the extremely opposite genetic correlations with Δ are to be found in IPCA2 score differences. Thus genotypic adaptation to timing and intensity of environmental constraints should have played a major role in determining such differences and, probably, more information is needed with regard to cultivar development along the growing cycle in order to understand the inherent complexity associated to IPCA2.

Based in our results, no definitive conclusions arise about the efectiveness of Δ in screening drought tolerant barley lines, although the suitability of AMMI and cluster analyses to partition G×E and minimize interaction patterns within groups must be emphasized when attempting to augment yield-based selection.

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A NOVEL SYSTEM TO STUDY THE REGULATION OF GENE EXPRESSION IN BARLEY.

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Introduction. Anthocyanins, as colorful pigments, are visible in flowers, fruits, leaves and other parts of higher plants. The biosynthetic pathway has been intensively studied in various plants such as *Antirrhinum, Petunia,* maize and barley. Several genes related to anthocyanin production have been isolated and characterized (1).

Materials & Methods.

1. Plant materials. Grains from barley Gula and its homozygous mutant ant18-162 were obtained from the Carlsberg collection. They were germinated at 22°C for six days, then transferred to 16h light 15°C/8h dark 10°C. Stem tissue with leaf sheath in 2 cm long pieces was harvested from the seedlings and used for bombardment. The plant segments were then placed in a 5 cm Petri dish on filter papers wetted in liquid media.
2. Plasmid DNA and microprojectile bombardment. The following plasmids were applied in this study and see (8) for detailed information.

pEmuGN--the GUS gene driven by the pEmu promoter.
pgDFR ▵ (I2+I3)--Ant18 gene with the second and third introns removed.
The plasmid DNA was delivered into the plant tissue by the helium-driven particle delivery system PDS 1000/He. The gold particles in 1.5-3.0 μ m were coated with DNA (9). The samples were incubated for one week at 15°C with 16 h illumination after bombardment. Anthocyanin production was determined by rating the visually pigmented red cells under a stereo microscope.

Results and Discussion.

1. Optimization of anthocyanin accumulation in cv. Gula.
   a) Growth conditions. The seedlings were grown under three alternative conditions for anthocyanin production. Among them, 22°C for the first 10 days followed by 15°C 16h light/10°C 8h dark for 11 days resulted in the highest intensity of anthocyanin pigmentation (Table 1).

Table 1. Effect of growth conditions on anthocyanin accumulation in barley cv. Gula.

	Light	Light / Dark	Light / Dark
Day 1-10	22°C 24h	15°C 16h / 10°C 8h	22°C 24h
Day 11-21	22°C 24h	15°C 16h / 10°C 8h	15°C 16h/10°C 8h
Appearance of pigmentation	18 days	14 days	12 days
Intensity of pigmentation	+	++	+++

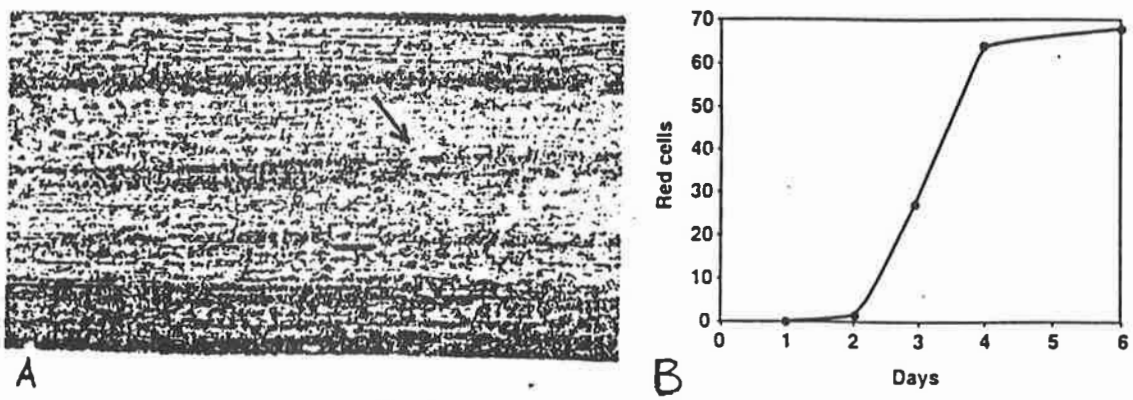
b) Medium composition and pH. The plant segments were incubated on the filter paper supplied with different media (Table 2). The most intense level of anthocyanin production was achieved when the tissue was incubated with MS salts plus 4% maltose at pH 7.0.

Table 2. Anthocyanin accumulation in leaf sheath segments of cv. Gula barley over seven days in media of different composition. (Score used: 0, no pigment; 1, weak pigmentation; 2, medium pigmentation; 3, strong pigmentation. Ten tissue segments were used to get the mean value.)

Medium	Mean score
MS salts, 4% maltose, pH 7.0	2.8
MS salts, 4% maltose, pH 5.7	1.4
MS salts, 4% sucrose, pH 7.0	2.4
4% maltose, pH 7.0	2.0
4% sucrose, pH 7.0	1.4
water, pH 7.0	0.4

2. Anthocyanin production in *ant18*-162 after bombardment with pgDFR$_\Delta$ (I2+I3). The red pigments were visualized two days after the Ant18 gene was received by the cells (Figure 1A) indicating the gene expressed in ant18-162. The number of red cells increased during the six day incubation (Figure 1B). The highest score of 26 red cells in an area of 0.4 cm^2 was detected under these conditions.

These results demonstrate a novel system to study gene expression in barley by Ant18 gene and its recessive mutants. In this system gene expression can be studied in real biological environment. It also offers a simple way to monitor the expression process by a visible marker.



A B

Figure 1.A. Anthocyanin has been synthesized in individual cells of leaf sheath segments of ant18-162 (see the arrow). B. Number of red cells accounted over a period of six days in leaf sheath of ant18-162 bombarded with pgDFR$_\Delta$(I2+I3).

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An assessment of the effect of genotype and environment on kernel discolouration of barley in Western Australia. K.J. YOUNG, Esperance Agricultural Centre, Agriculture Western Australia, PMB 50, Esperance, 6450, Australia.

Introduction. The southern cereal growing region of Western Australia produces about 0.5m tonnes of barley annually. On average only 65% of the production of the malting barley varieties is accepted into the malting grade. A recent survey of grower deliveries (K.J. Young unpublished) revealed that the major reason for downgrading is kernel discolouration (KD) due to weather staining. KD has been observed to take two forms. One form is carameling caused by heavy dews and light showers which discolour the grain without, it seems, the involvement of fungi. The second, more severe form, appears after heavier rain and is the grey discolouration, as well as black and brown point that is associated with a number of fungal pathogens. In 1970 a breeding program commenced at he University of Minnesota to incorporate KD resistance genes into midwestern malting barley. It has been shown that useful levels of KD resistance can be conferred (Miles *et al.* 1989) using the six row variety Chevron as the source of resistance. This article reports on a project that commenced in 1995 which aims to determine the effects of genotype, development stage and environment on KD in Western Australia.

Materials and Methods. Four hundred and fifty barley lines consisting of Australian and overseas cultivars and elite crossbreds were sown in 0.71m by 2m plots at Esperance on the south coast of Western Australia in 1995. The popular local malting barley, Stirling, was sown as the control at seven sowing dates at weekly intervals. At Zadoks Growth Stage 49 (awn emergence) (Zadoks *et al.* 1974) ten tillers of each line were tagged and Stirling tillers of the same development stage tagged on the same day. Development was recorded on each plot until physiological maturity, which was considered to be complete loss of green from the peduncle. Two weeks after this five heads were harvested (H1) and oven dried. A further four weeks later the second five heads were harvested (H2). Heads were hand threshed and scored visually for discolouration on a 1 to 9 scale with 1 being the brightest grain and 9 the most heavily discoloured. An automatic weather station set up at the site recorded temperature, solar radiation, relative humidity and rainfall. Data from twenty five lines which were sown in two replications was analysed by analysis of variance to assess genotypic differences in KD and gain an estimate of site error.

Results and Discussion. Rainfall for the grain-filling period and thereafter is summarised in Figure 1. From October 17th to the 19th, 45mm of rain fell with Stirling plots at the time ranging from awn emergence (Z49) to the hard dough stage (Z87). A plot of grain colour at the first harvest date (Fig. 2a) showed that KD was highest for grain that had reached the soft dough stage (Z85). A further delay in harvest date caused a significant increase in colour when rainfall for the period exceeded 10mm (Fig. 2b).

Analysis of the replicated lines showed a highly significant difference between genotypes ($P<0.001$) and produced an LSD (0.05) of 1.1 colour units. Introduced lines were compared to the Stirling barley control that had undergone grain filling at the same time. A difference of 2 colour units or greater was therefore statistically significant. Within each geographic

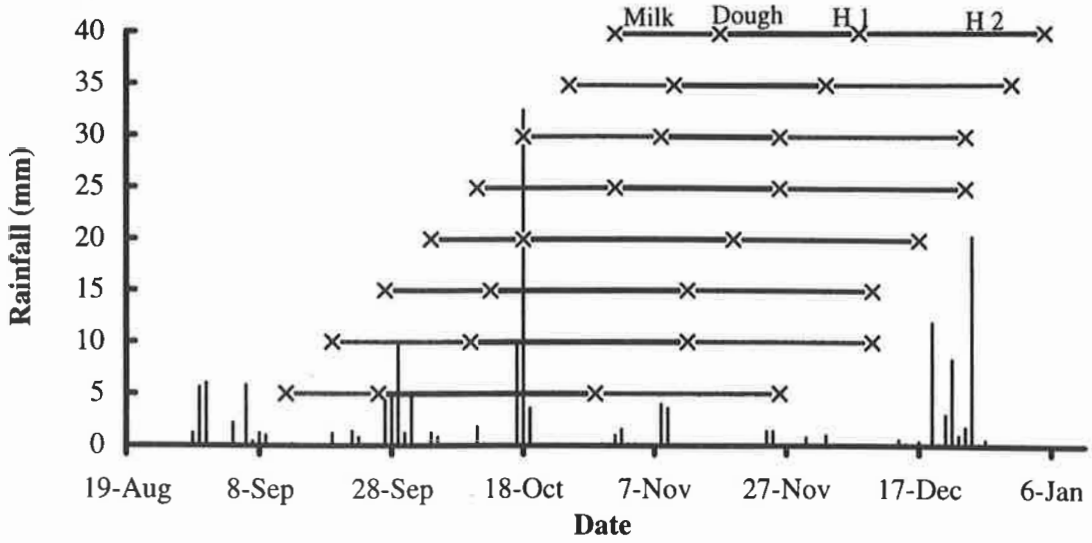

Figure 1: Rainfall during the grain filling period (milk and dough stages)and the 28 day period between the two harvest dates (H1 and H2). Bars represent grain development for the eight headings dates of the control variety Stirling.

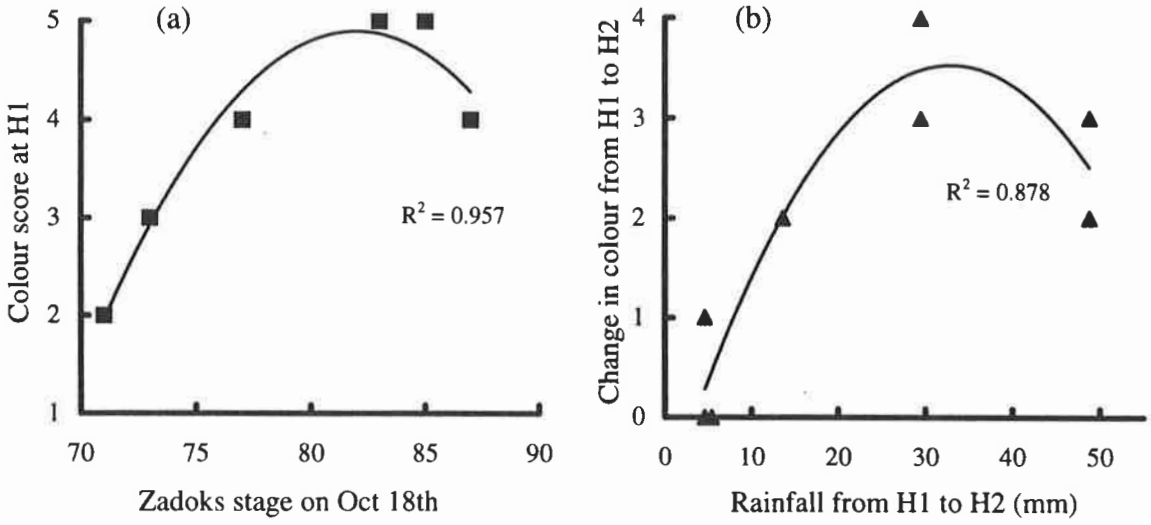

Figure 2:(a) The effect of development stage at the time of a 45 mm rainfall event on on the grain colour at the first harvest date (H1) and (b) the subsequent increase in grain colour from further rainfall between the first and second harvest dates H1 and H2).

region, lines ranged from those which were brighter to significantly more discoloured than the control (see table 1 for a sample from each region). Only a very small number of the 450 lines tested showed any significant improvement over Stirling. The most promising lines originated from Canada and Japan with the some of them a bright colour after a month's delay in harvest. Lines from Australia, Europe, ICARDA and the USA were generally similar to Stirling with only minor improvements in a few lines. The KD

Table 1: Kernel discolouration scores for a range of Australian and overseas barley cultivars with the colour score relative to the corresponding Stirling barley control.

Variety and origin	Colour score		Score relative to Stirling		Variety and origin	Colour score		Score relative to Stirling	
	H1	H2	H1	H2		H1	H2	H1	H2
Australian					**European**				
87S671-35-23	3	4	**-2***	**-2***	**Dzugjaj**	4	4	-1	**-2***
Onslow	4	4	0	**-2***	**Chariot**	4	4	0	**-2***
Arapiles	4	6	0	0	Cheri	5	6	0	0
Schooner	4	6	0	0	Rovenskii 37	5	6	0	1
Franklin	5	5	0	0	Clarine	4	6	2*	1
ICARDA					Pastorale	5	9	3*	3*
90IWFBON 105	3	5	**-2***	-1	**Canadian**				
91BCB 11	4	4	0	**-2***	**SB85738**	2	2	**-3***	**-4***
91IWFBCB 21	5	6	0	0	**TR118**	3	3	**-2***	**-3***
91IBON 6	7	8	2*	2*	Harrington	4	5	0	-1
91IBON 45	7	6	3*	0	Stein	5	5	0	0
Japan					**USA**				
Kino Nijo 7	2	3	**-2***	**-3***	**Bearpaw**	4	4	-1	**-2***
Kinuyutaka	2	4	**-2***	**-2***	**Klages**	4	4	0	**-2***
Nasu Nijo	3	3	-1	**-3***	Robust	7	7	3*	1
Kino Nijo 22	4	5	0	-1	Stander	7	7	3*	1

resistant lines from the University of Minnesota were not available for testing in the early stages of the project. The standard midwestern six row barleys were heavily discoloured at H1 and similar to the control at H2. The best lines from Canada are two row lines, TR118 (a malting line) and SB85738 (a naked type, although the scores relate to its hull colour).

Future Plans. In the ensuing four years of the project additional lines will be imported and screened in field trials. Further to this, nursery tunnels will be erected and the most promising lines irrigated with using overhead sprinklers to ensure conditions that predispose the grain to kernel discolouration. Work has commenced to identify the major pathogens involved and quantify grain colour using digital image analysis. Studies on the relative susceptibility of stages of grain development to rain and high relative humidity will be also conducted in nursery tunnels. In the later years of the project it is hoped to assess the progeny of crosses between local varieties and the most promising lines showing resistance to kernel discolouration including lines from the University of Minnesota.

Acknowledgments. The technical support of David Dodge and the financial support of the Grains Research and Development Corporation of Australia is gratefully acknowledged.

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Section XII: Pathology and Pest Resistance

Resistance of Oat Stem Rust in Australia

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Introduction A study, forming part of a PhD program, was conducted

1. to estimate the frequency and distribution of stem rust resistance genes in various germplasm of oats,
2. to study the mode of inheritance of resistance and
3. to examine the relationships of new genes to the currently used genes.

Materials and methods One hundred and ninety-two elite lines and cultivars were selected from various Australian oat breeding programs and the International Quaker Oat Nursery; they were tested with 203 isolates of *P. graminis avenae*. The inheritance of stem rust resistance was studied in thirty-five crosses involving selected resistant materials with susceptible cultivars Swan or West and 30 intercrosses involving resistant materials.

Results and discussion

Gene Postulation Most of the commercial cultivars were susceptible and appeared to carry *Pg-2* and *Pg-4* singly or in combination, whereas many of the elite lines were resistant to all cultures and gave similar infection types. Since they displayed similar reactions and none of them were susceptible to any of the cultures, it was concluded that they could have a common gene. The infection types were similar to that of Omega possessing *Pg-a*, also resistant to all the cultures, suggesting that all lines possessed *Pg-a*.

Inheritance Studies An extensive study of the inheritance of stem rust resistance made by crossing resistant/susceptible lines gave similar inheritance patterns. The resistance was governed by a complementary recessive gene. Similarly, the inheritance of *Pg-a* conformed satisfactorily with the complementary model as suggested by Martens *et al* (1981). However, *Pg-12* hypothesised to be one of the components of *Pg-a*, was not recovered in this study.

The progeny of resistant/resistant crosses, and Omega and other resistant lines failed to segregate, thus substantiating the hypothesis that they all shared *Pg-a*.

These results showed the genetic uniformity in oats for resistance to stem rust in Australia. On the other hand, many pathotypes of *P. graminis avenae* are recovered from surveys possibly due to the abundance of wild host populations. In recent years the pathogen population in Australia has changed rapidly for increasing virulence. Virulence for *Pg-a* has also been identified in greenhouse studies. This is the only gene conferring field resistance in this country.

Amagalon, derived from interspecific cross of diploid *A. longiglumis* and tetraploid *A. magna*, was resistant to all pathotypes of stem rust. Since the origins of Amagalon and Omega (*Pg-a*) were quite different, it was thought that they had a different source of resistance. However, genetic studies showed that Amagalon also had *Pg-a*. This was further substantiated by induced susceptibility response and the temperature sensitive resistance in Amagalon; both phenomenon are described below.

Induced susceptibility. A unique characteristic of *Pg-a* was observed and further investigated. A mixed infection of an avirulent pathotype of stem rust and a virulent pathotype of leaf rust induced susceptibility to the stem rust pathotype. This phenomenon did not occur with any known gene conferring resistance to stem rust other than *Pg-a*. The induced susceptibility was observed regardless of prior or post inoculation of the stem rust pathogen, provided a virulent leaf rust pathogen was present. This phenomenon provides a bioassay method for screening lines for the presence of *Pg-a*. However, it would be restricted to lines susceptible to leaf rust.

Temperature Sensitivity. The expression of certain genes were observed to be sensitive to temperature. Lines possessing *Pg-8* and *Pg-16* became susceptible at 21.5^{0}C, but were resistant at 17^{0}C. By contrast, lines with *Pg-4*, *Pg-12* and *Pg-a* were effective at 21.5^{0}C, but lost their effectiveness at 26^{0}C and above. The resistances conferred by *Pg-1*, *Pg-2*, *Pg-10*, *Pg-13* and *Pg-Sa* did not change between 17^{0}C and 30^{0}C.

Conclusion. These studies, involving a wide range of germplasms present in Australia, failed to isolate sources of resistance other than *Pg-a* and showed that many advanced lines and cultivars from a range of breeding programs have the same source of resistance. This is an alarming situation and it is a matter of highest priority that Australian oat breeders find alternative sources of resistance to oat stem rust.

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EFFECT OF BARLEY YELLOW DWARF VIRUS ON ROOTS OF SOME CULTIVATED OATS.

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INTRODUCTION. Barley yellow dwarf virus (BYDV) is known to cause yield loss in most cereal crops throughout the world. The disease is characterized by several symptoms, including leaf discoloration, plant stunting, tiller mortality and grain sterility.
Recent studies have shown that BYDV can strongly reduce root growth of various cereal species. Eweida *et al.* (1988) found that oat roots represent the initial site of virus replication, just before the invasion of aerial parts. In studies with 4 oat cultivars that differed in tolerance, a significant reduction in root dry weight and rate of root elongation was observed (Kolb *et al.*, 1991). In barley, Haber and Comeau, (1989) observed that the first detectable effect of BYDV occurs at root level, reducing root meristem growth within 4 days after inoculation.
The goal of the present study was to assess root growth parameters of sixty cultivated oat, after BYDV inoculation with a severe PAV mixture isolate.

MATERIALS AND METHODS. An hydroponic culture apparatus was developed to allow the daily study of root growth. It consisted of four 120 X 100 cm superposed Plexiglas plates containing a total of 140 channels. Each channel received a modified Hoagland's solution and allowed root growth of an individual plant. Nutrient solution was supplied by a submersible pump (model #3E-12N, Little Giant Company, Oklahoma, USA) for 5 mn every half hour. The nutrient solution was renewed every week and the pH adjusted to 6 to 6.5, the electric conductivity was less than 2 mSiemens and temperature oscillated between 20°C and 26°C. The apparatus was placed in a glasshouse with a 18 hours photoperiod, 18°C day temperature and 16°C night temperature.
Seeds of sixty oat genotypes were germinated in "Oasis" rootcubes growth medium (Oasis® rootcubes® growing media #5000) placed in a growth chamber (photoperiod: 18 hours; temperature: 16°C). At 1-2 leaf stage, plants were inoculated by PAV mixture isolates, found extremely severe on cereal roots during a previous experiment. Aphids were killed 48 hours later by an insecticide spray of pirimicarb (Pirimor® 50 W, 0.5g/L of solution). Inoculated and control plants were immediately transplanted on the hydroponic apparatus. The experimental design was a split plot one with 4 replications, the "virus" was in the mean plot and the "genotype" was in the subplot, each individual plant constituted one experimental unit. The four replications were realized in the time because the limited capacity of the apparatus.
Root length was measured 7 time, at 3 days intervals, starting the 10th day after inoculation. The measure consisted of marking the length reached by the apex onto the transparent Plexiglas plates.

RESULTS AND DISCUSSIONS. Figure 1 showed root growth evolution of 20 genotypes from the 60 studied here. BYDV affect strikly root length and root dry weight of a susceptible oat cultivars. ANOVA analysis (table 1) showed that the "genotype x BYDV" interaction was highly significant at the 19th day after inoculation. For most genotype, BYDV effect on roots was very obvious, reduction of root length reached in some case 76% (figure 1). Root growth of the susceptible lines Clintland 64 and AC Hill for example, was very slowed by the virus during

the first days after inoculation and stopped later. For the tolerant genotypes, roots of the inoculated plants, even the observable effect of the virus on the length, continued to grow at the same rate as the controls (figure 1). Roots of the tolerant lines 76S6-1454 and CI 9311 were nearly unaffected by BYDV inoculation. Ogle's roots however were slightly affected even its known BYDV tolerance.

In general, BYDV effect on root growth is in accordance with the level of BYDV tolerance observed in field. Spearman correlation between symptom note of BYDV tolerance measured in the field and root growth of inoculated plants in the hydroponic is about 0.6**. This values is not very high but is sufficiently acceptable to consider root growth as an additional BYDV tolerance index. Symptoms observation on aerial parts remain however, the best way to evaluate BYDV tolerance due to the high correlation with yield reduction under inoculation (r = 0.94**).

As a selection tool for BYDV tolerance, the hydroponics system offers some advantages. The method is relatively easy, non destructive, and enough precise because we measure a quantifiable parameter. BYDV tolerance could be evaluated any time under glass house or growth chamber, where visual symptoms are often difficult to observe. Spring and winter cereals could be evaluated simultaneously, and wild resistant species could be also included, as a control or as a candidate for gene transfer to cultivated cereals. In term of economic cost, root growth measurement requires less space and less time, in comparison with visual assessment which take more space, more labor and usually more than one year to confirm visual scores.

In conclusion, the effect of BYDV on cereal roots is very damaging on susceptible plants. It could have a devastating effect when drought occurred. Plants inoculated earlier suffered more due to the active root development in the first stages. Rapid establishment of plant including rapid root formation, considered as a mechanism of drought avoidance, could be also considered as a BYDV avoidance. Special attention should be given to the effect of BYDV on root growth, especially if plant breeders are selecting plants for drought tolerance.

Table 1. ANOVA (mean square) of root lenght of 60 oat genotypes cultivated in hydroponics.

		Root lenght (days after inoculation)						
Source	d.f	10 days	13 days	16 days	19 days	22 days	25 days	28 days
BYDV	1	5381.6**	10066.3**	17386.8**	25326.8**	37327.3**	48080.5**	62372.7**
Genotype	59	61.5**	88.1**	123.2**	171.4**	226.2**	292.4**	361.4**
BYDV x genotype	59	30.0 ns	46.3 ns	68.7 ns	106.9**	149.5**	183.2**	235.4**

*, **, ns Respectively significant at level 0.05 et 0.01, and non significant at level 0.05.

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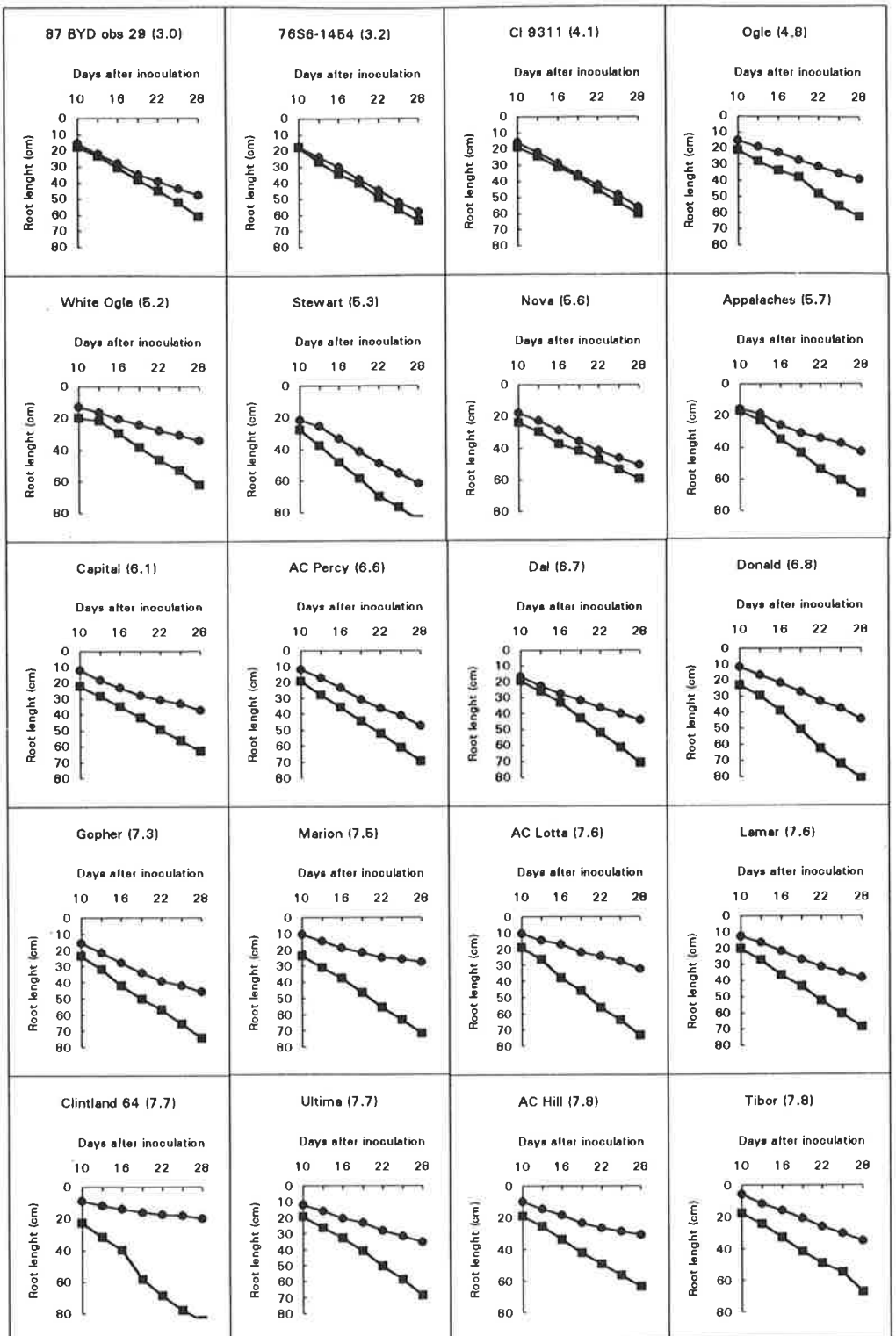

Figure 1. Root growth evolution of 20 different inoculated and non inoculated oat genotypes cultivated in hydroponics. In bracket, BYDV symptom note under field artificial inoculation.

Cereal cyst nematode resistance breeding in barley - a new technique for field screening. S.M. BHATNAGAR, B.N. MATHUR, R.S. SAINI, I. RAJVANSHI and S.D. SAXENA, Rajasthan Agricultural University, Agricultural Research Station, Durgapura, Jaipur, India.

Introduction. Cereal cyst nematode (*Heterodera avenae* Woll.) remains a most serious pest of barley in Rajasthan, India, where soils are sandy to sandy loam and frequently cropped. The yield losses in such soils always exceed 50 percent and immediately call upon devising viable management to abate the pest incidence. Existence and availability of resistant genes in barley cultivars obviously remains the best choice to transfer resistant gene through cross breeding in high yielding varieties. Resistance breeding is an integral part of Agricultural Research Station, Durgapura's barley breeding objective. The aim is to increase yield potential and adaption, maintain quality and simultaneously seek resistance to CCN, and if possible, to barley rust and other foliar diseases. The presently used technique, which is termed here as "Uprooting and transplanting (UT)", consists of gently taking out the plants from the soil with the help of a showel and examining their roots for deformity as well as white females of CCN and thererafter transplanting the desirable plants in pots maintained in a cagehouse to raise seeds from them (Mathur, 1969, Handa, 1983). Inspite of an intensive care of these transplants, due to their advance age and ambient high temperatures, a high plant mortality rate is observed. Further, this technique is demanding on space, labour intensive and deprive adult plant growth expression and is considered indequate. The present study was, therefore, aimed to develop a suitable field screening technique to enable breeders to handle a huge number of cross combinations and their progenies as also to simultaneously monitor the agronomical traits, and if possible, resistance to other diseases.

Materials and Methods. In resistance breeding programme monogenic dominant gene from Rajkiran (resistance was from Marocaine) and PL-101 (as indigenous resistant parent of unknown origin) was utilized in cross breeding with the most susceptible variety RD-103. The F_2 populations of crosses were further field evaluated in a uniformly CCN infested field carrying 8.0 l/ml soil at Agriculture Research Station, Durgapura, Jaipur. The test material was planted in 20 M^2 plot with 30 x 15 cm spacing, and were replicated 6 times. The normal package of practices were followed to raise the crop. To avoid possible escapes, susceptible cv. RD-103 was flanked between the test rows, which also maintained the inoculum level in the field for next crop season.

The minimum time for the development of first female in barley roots in Indian field conditions is about 60 days and the normal assaying would need 70-75 days after sowing (11.0 on Hann's scale and 59 of Zadok's growth scale). In the present study, two field screening techniques were compared. In UT technique, the plants were uprooted for root examination and resistant plants were transplanted in pots kept in cagehouse after 70-75 days of seeding. In the new technique `uprooting and drying' (UD) the agronomically desired plants were uprooted and assayed for CCN at 95 to 100 days after sowing (13.0 to 14.0 and 75 to 85 growth stage of Hann's and Zadok's scale, respectively). The desired resistant plants were allowed to dry up in nethouse to obtain seeds for next crop season. In this case, the screening was delayed by 20 days and harvesting was advanced by nearly the same period. The CCN resistant plants thus selected were subjected to retests for their resistant behaviour during next crop season. Both the techniques were compared for three years.

Results and Discussion. In the present study, two screening methods - "uprooting and transplanting" and "uprooting and drying" techniques of screening against CCN were compared and parameters studies are presented in tabular form (Table 1).

Table 1: Comparison of techniques for field screening against CCN

Parameters	Techniques	
	Uprooting and transplanting	Uprooting and drying
Transplanting	Required	Not required
Plant mortality (%)	35-40	Nil
One thousand kernel weight (g)	22-26	30-34
Germination (%)	50 to 65	60 to 85
Selection on Agronomical Trait	Not possible	Possible
Rust score	Possible in only early out break	Possible upto later stages

It is evident that the new technique of uprooting and drying has distinct advantages over the older method. In the new method, transplanting is not required, plant mortality is avoided, seed obtained is of comparatively better quality with improved germination. In uprooting and transplanting field test, the bio assays are demanding on space and labour intensive and does not permit adult agronomical expression of resistant plants. The new field screening method allows screening of greater number of test plants within the shortest span of time available. Moreover, in this method, selection pressure is restricted to agronomically superior plants, preferably having simultaneous resistance to rust etc., where as in UT technique, a large number of undesirable plants get selected on the basis of single character of CCN resistance. This new technique offers promise to be effective for screening against other soil borne root diseases of barley and other cereals.

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Host : Pathogen Studies of Oat Leaf Rust in Australia

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Introduction. Oat lines from a diversity of sources were screened with *Puccinia coronata* cultures to identify broadly effective sources of resistance. The cultures were isolated from collections received during the 1993-94 Australasian Oat Rust Survey and stored in the Plant Breeding Institute Cobbitty (PBI) culture collection.

Materials. The 155 host lines included the International and Australian Supplementary Differential Sets, a set of putative single gene lines used as differential testers in the United States of America, resistant Australian cultivars and lines selected from Quaker Nurseries, Brazilian oat breeding programs, the 1992 Australian Interstate Oat Variety Trial (IOVT) and the 1992 selection nurseries from the New South Wales (NSW) and South Australian (SA) Departments of Agriculture.

Screening with the survey cultures permitted identification of important, previously undetected races with virulence on cultivars (cvs.) Amby, Nobby and Riel. Races virulent on Enterprise were common and widespread.

A set of differential pathogen cultures was selected and used to postulate host resistance genotypes based on infection type (IT) scores. Forty five distinct genotypic groups were identified. Thirty five genotypes were distinguished among the 48 lines in the International and Australian Supplementary Differentials Sets and the putative gene lines. Three of these lines were resistant and two susceptible to all cultures. Sixteen genotypes were identified among the nursery lines, 10 of which were unique and not readily explained by combinations of known genes. Three cultivars were resistant to all cultures and the other five had genotypes based on known genes or combinations of known genes.

Results and discussion.

Pathogenicity Considerable geographic variation in pathogenicity for the host lines was identified and races virulent for several genes not previously utilised in Australian cultivars were detected. Virulence for some genes, such as *Pc67,* was widespread. The cultivars Bettong, Cleanleaf and Culgoa, the putative single gene lines PC68, H548 and WIX, and 21 nursery lines were resistant to all pathogen cultures.

Resistance Two sets of genes were responsible for the resistance of 60% of the nursery lines and five of the eight Australian cultivars. Genes *Pc58, Pc59* and *Pc61* formed the basis of resistance in 50% of nursery lines and in the cvs. Amby and Nobby. *Pc38* and *Pc39* were present in 10% of nursery lines and the cvs. Enterprise and Riel.

Environmental sensitivity Studies on environmental sensitivity showed that resistance conferred by some genes, including *Pc39, Pc67* and *Pc71,* became ineffective at higher greenhouse temperatures, whereas other genes, such as *Pc64*, became less effective at lower temperatures. Lowering the light level had a small but significant effect on the infection type (IT) produced on a number of lines. Temperature and light x race interactions were observed for some lines.

Environmental responses permitted differentiation of the resistances conferred by *Pc39* and *Pc55* and identified the gene carried by Enterprise as *Pc55.*

Genetic analyses Crosses between the susceptible cv. Swan and the universally resistant cultivars, putative single gene lines, six nursery lines (N147, Br80, Br102, Q200, Q300 and SA352) and cv. Enterprise were studied to determine the number of genes for resistance in each. Most lines were intercrossed to determine the genetic independence, or otherwise, of the genes that were identified.

Conclusion. The gene carried by PC68 was presumed to be *Pc68* and the gene carried by Enterprise was identified as *Pc55.* Bettong, Culgoa, Enterprise, PC68, H548 and N147 each carried single genes for resistance; temporarily designated ***PcBet***, ***PcCul***, ***PcH548*** and ***PcN147***, respectively.

Lines WIX, Br102 and SA352 carried two genes. One of the genes from Br102, temporarily designated ***PcBr102***, conferred resistance to a wide spectrum of races whereas the second gene, designated ***PcSA463***, was effective against fewer races. SA352 carried *Pc61* and a second gene, designated ***PcSA352***, conferring broadly effective resistance. The two genes carried by WIX were temporarily designated ***PcWIX1*** and ***PcWIX2***. The number of races against which each conferred resistance was not determined.

The resistance of Cleanleaf was based on *Pc38* and *Pc39* and a third gene designated ***PcCl***, which conferred broadly effective resistance.

Br80 carried four genes for resistance, *Pc39, Pc46, PcSA463,* and a gene designated *PcBr80. PcBr80* was broadly effective whereas the other genes each conferred resistance to a more limited range of races. Q200 and Q300 each carried a single gene, *PcQ200* and *PcQ300*, respectively, conferring resistance to race 384-*Pc58,59,61.* Whether the lines carried additional genes effective against other cultures was not determined. All genes identified were dominant or incompletely dominant.

Intercrosses between most of the resistant lines permitted the allocation of resistance genes to five arbitrary linkage groups.

Linkage group 1: Genes ***PcCul***, ***PcCl***, ***PcN147***, ***PcQ200***, ***PcQ300*** and ***PcSA352***
Linkage group 2: ***PcBet*** and ***PcBr102***,
Linkage group 3: *Pc68* and ***PcWIX1***
Linkage group 4: ***PcH548*** and
Linkage group 5: ***PcWIX2***.

With the exceptions of *Pc68* and *PcWIX1* in linkage group 3, the genes in each the other linkage groups may have been identical. *Pc68* and *PcWIX1* were differentiated by distinctly different ITs. The relationship of ***PcBr80*** to the other genes was not determined.

Reference

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Physiologic Specialization of *Puccinia hordei* in Israel and Ecuador in the years 1992-1994. U. BRODNY and M. RIVADENEIRA, Institute for Cereal Crops Improvement, Faculty of Life Sciences, Tel-Aviv University, Tel- Aviv 69978, Israel; and Estacion Experimental "Santa Catalina" INIAP, (Instituto Nacional de Investigaciones Agropecuarias), Quito, Ecuador.

Introduction. Barley (*Hordeum vulgare* L.) is the world's fourth most important cereal crop, after wheat, maize and rice. It is cultivated over a wide range of environmental conditions, some of them unfavorable for growing other cereals. Leaf rust (*Puccinia hordei* Otth) is an important barley disease, occurring wherever barley is cultivated. Leaf rust became predominant in Ecuador after the release of two susceptible varieties, Dorada and Teran. Both varieties are stripe rust resistant to race 24 of *Puccinia striiformis* f. sp. *hordei* but susceptible to leaf rust.

The fungus completes its life cycle on *Ornithogalum* species. The alternate host is of great importance for the perennation of the pathogen in countries like Israel, where *Hordeum* plants do not survive the rainless summer. Ornithgalum also supports genetic recombination of the leaf rust fungus resulting in the evolution of broad spectra of pathogen variability. Breeding for resistance is the best practical disease control measure. Its success requires abundant reservoirs of resistance factors, to match the wide spectrum of pathogen virulence. Such gene pools occur in natural populations of *Hordeum spontaneum* C. Koch (*H. vulgare* subsp. *spontaneum*) in Israel (2). Presumably, they are an outcome of coevolution of the host-rust obligate parasite system: *H. spontaneum* - *P. hordei* - *Ornithogalum* spp. over millennia at the center of origin of the components of this system. The process of coevolution is associated with reciprocal host-parasite selection pressure. Israeli populations of *H. spontaneum* have provided reliable sources of leaf rust resistance successfully utilized in the USA and Europe (2). A joint research project has been undertaken by Tel-Aviv University and the Estacion Experimental "Santa Catalina", INIAP, Quito, Ecuador, to provide Ecuador with accessions of *H. spontaneum* carrying various types of disease resistance to be utilized in barley breeding locally and in other countries of South America. The prerequisite for achieving this goal is recognition of parasitic attributes of *P. hordei* populations in Ecuador in comparison with those of Israel. Results of the study on these problems are summarized in this publication.

Materials and Methods. A total of 100 samples of *P. hordei* were collected from commercial barley in Ecuador at various stages of growth and spread over 20 locations in the northern, central and southern regions of the Andes. At the same time 100 collections of *P. hordei* were randomly sampled from diseased leaves of *Hordeum spontaneum* in 20 locations throughout Israel, from the northern Negev in the south to the Golan Heights in the north.
Samples of rust leaves were preserved in paper bags at 5C.
In the race identification tests, inoculum of monouredial origin was increased individually on seedlings of the very susceptible cultivar Nigrate (CI 2444) and subsequently used for inoculation of 1-leaf seedlings of 11 standard differential barley cultivars with specific resistance genes. The following differential hosts were used: their resistance genes (*Pa*) designation is according to Clifford (3) and CI numbers are given in parenthesis: Sudan (*Pa*, CI 6489), Peruvian (*Pa2* CI 935), Reka #1 (*Pa2*+ CI 5051), Quinn (*Pa2*+ *Pa5* CI 1024), Bolivia (*Pa2*+*Pa6* CI 1257), Estate (*Pa3* CI 3410), Gold (*Pa4* CI 1145), Magnif (*Pa5* CI 13806), Cebada Capa (*Pa7* CI 6193), Egypt 4 (*Pa8* 6481), and Abyssinian (*Pa9* CI 1243). The inoculation technique was described by Manisterski (6).

The development of uredia was recorded at 12 and 14 days after inoculation, and the reactions were classified according to Levine and Cherewick (5). The parasitic differentiation of *P. hordei* isolates was determined by recording the reactions incited on the differential cultivars.

Results and Discussion. The results obtained have demonstrated that in both countries the virulence spectra of the pathogen are broad. Only a few isolates from both Ecuador and Israel were virulent to gene Pa7 (cv. Cebada Capa). The following cultivars possessing the indicated resistance genes, were susceptible to a larger proportion of the Israeli isolates than to the Ecuadorian ones: *Pa2* (Peruvian) 69% vs. 53%, *Pa2+Pa5* (Quinn) 73% vs. 55%, *Pa2* + *Pa6* (Bolivia) 77% vs. 62%, *Pa4* (Gold) 58% vs. 45%, *Pa5* (magnif) 72% vs. 55%, *Pa9* (Abyssinian) 66% vs. 24% (Table 1). These results indicate a higher virulence potential of the Israeli isolates.

The broad spectrum of parasitic specialization of *P. hordei* isolates from Israel can be attributed to the fact that the pathogen survives the rainless summer by completing its life cycle on plants of Ornithogalum species common in the country. According to D'Oliveira (4) *H. spontaneum* - Ornithogalum sp., and *P. hordei* in the Mediterranean region have undergone a long process of convolution, which has resulted in a balanced polymorphism of the components in the host-parasitic system (1). The *P. hordei* populations in Ecuador, however, also show a broad range of pathogenic diversity despite the absence of the alternate host. This phenomenon is probably a result of the genetic differentiation of the barley source hosts cultivated in Ecuador. The broad virulence spectrum of *P. hordei* in Ecuador makes it highly advisable to utilize also partial (race non-specific) resistance in breeding programs.

Table 1. Percent of isolates of *Puccinia hordei* collected in Ecuador and Israel virulent to differential lines of *Hordeum vulgare* either with single genes (*Pa*) or lines with gene combinations used in the survey.

		Percent of virulent isolates	
Lines	Pa gene	Ecuador	Israel
Sudan	Pa	64	58
Peruvian	Pa2	53	69*
Reka #1	Pa2+	71	72
Quinn	Pa2+Pa5	55	73*
Bolivia	Pa2+Pa6	62	77*
Estate	Pa3	61	67
Gold	Pa4	45	58
Magnif	Pa5	55	72*
Cebada Capa	Pa7	2	3
Egypt 4	Pa8	84	83
Abyssinian	Pa9	24	66*
Mean		52.6	62.5

Percentage of virulent isolates followed by an asterisk (*) is significantly different from the corresponding percentage in the sample from the other country ($P<0.05$) χ^2 test.

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New sources of resistance to leaf rust in barley. O. CHICAIZA[1], J.D. FRANCKOWIAK[1], and B.J. STEFFENSON[2], Departments of [1]Plant Sciences and [2]Plant Pathology, North Dakota State University, Fargo, ND 58105, U.S.A.

Introduction. Potential new sources of genetic resistance to *Puccinia hordei* have been identified in landraces (Tu17, Tu27, and Tu34) from Tunisia (Yahyaoui et al., 1988) and in *Hordeum spontaneum* (PI 354937, PI 355447, PI 391024, PI 391069, PI 391089, PI 466245, and PI 646324) accessions (Jin et al., 1995). The reaction of the putative new resistance sources of *P. hordei* differed from that of previous known resistant genes; however, complete allelism tests have not been conducted to determine if the resistance genes differ from previously reported *Rph* genes and gene combinations. The objective of this study was to compare the new sources of leaf rust resistance to the set of barley differential lines carrying *Rph1* to *Rph14* genes.

Materials and Methods. The *Rph* genes from Tu17 and seven *H. spontaneum* lines were transferred to the susceptible cultivar 'Bowman' to develop backcross-derived lines. Tu17 was reported to have resistance gene(s) different from the previously known resistance genes (Yahyaoui et al. 1988). The accessions of *H. spontaneum* were selected as resistant to three isolates (ND89-3, BRS76-12, and BRS90-40), which together are virulent for all known *Rph* genes (Jin et al., 1995). The Bowman backcross-derived lines having *Rph* genes from Tu17 and seven *H. spontaneum* lines were intercrossed and crossed with twelve Bowman backcross-derived lines having *Rph1* to *Rph12* and the original sources of *Rph13* and *Rph14* (Jin and Steffenson, unpublished). Crosses were made in the greenhouse and the F_1 plants were grown in the greenhouse and/or in the field. F_2 progenies were planted in the greenhouse and evaluated for their leaf rust reaction. Six cultures of *P. hordei* (isolate ND8702 of race 8, Australian (AUS) 220, Netherlands (N) 202, race 4, 90-15, and Tel-Aviv), were used to evaluate the infection phenotype of the original sources of *Rph* genes and the Bowman backcross-derived lines. Isolate ND8702 was used to evaluate F_2 progenies from all crosses, except those involving Tu17 with *Rph1*, *Rph4*, *Rph10*, and *Rph11*, which were evaluated with isolate AUS220. Seedlings were inoculated following the procedure described by Jin et al. (1993). Infection types were assessed 9 to 12 d after inoculation using a 0 to 4 scale (Levine and Cherewick, 1952).

Results and Discussion. Because the genes in PI 354937, PI 355447, PI 391024, PI 391069, PI 391089, and PI 466245 were found to be alleles, only data from PI 355447 are presented. The segregation in F_2 progenies showed that the genes from Tu17a, PI 466324 and PI 355447 are different from each other (Table 1). Segregation was not observed in the progenies from the Tu17/Cebada Capa (*Rph7*) and PI 466324/Estate (*Rph3*) crosses. The reaction of these lines to different isolates of *P. hordei* (Table 2) produced infection types similar to those observed in the F_2 progenies. The two genes present in the original Tu17 line were separated based on the reaction types to isolates ND8702 and AUS220. Tu17 exhibited different infection types than Tu17a or Tu17b to these isolates. Allelism tests for Tu17b were not conducted.

Table 1. F_2 segregation for response to *Puccinia hordei* (isolate ND8702) of crosses between sources of the *Rph1* to *Rph14* genes with Bowman backcross-derived lines having *Rph* genes from Tu17a and two *H. spontaneum* (PI 466324 and PI 355447) accessions.

	F_2 segregation								
	Tu17a			PI 466324			PI 355447		
Gene tested	low IT	high IT	X^{2*}	low IT	high IT	X^2	low IT	high IT	X^2
Rp h1	295	85	1.40[+]	330	25	1.48	296	18	0.14
Rph2	323	24	0.26	272	18	0.00	150	13	0.83
Rph3	377	21	0.60	228	0	15.20	294	24	0.91
Rph4	92	38	1.24[+]	438	26	0.33	316	30	3.46
Rph5	424	33	0.73	250	11	1.84	494	22	3.47
Rph6	439	33	0.44	499	25	1.95	623	47	0.66
Rph7	248	0	16.53	373	18	1.80	88	3	1.35
Rph8	154	40	1.98	179	47	2.12	496	30	0.27
Rph9	491	36	0.30	180	8	1.27	493	29	0.43
Rph10	322	123	1.65[+]	386	28	0.18	500	27	1.14
Rph11	320	119	1.03[+]	181	10	0.33	226	14	0.07
Rph12	395	21	1.02	371	25	0.00	73	3	0.69
Rph13	322	18	0.53	185	7	2.22	440	37	1.85
Rph14	374	19	1.34	491	34	0.04	395	20	1.45
Tu17a				424	27	0.05	449	24	1.12
PI 466324							412	21	1.45

[*] $X^2_{0.05}(1) = 3.84$ and $X^2_{0.01}(1) = 6.63$. Expected ratios for X^2-testing are 3:1 for the *Rph1, Rph4, Rph10*, and *Rph11* crosses involving Tu17a and 15:1 for other crosses involving Tu17a and all crosses involving the *H. spontaneum* accessions.

[+] Progenies were screened with isolate AUS220, because the *Rph* genes are susceptible to isolate ND8702.

Conclusions. The gene in PI 355447 is different and non-allelic to the 14 described *Rph* genes; therefore, it probably is at a new *Rph* locus. Data for the PI 466324 and Tu17a genes indicate that multiple allelic series exist at the *Rph3* and *Rph7* loci, respectively.

Table 2. Infection types (IT) produced by six isolates of *P. hordei* on Tu17, Estate, Cebada Capa, and Bowman backcross-derived lines having *Rph* genes from Tu17 and seven *H. spontaneum* accessions.

Cultivar or pedigree	ND8702	A220	N202	Race 4	90-15	Tel-Aviv
Bowman (check)	34	$3^{+}4$	34	34	34	$3^{+}4$
Tu17	00;	0;	00;	00;	00;	00;
Bowman*5/Tu17a	$0;1^{-}$	3^{+}	3^{-}	23^{-}	0;	2^{-cn}
Bowman*2/Tu17b	3^{-}	0;12	$3^{-}2$	0;12	23^{-}	23^{-}
Cebada Capa	00;	00;	00;	0;	3^{-}	$10;^{n}$
Bowman*3/PI 354937	$0;^{cn}$	$0;^{cn}$	$0;^{cn}$	0;1	1^{cn}	1^{n}
PI 355447/3*Bowman	$0;^{cn}$	$0;^{cn}$	$0;^{cn}$	$0;^{cn}$	1^{cn}	$0;1^{cn}$
PI 391024/3*Bowman	$0;^{cn}$	$0;^{-n}$	$0;^{cn}$	1^{n}	1^{cn}	1^{cn}
Bowman*3/PI 391069	$0;^{cn}$	$0;^{cn}$	0;	$0;^{cn}$	$0;1^{cn}$	1^{cn}
PI 391089/3*Bowman	$0;^{cn}$	$0;^{cn}$	$0;^{cn}$	$0;^{cn}$	$0;1^{cn}$	1^{cn}
Bowman*4/PI 466245	$0;^{cn}$	$0;^{cn}$	$0;^{cn}$	1^{cn}	$0;1^{cn}$	$0;^{cn}$
Bowman*3/PI 466324	0;	0;	12^{-}	0;	00;	0;
Estate	00;	0;	3^{+}	0;	0;	00;

^{c,n} Denotes chlorosis and necrosis, respectively.
$^{+,-}$ Denotes more and less sporulation, respectively.

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Genetic basis of resistance to crown rust in different oat genotypes. R. CRUZ; L.C.FEDERIZZI; S.C.MILACH. Faculdade de Agronomia , Caixa Postal 776 , 90001-970 Porto Alegre , Brazil.

Introduction. The yield potential of oat varieties is being continually increased by the breeders , however the realization of this potential is continually restricted by evolution of pathogens able to overcome the resitance of the new varieties. Among the diseases which attack oat , crown rust (Puccinia coronata f.sp.avenae) is the most destructive , causing significant losses of yield in favorable years (MARTINELLI,1994). Chemical methods of rust control are disponible , besides raising up the production costs, they are harmful to the environment. The use of resistant cultivars is the best alternative. However, problems of resistance breakdown are relativelly commom and a continuos search for new genes of resistance are objectives of most oat breeding programs. Identification of new sources of resistance and its genetics basis are of great importance for defining the stategies been used for controling the effects of the rust.The objective of this work was to study the genetic mecanisms responsible for resistance to crown rust in different genotypes and to identifying different sources of resistance for our oat breeding program.

Materials and Methods. Experiments were performed in the field at Experimental Station (UFRGS) in Eldorado do Sul. Crosses utilized in the analysis are listed in table 1. F2 generations of all crosses were sown in 1993. The main panicle of each plant was harvested and thresheded individually. In 1994 , all panicles harvested in the previous year were sown in panicle /line. Plants were scored weekly , from the first occurence of pustules in susceptible parents , for the presence or absence of the disease. Genetic analysis were performed for each cross and segreganting populations. Genetic hypothesis of 1 or 2 genes were tested for each cross and for grouping of crosses depending in the performance of the parental genotypes.

Results and Discussion.Results obtained for each cross are related in table 1. Most of crosses did not fit to one gene model. However, when crosses were grouped in a way that all crosses with a parent were analysed together , the data fitted the proportion expected for one or two genes. (Table 2). Genotype UFRGS 881920 carry a single gene for resistance to crown rust , in contrast UFRGS 15 had a two gene difference with susceptible parents. Crosses involving the two resistant parents showed a two gene difference , indicating that these two genotypes have different genes for crown rust resistance (table 2).

The results obtained in this study cleary shows a very simple genetic basis for the resistance to the prevalent races of crown rust in Brazil , and that UFRGS 881920 and UFRGS 15 are different sources for the resistance and these genes can be recombined in a single parent. The manipulation of these genes in the program should be easy . The extensivelly use of these two major genes may be overcome easilly by the selection of new races of the pathogen.

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TABLE 1. Number of F2:3 lines resistant , segreganting and susceptible to crown rust of different oat crosses.

	N° lines			
Population	Resist.	Segreg.	Suscept.	X^2 (1:2:1)
UFRGS 7 x UFRGS 15*	7	79	77	133**
UFRGS 58 x UFRGS 15	26	104	68	13**
UFRGS 14 x UFRGS 15	5	26	36	32**
UFRGS 881920* x UFRGS 14	3	9	6	1
UFRGS 881920 x UFRGS 7	9	66	34	16**
UFRGS 881920 x UFRGS 8	23	28	16	3
UFRGS 881920 x UFRGS 15	6	59	47	30**

TABLE 2. Number of F2:3 lines resistant and susceptible, to crown rust in different populations.

	N° of lines F2:3			
Population	Resistant	Susceptible	X^2(9:7)	X^2(3:1)
UFRGS 881920 x Suscets.	138	56	-	1,55 NS
UFRGS 15 x Suscets.	247	181	0,37 NS	-
UFRGS 15 x UFRGS 881920	65	47	0,14 NS	-

VIRULENCE OF SINGLE SPORE ISOLATES OF *PUCCINIA CORONATA* FROM ONE SUSCEPTIBLE AND FIVE RESISTANT OAT GENOTYPES. M.B. DILKOVA and R.A. FORSBERG, Department of Agronomy, University of Wisconsin, 1575 Linden Dr., Madison, WI 53706, USA, and K. LEONARD, Cereal Rust Laboratory, USDA-ARS, University of Minnesota, 1551 Lindig Street, St. Paul, MN 55108, USA.

INTRODUCTION. Breeding for resistance to *Puccinia coronata* Corda var. *avenae* (Erikss.) requires extensive knowledge of the genetic basis of host-plant resistance. The mechanism behind resistance includes two coevolving genetic systems, in host and pathogen, which interact with each other and with the environment. Nof and Dinnor (4) confirmed that the gene-for-gene concept applies to *Avena* and crown rust.

Harder and Haber (3) stated that the relative stability of a *P. coronata* population within a geographically defined region allows proper characterization on the basis of a standard set of differential host lines. Oates et al. (5) measured the degree of virulence by the number of differentials successfully attacked by a particular crown rust isolate.

Our main objectives were to analyze the virulence patterns of single spore isolates of *P. coronata* from six different oat genotypes, and to compare the pathogenicity profiles of the isolates from the diverse sources of host resistance.

MATERIALS AND METHODS. The Wisconsin 6x-amphiploid program and the Derived tetraploid C.I.7232 project yielded breeding stocks with desirable degrees of resistance to *P. coronata* and good durability (1, 2). The genes for resistance had wild diploid *Avena strigosa* origin. For this study spores from occasional lesions were collected from resistant lines N770-165-2-1, DCS1789, JR2-3-3-B, MAM17-4, and MAM17-5 grown in West Madison Agricultural Research Station oat nursery in 1993. Isolate Pacer F originated from an adult plant of susceptible cultivar Pacer in the field nursery, and isolate Pacer GH from Pacer seedlings grown in the greenhouse in 1994 and inoculated with a field collection of uredospores from Pacer. The pathogenicity profiles of the seven single spore isolates were constructed using the rust reaction of 29 standard crown rust differential lines, carrying single Pc genes for resistance. In the USDA Cereal Rust Laboratory, St. Paul, MN, 10 seedlings from each differential line were inoculated at the second leaf stage with oil suspension of uredospores with concentration 5 mg/ml, and the rust reactions were examined 12 days later. A scale including five crown rust reaction types was used for rating the interaction phenotypes: Highly Resistant (HR), Resistant (R), Moderately Resistant (MR), Moderately Susceptible (MS), and Susceptible (S). For each isolate an avirulence/virulence formula was derived using only sharply defined interaction phenotypes as a key for translation, i.e., HR/S = Effective/Ineffective Pc genes. Collected data were subjected to correlation analysis.

RESULTS AND DISCUSSION. Host-pathogen interactions are presented in Table 1, allowing the range of resistance possessed by the host breeding stocks to be measured. In terms of isolate virulence, isolates N770, DCS, MAM17-4, MAM17-5, and Pacer F demonstrated higher virulence with an average of 10 genes, compared to isolates JR and Pacer GH, which had two and four genes for virulence, respectively (Table 2). Nineteen out of 29 differentials gave the same disease response to the DCS and JR isolates while N770 shared 12 with JR. Although isolates MAM17-4 and MAM17-5 possessed 10 virulence genes each, only six of those Pc genes were identical for both lines (Table 2). The MAM isolates were more virulent than the JR isolate, with only two ineffective Pc genes, even though selection JR2-3-3-B was one of the direct parents of the MAM lines (1). Because lines N770-165-2-1 and DCS1789 were the parents of line JR2-3-3-5 (1), the relationship between the isolates MAM, N770, and DCS was of a special interest. Isolate MAM17-4 correlated with N770 at $r=0.73$, $P=0.05$, and with DCS at $r=0.41$, $P=0.01$, while results for isolate MAM17-5 were quite opposite: $r=0.41$ with N770 and $r=0.63$, $P=0.05$, with DCS.

Only 11 differentials showed the same rust reaction to both of the Pacer isolates (Table 1). There were 12 differential lines whose Pc genes were effective against isolate Pacer GH, but which demonstrated complete ineffectiveness against the corresponding genes for virulence possessed by isolate Pacer F (Table 2). Isolate Pacer GH was nearly three times less virulent than Pacer F.

Overlapping in pathogenicity profiles of isolates N770 and DCS was rarely seen, indicating that they possessed different genes for virulence and avirulence. Data demonstrated that the genetic basis of resistance of lines DCS and N770 was different, with DCS having a higher level of resistance. Although MAM17-4 and MAM17-5 were sister lines, they hosted slightly different pathogen populations that could be a result of line differentiation through selection.

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Table 1. Crown rust reactions of 29 differential lines against single spore isolates of *Puccinia coronata* from one susceptible and five resistant oat genotypes.

Differential lines with (Pc)genes	Crown rust isolates						
	N770	DCS	JR	MAM17-4	MAM17-5	Pacer F	Pacer GH
Pc14	S	S	S	S	S	S	S
Pc35	S	HR	HR	MS	S	R	R
Pc36	S	R	R	R	R	S	MR
Pc38	S	R	R	R	R	MS	R
Pc39	MS	R	R	S	R	S	R
Pc40	S	S	MS	S	S	MS	S
Pc45	R	HR	HR	R	HR	R	R
Pc46	MS	R	R	R	R	R	MS
Pc48	HR	HR	HR	HR	HR	R	R
Pc50	HR	HR	HR	HR	S	HR	R
Pc51	S	S	R	S	S	S	R
Pc52	HR	R	HR	HR	HR	HR	R
Pc53	HR	HR	R	R	HR	HR	HR
Pc54	R	R	R	R	HR	R	MR
Pc55	MS	R	R	S	R	S	R
Pc56	MS	MR	R	R	R	S	MS
Pc57	R	S	R	R	S	MS	MR
Pc58	R	HR	R	MS	MR	R	R
Pc59	MS	HR	HR	S	HR	R	HR
Pc60	MR	S	MS	S	S	MS	MS
Pc61	R	S	R	MR	S	MS	MR
Pc62	R	R	R	R	R	MS	R
Pc63	MS	R	R	R	R	S	R
Pc64	R	R	R	R	R	S	MR
Pc67	S	S	R	MS	MS	S	S
Pc68	HR	HR	HR	HR	HR	HR	HR
Pc70	S	R	R	S	R	R	R
Pc71	S	R	R	S	S	S	R
Marvellous	S	S	S	S	S	S	S

Table 2. Key to the seven isolates of *Puccinia coronata* based upon only well defined (HR/S) rust reactions on seedlings of 29 differential oat lines.

Isolate	Effective / Ineffective host resistance Pc genes
N770	Pc 48,50,52,53,68 / Pc 14,35,36,38,40,51,67,70,Marvellous
DCS	Pc 35,45,48,50,53,58,59,68 / Pc 14,40,51,57,60,61,67,Marvel.
JR	Pc 35,45,48,50,52,58,68 / Pc 14,Marvelllous
MAM17-4	Pc 48,50,52,68 / Pc 14,39,40,51,55,59,60,70,71,Marvellous
MAM17-5	Pc45,48,52,53,54,59,68/Pc14,35,40,50,51,57,60,61,71,Marvel.
Pacer F	Pc 50,52,53,68 / Pc 14,36,39,51,55,56,63,64,67,71,Marvel.
Pacer GH	Pc 53,59,68 / Pc 14,40,67,Marvellous

Diversity of the Czech and Slovak spring barley cultivars to powdery mildew and leaf rust

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Introduction. The importance of diseases on spring barley was investigated in the Czech Republic. Based on the unified parameters 185 field experiments of the State Variety Trials were evaluated in 1989 to 1995. High infection severities in powdery mildew [*Erysiphe graminis* f. sp. *hordei* (*Blumeria graminis*)], leaf rust (*Puccinia hordei*), leaf blotch (*Rhynchosporium secalis*), and net blotch (*Pyrenophora teres*) were found in 55, 32, 8 and 1 experiments, respectively (DREISEITL and JUREČKA, unpublished). That is in accordance with long-term experience. Therefore, breeding cultivars has focused particularly on powdery mildew and leaf rust resistance through the last decades.

Materials and Methods. Results obtained for 37 Czech and Slovak spring barley cultivars during the period from 1981 to 1996 were evaluated. To identify specific resistance genes 17 isolates of powdery mildew and 12 isolates of leaf rust with known virulences were used. The experiments with powdery mildew were carried out mainly at the Agricultural Research Institute Kroměříž and those with leaf rust at North Dakota State University Fargo. The plants at the primary leaf stage were inoculated separately with the mildew isolates by shaking infected plants over the cultivars and with the leaf rust isolates by spraying the oil-uredospore suspension on primary leaves. Seven days after inoculation in the mildew experiments and ten days in the leaf rust experiments the infection types (i.t.) were scored using the 0-4 scale (TORP et al. 1978). This scale (in a modified form) was also used for scoring i.t. in leaf rust. Resistances of the cultivars (Table 1) are described using the gene symbols, and in the case of powdery mildew also the accepted European codes (BOESEN et al. 1996). The resistance spectra of cultivars always within a respective pathosystem were compared with those of standard cultivars and lines possessing known resistance genes. The method is based on the gene-for-gene system (FLOR 1955).

Results. Twelve known and one unknown resistance genes to powdery mildew have been found. They are present as single genes or combined with others (genes of the *Mla* locus with *Mlg, MlLa* or *Mlat*) in each of the tested cultivars. Some of them are composed of two lines with different resistances (resistances of lines are designated with '+' in Table 1). No specific resistance genes have been detected in nine cultivars tested for leaf rust resistance. Three cultivars were always composed of a line with a resistance gene and a susceptible line. Four specific resistance genes to leaf rust have been found. One of them (in 'Kredit' and together with a susceptible line in 'Jubilant' and 'Viktor') has not been identified until now. Three cultivars show a combination of genes *Rph3,Rph12*.

Discussion. In 1981, spring barley cultivar 'Karat' was registered in former Czechoslovakia (BRÜCKNER 1982). It was the first cultivar that possessed, besides the

fully effective resistance to **powdery mildew** (*Mla13*), resistance to leaf rust (*Rph3*) of the same effectiveness at that time. The cultivars carrying *Mla13* have been grown since 1978. The gene was defeated by the adapted powdery mildew population during the epidemics in 1985. Till that time, the cultivars carrying *Mla13* had been sown on the area of 1.5 mln ha in former Czechoslovakia and did not need chemical treatments against powdery mildew (DREISEITL 1993). After the *Mla13* resistance had been broken, cultivars combining genes of the *Mla* locus with *MlLa* and *Mlat* began to be grown. However, an important change in growing barleys resistant to powdery mildew took place in 1993 when 'Forum' was registered. This is the first cultivar of domestic ones which possesses the fully effective gene *mlo* to the present population (BRÜCKNER 1993). Other cultivars carrying this gene have been registered since that time. A number of spring barley cultivars resistant to powdery mildew is supposed to increase in the future. This assumption is supported by many *mlo* genes (JØRGENSEN 1994) with other defensive mechanisms and the identification of a number of new genes coming from *Hordeum spontaneum* and located particularly in the *Mla* locus (JAHOOR and FISCHBECK 1987, 1993).

Leaf rust occurred on the cultivars carrying *Rph3* for the first time in 1985, and three years later, they were infected similarly to those susceptible to this pathogen (DREISEITL 1990). Other genes found in the tested cultivars are not effective in the field either. Gene *Rph7*, still effective in the Czech Republic, has been identified in three new lines which were tested in the State Variety Trials in 1994 and 1995 (DREISEITL and STEFFENSON, unpublished). Similarly to powdery mildew, the variability for important resistances to leaf rust within *Hordeum vulgare* seems to be exhausted. Therefore, the interest has focused on *Hordeum spontaneum* again, which has resulted in detecting a lot of resistant samples (JIN et al. 1995). Although their inheritance and relationships have not been studied so far, they are expected to enable developing resistant cultivars.

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Table 1. Specific resistance genes to powdery mildew and leaf rust in chosen Czech and Slovak spring barley cultivars (Czech Republic, 1996)

Cultivars	Resistance to powdery mildew		Resist. genes to leaf rust
	Genes	European codes	
Karat [1)]	Mla13	Ru	Rph3
Zefir [1)]	Mla12, Mlg	Ar We	none
Krystal	Mla13, Mlg	Ru We	Rph2
Horal	Mla7	Ly	none
Rubin	Mla1	Al	Rph12
Mars [1)]	Mla3,Mlg	Ri We	Rph2
Kredit [1)]	Ml(Kr)	Kr	U
Bonus [1)]	Mla13	Ru	none
Zenit [1)]	Mla13	Ru	Rph3
Jaspis	Mla6,Mlat,MlLa	Sp At La	none
Orbit	Mla6,Mlat,MlLa	Sp At La	none
Jarek	Ml(Kr),MlLa	Kr La	none
Perun	Mla13	Ru	Rph3
Novum	Mla13,Mlg	Ru We	Rph2
Profit	Mla6,MlLa	Sp La	Rph3
Malvaz	Ml(Kr)	Kr	Rph3
Galan	Mla13	Ru	Rph12
Jubilant	Mla12,U	Ar U	none+U
Terno	Mla9,MlLa	MC La	Rph3
Akcent	Mla7,U	Ly U	Rph12
Heran	Mla13	Ru	Rph12
Ladik	Mla12	Ar	Rph3
Sladko	Mla7,U	Ly U	Rph12
Svit	Mla13,Mlat	Ru At	none
Donum	Mla7+Mla1	Ly+Al	Rph12
Forum	mlo	Mlo	Rph3
Stabil	Mla6,MlLa+Mla13,MlLa	Sp La+Ru La	Rph3
Pax (Kosan)	Mla13,MlLa	Ru La	none+Rph3
Viktor	Mla13,MlLa,Mlg	Ru La We	none+U
Amulet	Mla13,MlLa	Ru La	Rph3,Rph12
Kompakt	Mla13,MlLa	Ru La	Rph3,Rph12
Lumar	Mla1,Mlg	Al We	Rph12
Primus	Mla6,Mlat,MlLa	Sp At La	none
Atribut	mlo	Mlo	Rph3,Rph12
Famin	Mla7,U	Ly U	Rph12
Olbram	mlo	Mlo	Rph12
Pejas	Mla13,Mlat	Ru At	none

[1)] withdrawn from growing

Investigation of Tolerance to Barley Yellow Dwarf Virus in cultivated Oats

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Introduction

Oats (*Avena sativa* L.) are mostly very susceptible to Barley Yellow Dwarf Virus (BYDV) and react with severe stunting, reddening and yield reductions (OSWALD & HOUSTON 1953;). The creation of tolerant or resistant cultivars is the most economical way of controlling the disease. From an epidemiological point of view the resistant cultivars should be preferred because they may stop the spreading of the virus (GRAY et al. 1993; JEDLINSKI et al. 1977).

Among the *A. sativa* genotypes and their wild relatives a number of tolerant or resistant accessions have been found (COMEAU & DUBUC 1978; COMEAU 1984; ENDO & BRAUN 1964). The level of the most tolerant A. sativa genotypes provides approximately 30% of the yield of the control plants after inoculation in the one to two-leaf stage, whereas susceptible genotypes often die after such early infection (COMEAU 1987).

The following trials were made to examine the tolerance to BYDV in some accessions from the European Oat Disease Nursery (EODN)(SEBESTA 1994) and to investigate the tolerance level with an German PAV-isolate of BYDV.

Materials and Methods

Oat genotypes have been chosen according to the results of the EODN and other recordings, so that the first Screening in 1994 was concentrated on 51 genotypes. In the following year the best 20 lines were investigated a second time.

Viruliferous *Rhopalosiphum padi* L aphids were reared in a glasshouse on oat-plants cv. Gramena infected with PAV-1 isolate of BYDV, provided kindly by Dr. A. Habekuß. Inoculation was done by transferring the aphids on short leaf segments containing 5-15 aphids to every single seedling. After a period of 48 hours the aphids were killed with Decis.

In 1994 the seedlings were inoculated in the one-leaf stage and in 1995 the inoculation was done in the three- to four-leaf stage. At the same time control plants have been cultivated under the same growing conditions.

Some days after inoculation seedlings have been planted in to the field in a completely randomised block design with 4 replications. Each replication consisted of one row inoculated plants (8 plants in 1994 and 10 plants in 1995) and one row control plants with a distance between the rows of 20 cm and between the plants 10 cm.

The border-plants were not taken into account for evaluations. Following data were recorded or calculated:

- Number of panicles per plant were counted from the centre plants.
- Plant height was measured from the ground of the main culm to the top of the panicle.
- Grain yield of the single plants, calculated by deviding the yield of the whole bulk through the number of plants, including the death ones.
- Kernel number per panicle was calculated by deviding the kernel number of the hole bulk through the number of panicles.
- 1000-kernelweight was calculated using the kernel number and the weight of the hole bulk.
- Discolouration: Visual Scoring with 1 = no discolouration; 9 = the most severe discolouration (reddening), mostly combined with necrotic leaves

All data with the exception of the discolouration scoring have been subjected to an analysis of variances with the model „fixed" for genotypes and „random" for blocks. To calculate the single linear correlation coefficient and all other calculations the software MS Excel, version 5.0 have been used.

Results and Discussion

In 1994 all genotypes showed strong symptoms like stunting, reddening and early death of many plants before heading, especially in the most susceptible lines. Most of the plants developed only

Table 1 Response of different oat genotypes to BYDV inoculation in the experiments of 1994 and 1995

Genotype	Grain yield per plant (g)				No. kernels per panicle				1000-kernel wt.(g)				No. panicles per plant				plant height (cm)				discolouration score***	
	1994		1995		1994		1995		1994		1995		1994		1995		1994		1995		1994	1995
	Inocul.	Contr.	Inocul.	Contr.	Inocul.	Contr.	Inocul.	Contr.	Inocul.	Contr.	Inocul.	Contr.	Inocul.	Contr.	Inocul.	Contr.	Inocul.	Contr.	Inocul.	Contr.	Inocul.	Inocul.
IL 86-4189	1,2	6,9	3,8	8,8	15	36	42	67	28	30	29	34	3,0	6,6	3,8	4,6	50	68	74	95	4,3	4,3
IL 86-6404	1,0	6,2	2,0	9,5	15	34	30	76	33	34	31	42	2,1	5,5	2,7	4,3	55	80	72	98	4,8	4,9
Pc 61	0,9	5,2	1,2	4,8	10	18	12	23	30	31	27	40	2,8	9,8	4,1	7,0	46	65	53	83	5,0	7,0
IL 86-5698	0,8	4,2	1,2	6,8	7	26	17	47	36	32	35	44	2,8	4,9	2,6	4,7	55	75	65	92	4,8	6,1
Pc 68	0,7	5,4	2,1	11,4	20	32	35	78	30	34	35	42	1,9	5,1	2,9	4,2	50	76	67	98	5,0	6,6
Jostrain	0,5	3,2	0,6	10,0	24	22	27	75	22	26	22	33	1,3	5,7	1,5	5,9	48	78	41	101	5,1	7,3
Wiesel	0,5	6,5	2,0	11,8	10	34	30	85	27	29	28	39	1,7	6,5	2,9	5,8	40	70	57	88	5,7	7,3
EPNGr.	0,5	6,6	1,1	14,7	11	46	25	100	27	31	27	37	1,4	4,8	2,4	4,8	43	78	54	93	5,7	7,4
Pc 64	0,4	4,1	1,3	11,0	17	31	37	88	22	28	21	30	2,3	4,8	2,1	6,2	58	78	60	105	4,8	6,8
Pc 50-2	0,4	4,7	0,5	11,3	11	29	24	105	25	25	19	30	1,5	6,8	1,8	4,9	48	78	49	97	4,9	7,3
Pc 58	0,4	3,3	0,2	4,1	11	20	11	35	27	29	24	43	1,5	5,5	1,9	3,8	51	69	34	87	5,1	8,0
Gramena	0,4	8,7	1,4	15,4	13	52	29	120	25	29	27	35	1,2	5,8	2,5	4,5	38	79	61	98	6,1	7,3
Pc 59	0,4	2,9	1,0	4,3	6	20	15	32	34	32	34	45	2,0	4,8	2,6	3,2	45	63	51	81	5,0	6,2
IL 85-2069	0,4	4,1	1,1	6,9	7	25	18	51	29	34	28	40	1,9	4,7	2,4	4,0	45	65	61	92	5,7	7,4
IL 85-6467	0,3	2,6	2,7	7,9	6	18	34	60	27	32	32	42	1,8	4,5	2,8	3,8	36	61	63	80	4,8	5,7
APR 122	0,2	3,1	0,1	11,9	10	23	20	102	24	27	15	30	1,3	4,8	1,1	5,4	34	74	23	98	5,8	8,5
Melys	0,2	5,4	2,2	11,2	10	41	46	137	27	27	25	33	1,2	4,8	3,3	4,5	40	73	64	89	4,8	5,7
Cc 4146	0,2	2,9	0,3	4,6	8	25	23	83	23	29	20	32	0,9	4,1	1,2	3,0	59	118	24	121	5,8	7,3
Erbgraf	0,1	4,5	0,5	11,8	8	24	18	77	24	30	24	36	0,6	6,3	2,2	5,3	30	70	51	93	6,7	8,0
Saia			3,3	8,1			43	73			20	24			5,1	6,5			120	140		4,8
GD Tukey (p=0,05)	0,7	4,3	1,1	6,5	16,4	27,2	21,7	39,6	11,2	5,5	8,6	6,1	1,4	2,5	1,9	2,2	31,9	15,9	33,3	15,0		
mean (without Saia)	0,5	4,8	1,3	9,4	11,5	29,2	26,0	75,8	27,3	29,9	26,5	37,2	1,7	5,6	2,4	4,7	45,7	74,4	53,8	94,0	5,2	6,8
% reduction*	89,4		85,7		60,6		65,8		8,8		28,8		68,9		48,1		38,5		42,8			
correlation coefficient between both years**	0,56	0,61			0,41	0,72			0,82	0,75			0,62	0,65			0,07	0,90			0,73	

* reduction = (control - inoculation) x 100 / control
**underlined coefficients are significant for alpha = 0,05
*** Score 1....9 with 1= no discolouration

one to three culms with poor panicles. This has led to severe yield reduction with a mean single plant yield reduction over all genotypes of 89 % (Tab. 1). Among the yield components the 1000-kernel weight showed the lowest reduction of 8,8 %, the number of panicles per plant showed the highest reduction of 68,9 % .

In 1995 the grain yields and plant height of the inoculated and control plants were higher than in 1994. This was mainly realised about higher tillering and higher kernel number per panicle (Tab. 1). The reasons for the better performance in 1995 are at first the favourable weather conditions. The second reason may be the later inoculation in 1995, so that especially the number of panicles per plant was less reduced than in 1994. On the other hand the 1000-kernel weight was more reduced in 1995, explainable with the high kernel number per panicle, which makes it difficult for the infected plant to fill well the kernels. Similar results were described by COMEAU (1987), who investigated the influence of various dates of inoculation with BYDV in oats, barley wheat, rye and triticale.

In the two years significant differences between oat lines in yield and its components have been found. The highest grain yields per plant about both years were measured in the lines IL 86-4189; IL 86-6404; IL 85-6467; IL 86-5698, Wiesel, Melys, Pc61 and Pc68 (Tab. 1). The tolerant *A. strigosa* cultivar „Saia" has been included for the first time in 1995 showed a high tolerance level like IL 86-4189. These findings confirm the results from other scientists, especially concerning the yield reductions and reactions of IL 86-4189, IL 86-6404 and Saia (OSWALD & HOUSTON 1953; KOLB et al. 1991). Although IL 85-6467 and Melys have shown different performances in both years, there was in general a good correspondence between the ranges in both years, documented by significant correlation coefficients (Tab. 1). Therefore an evaluation of oat lines with the used specific BYDV-strain seems to be reliable, but there are other variants of BYDV, which may alter the resistance ranking and makes it necessary to examine the oat genotypes against different BYDV variants (COMEAU & DUBUC 1978).

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Present Status of localisation of mildew resistance genes in barley and their synteny among cereals.

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Powdery mildew caused by *Erysiphe graminis D.C. f. sp. hordei* is an obligate parasite and one of the most important diseases of barley at temperate climates. Based on the gene-for-gene hypothesis of Flor (1955) that was confirmed for powdery mildew of barley by Moseman (1959), many race-specific powdery mildew resistance genes from different origins have been recognized in barley (Moseman 1955). As far as they have been localized, they were mapped on chromosomes 4 (4H), 5 (1H), and 6 (6H). Recently, the *MlLA*-mildew resistance gene was mapped on chromosome 2 (2H) by means of RFLP markers (Hilbers et al. 1992; Giese et al. 1993).

Loci for resistance to powdery mildew of barley such as *Mla* (Schüller et al. 1992), *MlLA* (Hilbers et al. 1992), *mlo* (Hinze et al. 1991), and *Mlg* (Görg et al. 1993), which are widely used in barley breeding, have also been marked with RFLP clones. One of the far reaching aims is to isolate these genes by map-based cloning (Paterson und Wing 1993). Accessions of *H. vulgare ssp. spontaneum* lines from Israel have repeatedly been described as a very rich gene pool for powdery mildew resistance (Moseman 1955; Fischbeck et al. 1976). Many resistances were identified but only for some of them allelism or close linkage with already known loci for mildew resistance has been determined (Jahoor und Fischbeck 1987).

In three barley lines 'RS42-6*O.', 'RS137-28*E., and 'HSY-78*A.' derived from crosses from wild barley (*Hordeum vulgare ssp. spontaneum*) new major, race specific resistance genes to powdery mildew (*Erysiphe graminis f. sp. hordei*) were identified. The resistance gene originating from wild barley of line 'RS42-6*O.' shows a recessive mode of inheritance, the other both wild barley genes a dominant one. RFLP (**R**estriction **F**ragment **L**ength **P**olymorphism) mapping of these three genes was performed in segregating F2 populations. The recessive gene of line 'RS42-6*O.' was localized on barley chromosome 1S (7HS), while the dominant genes of the lines 'RS137-28*E.' and 'HSY-78*A.' were localized on the chromosomes 1L (7HL) and 7L (5HL), respectively. Closely linked RFLP clones mapped at distances between 2,6 cM and 5,3 cM. Hitherto, specific loci for powdery mildew resistance in barley remained unknown on these chromosomes. Furthermore, tests for linkage to the unlocalized resistance gene *Mlp* revealed free segregation. Therefore, new designations are suggested: *mlt* ('RS42-6*O.'), *Mlf* ('RS137-28*E.'), and *Mlj* ('HSY-78*A.'). Comparisons with mapped QTLs for mildew resistance are made and discussed in the context of homoeology in the genomes of barley (*H. vulgare*), wheat (*Triticum aestivum*), and rye (*Secale cereale*). Duplications of RFLP bands detected in the neighbourhood of *Mlf* and *mlt* seem to indicate evolutionary interrelationship to the *Mla* locus for mildew resistance (Fig.1).

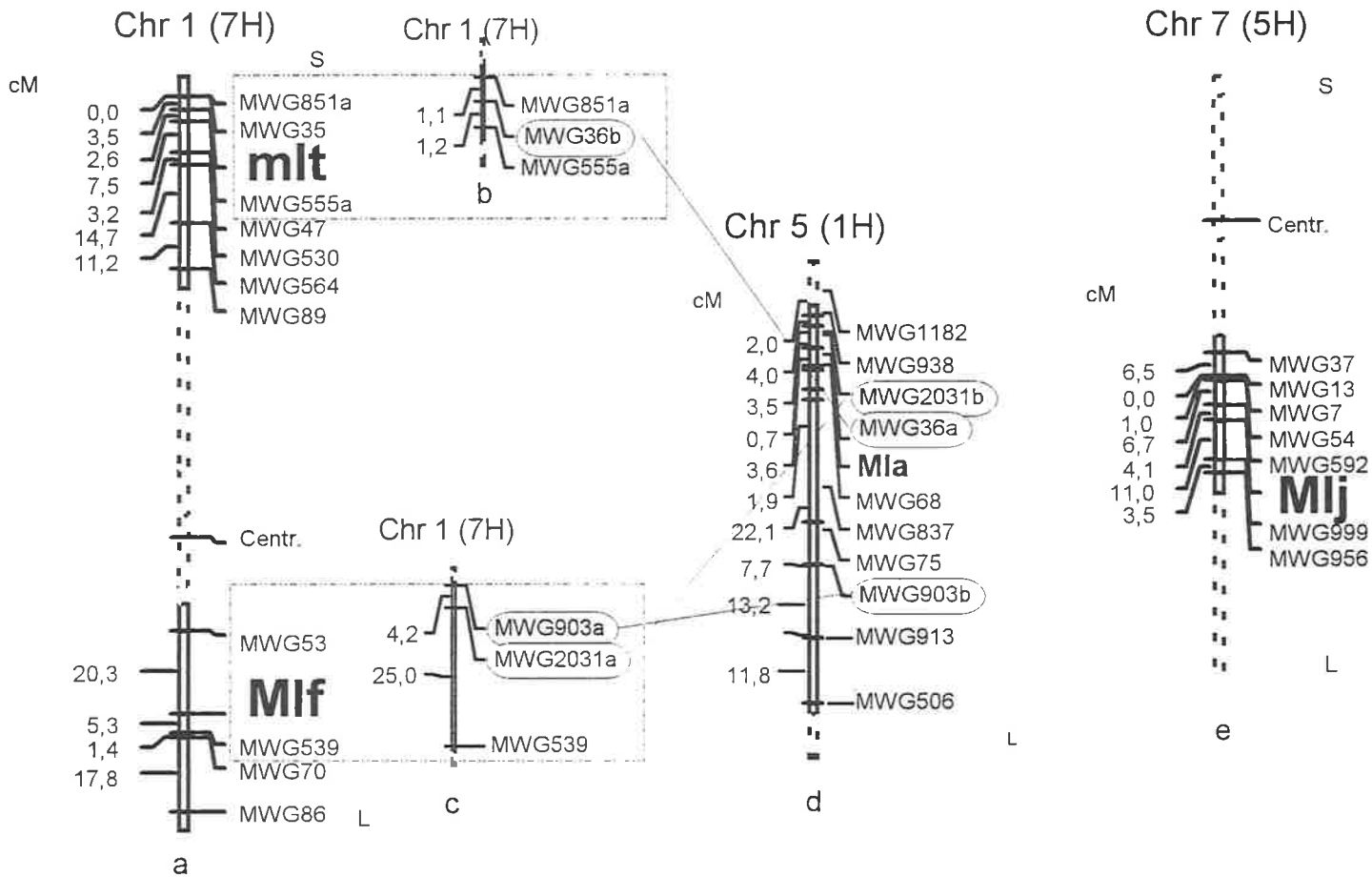

Figure 1: Centr.=centromere, S=short chromosomearm, L=long chromosomearm, (**a**,**e**) RFLP mapping the loci *mlt*, *Mlf* and *Mlj* (**b**) Kilian et al. (1995) (**c**) Graner et al. (1993) (**d**) RFLP probes linked to chromosome 5 (1H) of the F2 mapping population '(HSY-78*Ar.)*Pallas
[.....] cuts of chromosomes of different mapping populations with one common marker at minimum

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Screening for resistance to *Rhizoctonia solani* in barley. V.A. JITKOV[1], S.E. ULLRICH[1], and R.J. COOK[2], [1]Departments of Crop and Soil Sciences and [2]Plant Pathology, Washington State University, Pullman, WA 99164, USA.

Introduction. *Rhizoctonia solani* Kühn is a fungus capable of affecting a large number of plant families and causes various plant diseases. In barley (*Hordeum vulgare* L.) *R. solani* causes a root rot that is often called "bare patch". Thus far no genes for resistance to *R. solani* have been identified and reported in the literature. However, reports that genetic transformation of tobacco (*Nicotiana tabacum*) plants with the chitinase gene and the barley ribosome inactivating protein (RIP) gene result in increased resistance of the transgenic plants to *R. solani* (Broglie et al., 1991; Logemann et al., 1992), indicate the possibility of the existence of "natural" plant genes for resistance to this ubiquitous pathogen.

Materials and Methods. *Plant material.* Screening was performed on a diverse set of *Hordeum vulgare* germplasm. A subset of 1,100 accessions from a "preliminary core" collection of the USDA-ARS working collection was used (provided by H.E. Bockelman).

R. solani Inoculum Preparation. Inoculum of the *Rhizoctonia solani* strain C-1 was stored at 4°C in Petry dishes on 1% potato-dextrose-agar (PDA). Upon need, 5 mm agar plugs from the edge of the colony were transferred to new Petry dishes, containing fresh PDA, and grown at room temperature for 7 days. After the incubation period was completed, colonized agar was cut into 4 cm^2 piece's and transferred to sterilized oat medium (twice sterilized oat kernels). Inoculum was then incubated for 21 days at room temperature. After the incubation period was completed, oats, colonized with *R. solani* were air dried and stored at 4°C (Weller et al., 1986).

Screening procedure. Screening for *R. solani* resistance was performed under growth chamber conditions. Plastic conetainers (30 mm diameter) were filled 2/3 with vermiculite and 1/3 with soil. Soil was inoculated with *R. solani* by adding two colonized oat kernels to moist planting media 2 days prior to planting. Two to three barley seeds were planted in three replications and covered with 1 cm of vermiculite. Cones than were placed in the growth chamber at 12°C and 12 hr. light/dark photoperiod, and barley seedlings were allowed to develop for the period of 3 weeks. After 21 days of incubation barley roots were examined for reaction to *R. solani.* Visual ratings were given as follows: 0-Tr (trace) = 0-2 minor lesions; 1 = at least 1 major lesion on root axis (root is stubbed off), no lesions on the coleoptile ; 2 = 2-3 seminal roots stubbed off, no lesions on the coleoptile; 3 = 4-5 roots are stubbed off, possible lesions on the coleoptile; 4 = all roots are stubbed off, some lesions on the coleoptile; 5 = all roots are stubbed off, intensive lesions on the coleoptile and discoloration of the seedling. Genotypes showing resistance and extreme susceptibility were retested to confirm results.

Results and Discussion. Screened accessions were grouped into six categories: Highly resistant (0-Tr), Resistant (1), Moderately resistant (2), Moderately susceptible (3), Susceptible (4), and Highly susceptible (5). Distribution of the 1,100 screened accessions

among these categories is depicted in the Figure 1. Based on this study 8% or 81 accessions were highly resistant, 14% or 154 accessions were resistant. The highly resistant group consisted of 63% (51 accessions) landraces, 31% (25 accessions) cultivated varieties, and 6% (5 accessions) breeder lines (Figure 2). The original set of accessions consisted of 46% landraces, 41% cultivated varieties, and 13% breeder lines. The geographic origin of the resistant accessions is spread around the globe (Figure 3). However, 43% (104 accessions) originated in Asia and 29% (70 accessions) in Europe, while only 35% of the screened accessions have their origin in Asia and 20% in Europe.

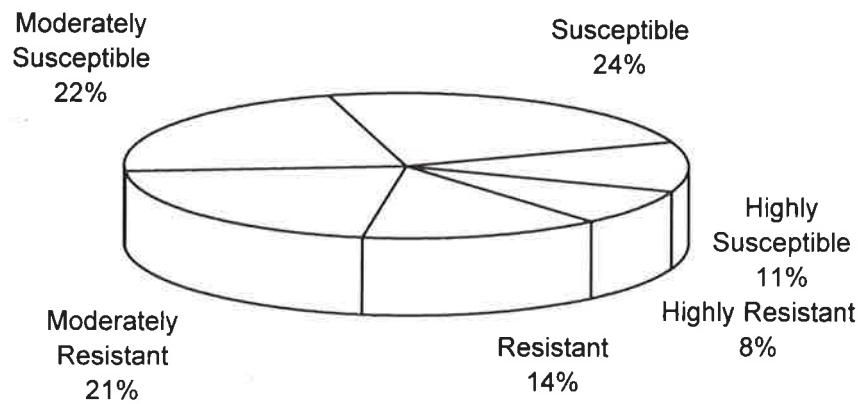

Fig. 1. Distribution of reaction to *Rhizoctonia solani* in screened barley accessions.

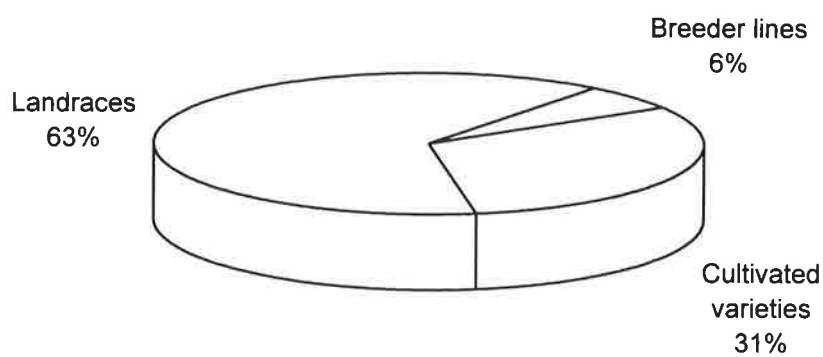

Fig. 2. Composition of the barley group highly resistant to *Rhizoctonia solani*.

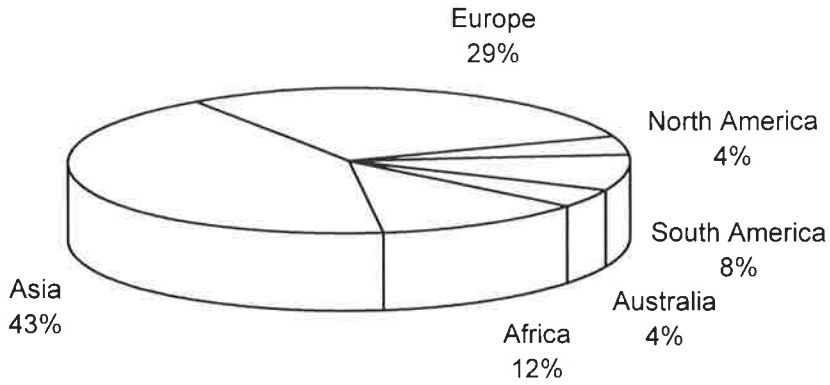

Fig. 3. Geographic origin of *Rhizoctonia solani* resistant barley accessions.

In order to determine the inheritance characteristics of resistance and susceptibility, several lines each expressing high resistance or high susceptibility have been crossed in various combinations. F_2 progeny derived from these crosses will be screened for resistance to *R. solani*.

This should be considered a preliminary report. However it appears that for the first time resistance to *R. solani* in barley has been identified.

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Necrosis Inducing Peptide 1 involvement in a gene-for-gene relationship with *Rh3* of barley cultivar Atlas 46.

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Introduction

The gene-for-gene hypothesis explains specific host-pathogen interactions as involving two genes (Flor, 1942), a resistance gene in the host and an avirulence gene in the pathogen. If there is recognition between these gene products then an incompatible reaction occurs and infection is prevented. Necrosis inducing peptide (NIP) 1 is an avirulence gene product from *Rhynchosporium secalis* that interacts specifically with the *Rh3* resistance gene in barley cultivar Atlas 46 (Wevelsiep et al., 1991). When NIP 1 is not recognized, it behaves as a virulence factor and acts to stimulate the host cells' plasmalemma ATPase and cause cell collapse. NIP 1 must be present and active in the *R. secalis* genome to cause avirulence on Atlas 46. Pathotypes that are virulent on both Atlas and Atlas 46 have been isolated in western Canada. Primers derived from the DNA sequence of the NIP 1 gene were used to characterize Canadian *R. secalis* isolates. DNA sequence analysis shows relevant differences between isolates that may be the cause of virulence or avirulence on barley cultivar Atlas 46.

Materials and Methods

DNA was extracted from *R. secalis* isolates using a modified method of McDermott et al. (1989). Cultures were grown for 2-4 weeks in Fries medium then mycelia were harvested by filtering through Miracloth in a Buchner funnel. 1-1.5 g of filtered mycelia were ground to a powder in a mortar and pestle in liquid N_2 then suspended in 3 ml extraction buffer (150 mM EDTA, 50 mM Tris pH8.0, 1% sarcosyl, and 300 mg/l proteinase K). This suspension was centrifuged at 2100 rpm for 10 min. and the supernatant extracted twice with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1). The aqueous phase was precipitated by addition of two volumes of 95% ethanol and sodium acetate at a final concentration of 0.25 M. After centrifugation for 10 min. at 2100 rpm the pellet was washed in 70% ethanol, dried and resuspended in 200-500 ul TE buffer.

Polymerase chain reaction amplifications were performed in a total volume of 25 ul consisting of 20 pmoles of each primer, 25 ng genomic DNA, 0.2 mM of each dNTP, 2.0 mM $MgCl_2$ (Perkin-Elmer), 1x Taq activity buffer (Perkin-Elmer) and 1 unit Taq polymerase. Thermolyne temptronic thermocyclers were used for all amplifications. Programs started with 5 min. at 95°C followed by 35 cycles of: 95°C for 5 sec., 52°C for 30 sec., and 72°C for 60 sec. Products were visualized on 1.6% agarose gels stained with 4.2 ug/ml of agarose and photographed with Polaroid film.

DNA fragments were extracted from agarose gels using either the freeze and squeeze method or by poking the band of interest with a 200 ul pipette tip and

immediately swirling it in a fresh PCR mix for a second amplification. Purified fragments were sequenced at the Plant Biotechnology Institute (NRC, Saskatoon). Comparison of sequences was done using ALIGN.

Results and Discussion

All of the Canadian isolates were found to carry the NIP 1 gene except for WRS1860. Sequencing reactions were performed with isolates WRS1389, WRS1860 (x2), WRS1391, WRS837, WRS1493, WRS1824, and WRS1864 (Figure 1). Comparison of sequence showed that WRS 1860 had the same sequence as WRS 1864, WRS1824, WRS1493, and WRS837 even though the NIP 1 gene does not visibly amplify with the NIP 1 primers while the others do. It is not possible to compare sequence at the conserved primer binding sites where it is likely that WRS1860 differs from the other isolates. Some other sequence differences coded for a change in amino acid sequence or in the case of WRS 1391 and WRS1389 the difference was at the last base of the intron.

The virulence of an isolate on Atlas and Atlas 46 did not always agree with molecular evidence of the presence of NIP 1. Isolates WRS1391 and WRS1380 were virulent on Atlas 46 although NIP 1 could be amplified from both genomes. This can be explained if there is a sequence change within the amplified fragment that renders the peptide inactive. An alternative explanation is the requirement for a minimum concentration of the peptide to be present to be functional. Wevelsiep et al. (1991) found that a much higher concentration of NIP 1 was required to cause necrosis than the NIP 2 or NIP 3 peptides. The presence of the NIP 1 fragment on the gel could be used in most cases to predict avirulence on Atlas 46. It is possible that primers based on sequence variations could be used to extend the predictability of this test and provide a useful tool for future research into this gene-for-gene relationship.

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US238 -240
ATCCTCAGCTTCTGTGAGTATACTTGGTTTCCAGGATAATTAAGCCATTGCCATCCACA
-180
GGGATCCTGATAGAACTATTCCCTGCAGTAACAAGTTCTATCAGGGTCGACCGTCTCTAT
-120
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-60
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1
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61
ACGCCGGATCGATGCAGATACACCCTTTGTTGCGATGGAGCTCTCAAAGCTGTTTCCGCA
1860                                                            a
1389                                                            a
1391                                                            a
AU2                                                             a
121
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1860    a a                     t
1389    c t                     c
1391    c t                     c
AU2     a a                     t
181
TTGACGCTTTGCTCATATGGTGAAGGTGGCAATGGCTTCCAATGCCCGACGGGATACGTA
1860            g                               a
1389            g                               c
1391            g                               c
AU2             c                               a
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  g  1860
  g  1389
  g  1391
  a   AU2

361
TAGGCGAACGGATCACTCCAAAGGGTCTCGCTCTAGCTGAGAATTGACAAAGAAAGAAAT
421
GAAACATGACATTGAGCTAAACACTGAAATACATCGGTGCATCAATTTAAATGTCATGCC
481
TTTCAGGGACGCGGGACCTGGACCCACTCTGTGCTGCTACATGAACTAAATCTGAACCATT
541
GTCCCTGCCATATAGCAATAATGCAATTCATATACTATCTATCGTGGCGGCTTT 595
```

Figure 1. NIP 1 sequence

The start and stop codons are underlined, the intron is marked with the dotted underline and the NIP 1 primer binding sites are double underlined. The putative TATA box is in bold print.

RAPD analysis of *Rhynchosporium secalis* pathotypes

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Introduction

Leaf scald of barley is caused by *Rhynchosporium secalis* (Oud. Davis). *R. secalis* populations are highly variable for virulence both in Canada (Tekauz, 1991) and worldwide as studies in Australia (Brown, 1990) and the southwest United States (Jackson and Webster, 1976) have shown. A sexual stage in the life cycle has not yet been identified therefore variation is not due to recombination events during meiosis. The high level of variability may come from either a high mutation rate or from parasexual recombination (Goodwin et al., 1994). Knowledge about the molecular mechanism of this variability would be useful to evaluate host resistance gene deployment strategies. The lack of a universal single resistance gene differential set combined with difficulties encountered in maintaining virulence in cultures of *R. secalis* have made understanding population dynamics difficult. Studies comparing isozymes (Goodwin et al., 1993), colony colour and rDNA sequence variation (McDermott et al., 1989) to virulence or pathogenicity have not been effective in determining a correlation between the two. The objective of this study was to determine whether the wide range of virulence exhibited by *R. secalis* isolates in Canada is a reflection of genetically distinct populations carrying different virulence/ avirulence genes, or simply the result of variation throughout the genome. RAPD analysis (Williams et al., 1990) was used to detect random sequence variation in the *R. secalis* genome. RAPD analysis has been used to identify pathotypes of *Fusarium graminis* (Grajal-Martin, 1993) and wheat leaf rust (Kolmer, pers. commun.).

Materials and Methods

R. secalis isolates were selected on the basis of variation in virulence and viability (Tekauz, 1991; Tekauz, unpublished). Scald infected barley leaves were collected from farm fields and research plots throughout Canada. Isolations were made from leaf tissue (2mm x 5mm) comtaining a single discrete scald lesion. Following initial differential screening, isolates were stored under oil at 4°C. Inoculum was increased by transferring a piece of agar from oil storage into 40 ml of Fries liquid medium, and incubated for three weeks at 16°C with 12 hr. light/24 hr. period. Inoculum was further prepared, quantified, and inoculated as described by Tekauz (1991).

DNA was extracted according to McDermott et al., (1989) with the following modifications. Mycelia were harvested by filtering through Miracloth in a Buchner funnel. Filtered mycelia (approx. 1-1.5g) were ground in a pre-chilled mortar and pestle with liquid N_2 and suspended in 3 ml of extraction buffer (150 mM EDTA, 50 mM Tris/pH8.0, 1% (w/v)sarcosyl, and 300mg/l proteinase K). Suspensions were vortexed for 45 sec. and centrifuged for 10 min. at 2100 rpm. The supernatant was removed to a fresh tube and protein was removed with two

washes of an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1). DNA was precipitated through addition of sodium acetate to a final volume of 0.25 M and two volumes of pre-chilled 95% ethanol, followed by incubation at -20°C for a minimum of 20 min. After centrifugation for 5 min. the supernatant was decanted and the pellet was washed with 70% ethanol. Pellets were then vacuum dried and resuspended in 200-500 ul of TE bufer (pH7.5).

Polymerase chain reaction amplifications were performed in a total volume of 25 ul composed of 20 pM primer, 50 ng of genomic DNA, 0.2 mM of each dNTP (Pharmacia) 2.0 mM $MgCl_2$ (Perkin-Elmer), and 1 unit of Taq polymerase. Thermolyne Temptronic thermocyclers were used for all amplification experiments. Temperatures were set at 94°C for 5 sec., 36°C for 30 sec., and 72°C for 60 sec. for a total of 35 cycles. PCR products were visualized on 1.6% agarose gels containing 0.24 ug of ethidium bromide per ml of agarose. Gels were run at 70 volts (constant voltage) for four hours. Photographs were taken with a Polaroid camera with 1 to 1.5 sec. exposures using a UV light box as an illuminator. Arbitrary ten base primers were purchased from either the University of British Columbia (UBC) or Operon Technologies (OP).

Polymorphisms were scored as '1' strongly present, or '0' absent after a minimum of three replications. Bands that were monomorphic across all isolates, or were weakly amplified, or were difficult to score were not tabulated. Data matrices were analyzed using the NTSYS program. Similarity was assessed using simple matching and SAHN clustering performed using the UPGMA method. Dendrograms were constructed to graphically illustrate relationships.

Results and Discussion

The virulence spectrum of fifteen isolates was determined by inoculation on a standard differential set of resistant barley lines. A virulence matrix was constructed solely on a line/line basis. A dendogram based on this matrix (Figure 1) showed two definite clusters which may be correlated to virulence on the *Rh* and *Rh2* resistance genes. These two genes probably form the basis of the majority of scald resistance in Canada (Penner et al., 1996) and may exert selection pressure on scald populatons.

Three hundred and twenty arbitrary sequence ten base primers were used to amplify genomic DNA from two scald isolates (WRS1391 and WRS1488). Of these, 157 amplified fragments from both genomes and 80 amplified polymorphic fragments. These 80 primers were applied to a further five isolates resulting in 477 amplified fragments, of which approximately 50% were polymorphic between at least two of the seven isolates. A subset of 27 primers, selected on the basis of clarity of bands amplified, were applied to a further eight isolates and 72 polymorphisms were found.

The dendrogram constructed using RAPD data showed no significant clusters (Figure 2). Although the isolates collected from Alberta grouped together as did the Ontario isolates with the exception of WRS 1380, the small number of isolates included along with relatively weak clustering precludes making any assumptions about a relationship between geographic origin and RAPD

fragments. However, the genomic sequence variation assessed by RAPD analysis does show that the phenotypic clusters seen in the dendrogram based on virulence are artificial. The RAPD data suggests that DNA sequence variation is spread throughout the entire R. *secalis* genome and is not confined to regions responsible for virulence/avirulence. The lack of clustering based on sequence similarity provides evidence for high rates of change in DNA sequence as being the reason for high variability in virulence. This conclusion should serve to confirm that incorporation of single resistance genes into barley cultivars will not be effective over time.

Figure 1 Dendrogram based on the similarity of virulence among *R. secalis* isolates.

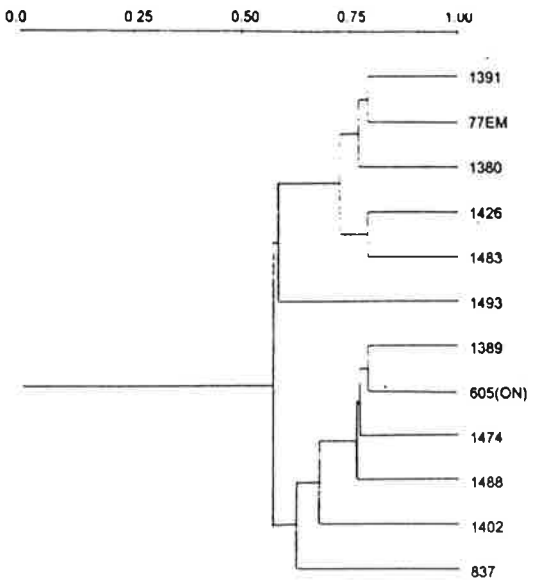
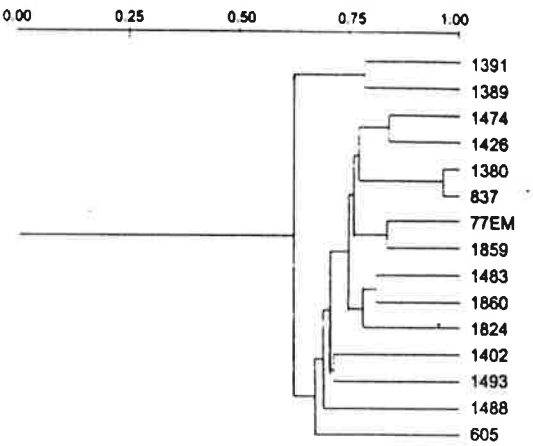


Figure 2 Dendrogram based on the similarity of RAPD polymorphic fragments among *R.secalis* isolates.



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A virulence survey of Swedish net blotch isolates (*Drechslera teres*) and identification of resistant barley lines. R. Jonsson, Dept of Plant Breeding, Swedish University of Agricultural Sciences, Svalöv, S-268 31,

Introduction. *Drechslera teres* (Sacc.) Shoem. (teleomorph *Pyrenophora teres* Drechs.), the causal agent of net blotch of barley, is an important pathogen on barley and occurs wherever barley is grown. Understanding of the variation and distribution of virulent isolates is important for successful resistance breeding. A large number of pathotypes have been found in virulence studies of *D. teres.* (*e.g.* Tekauz, 1990; Steffenson & Webster, 1992 and Jones & Clifford, 1995). Regional differences in virulence distribution have been demonstrated (Harrabi & Kamel, 1990) emphasising the importance of surveys of the growing region of interest.

Only limited information about the variation and distribution of virulent isolates of *D. teres* in Sweden is available (Sandnes & Leijerstam, 1988). The present paper describes a screening of barley varieties, germplasm lines and breeding material with 7 isolates of the net form of net blotch (*D. teres* f. sp. *teres*) in order to identify potential sources for resistance breeding and to select a useful set of differential barley lines. The resulting differential set was used to investigate the virulence of Swedish isolates of net blotch.

Materials and Methods. Single spore isolates were prepared from dried leaf samples and from infested seeds. The samples were collected from the major barley growing areas in Sweden. In addition, two Canadian net form isolates isolated from spring barley in western Canada were used (102 WRS and 1607 WRS). Ten-day old cultures from single spores were flooded with 6 ml of sterile water and the resulting suspension of spores and mycelia was mixed with 2 ml of glycerol and stored in 1 ml aliquots at -80 °C. The spore suspension was multiplied on V-8 agar as needed. Cultures were grown in near UV light with a photoperiod of 12 h. All inoculation experiments were performed in the glasshouse on first leaf seedlings. The plants were spray-inoculated twice with a one-day interval, using a water suspension of spores and mycelia (approx. 1000 infective units/ml). Gelatine (3g/l) was added to improve adhesion. The plants were grown in soil and covered with polyethylene from the first inoculation until scoring. The degree of necrosis and chlorosis was recorded on a scale 0-9 (Tekauz, 1985) but modified for the purpose of scoring necrosis and chlorosis separetely. For necrosis the value of six was used when clear netting started to develop.

In the initial screening, a collection of 109 barley varieties, breeding lines and germplasm collections was tested for resistance against six Swedish and one Canadian isolate of the net form of net blotch. Using the results from the initial screening a differential set of 18 barley lines was selected.

Results and Discussion. The 109 barley lines could be separated into four groups showing different resistance reactions to the seven isolates (differential reactions or resistant, susceptible or intermediate reactions to all isolates). Some of the most resistant lines and their resistance reactions are shown in Table 1. Several of the lines with European origin were resistant to the Canadian isolate but susceptible to the 6 Swedish ones.

The differential set was constructed using barley lines with different

resistance reaction but also being of different geographic origin. Twenty-seven isolates, including the two Canadian isolates, and one spot form isolate were separated in 14 pathotypes by the differential set. Three pathotypes comprised 59% of the isolates. The results indicate that host selection on the pathogen is present. All six isolates produced from cv. Golf belong to the same pathotype and four out of five isolates from cv. Karin have similar virulence pattern.

Twenty-six leaf or seed samples yielded *D. teres.* Twenty-five samples produced net form isolates and only one spot form (*D. teres* f. sp. *maculata*) isolate was isolated indicating the predominance of the net form in the sampled area.

Table 1. Degree of necrosis (0-9; after Tekauz, 1985) for the 11 most resistant barley lines in inoculation tests of 109 barley lines on first leaf seedlings with six Swedish (1-6) and one Canadian (7) single spore isolates of *D. teres* f. sp. *teres.*

Barley lines	CI no.	Degree of necrosis							
		Isolate:							
		1	2	3	4	5	6	7	Mean
Alexis (check)		7	7	8	9	7	8	7	7.4
Rabat 071	9776	2	2	2	3	4	1	1	1.9
SW 1114-93		2	1	2	2	3	3	3	2.1
Heartland		2	1	1	2	4	3	4	2.3
TR 473		3	1	4	4	3	0	3	2.3
	4976	4	1	2	3	5	2	2	2.5
Virden		5	1	2	2	3	3	3	2.6
Abyssinia	5822	3	2	3	3	5	3	3	3.0
CDC Guardian		3	2	3	4	5	3	3	3.0
Manchu	4795	6	3	3	3	2	2	5	3.1
Cebada Capa	6193	4	2	4	3	5	3	3	4.1
	4502	6	2	3	3	3	2	5	3.2

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Studies of Powdery Mildew (*Erysiphe graminis* f. sp. *hordei*) in Barley.

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Introduction. The powdery mildew resistance in this study came from *Hordeum vulgare* spp. *spontaneum* accessions which were originally selected by Hannu Ahokas (1985) in Finland for high protein content. Several accessions were found to have mildew resistance and were crossed first with either Golf or Triumph. The F_1's were then crossed to other cultivated barleys. In the F_3 and F_4 generations, non-segregating lines were selected for mildew resistance. The selected lines were tested with 19 isolates of powdery mildew. Isogenic lines for identified mildew resistance showed different patterns of resistance to the isolates than the selected lines. From this it was deduced that the resistance genes in the selected lines were different from those in the isogenic lines.

Material and Methods. 23 accessions were selected in the original lines from 1985. These lines were tested with a virulent race of powdery mildew. The resistant accessions were crossed with Golf or Triumph. The progenies and resistant selected plants were then backcrossed five times to modern barley lines. After the last crosses, the material was taken to the F3 and F4 generations. Lines with a "0" reading on a scale of 0-4 (resistant-susceptible) for all isolates were eliminated from this study because the "0's" could come from ml o resistance which was present in some of the parents used in the crosses. Other lines which were not segregating for resistance were tested with 19 different isolates of powdery mildew. Lines which did not show the same pattern as the isogenic lines with known resistance genes were crossed with a tester set of seven isogenic lines including one cultivar without resistance (Ml a12, Ml a23, Ml p, Ml g, ml o, Ml La and Ingrid). About 100 seedlings of each F_2 were tested with a virulent isolate (93-1) and a less virulent isolate (14-77) of powdery mildew. The F_2's were scored and a X^2 value was calculated for each combination for 3:1, 15:1, and 13:3 segregations.

Results and Discussion. The selected 23 lines did not segregate for powdery mildew resistance and they did not fit the patterns of resistance of the isogenic lines (Table 1). Due to lack of space the whole table is not shown in the abstract. The F_2's from crosses with the tester set segregated. This would prove that the resistance or part of the resistance is not the same as that of the tester set. If it were the same gene, there should be no segregation in the F_2. Most of the F_2's from crosses with the susceptible cultivar "Ingrid" segregated 3:1 which would confirm that most of the resistance is due to a single gene. Two of the selected lines 93-3303 and 93-4110 were susceptible to the mildew isolate 93-1. This was also seen in the F_2 from their crosses with Ml p and Ml a23 which are resistant to this isolate and gave a 3:1 segregation. Some linkage studies with molecular markers, such as used by Hinze et al. (1991) or Kintzios et al. (1995), could give us a better picture as to where the genes are located and if they are possibly the same as have been shown by other barley workers. Possibly the use of other allele studies with isolated genes from *spontaneum* (Islam, et al. 1992, Masterbroek et al. 1995) might show that these lines contain some of the same genes. By using the isolates which were used in the study of

Islam, et al. (1992) or Masterbroek, et al. (1995), the genes could possibly be determined by comparing the pattern of resistance. Lines derived from backcrosses to one and the same susceptible cultivar could have given better information about the resistance found in *spontanuem* than the selected lines which were derived from the multiple crosses with cultivars containing other genes for resistance.

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Table 1. The scores of selected lines, cultivars, and isogenic lines for resistance to isolates of Powdery Mildew (*Erysiphe graminis* f. sp. *hordei*). Scoring scale is 0-4. i=immune reaction. 0-2=resistant. 3-4=susceptible. n=necrosis. c=chlorosis.

Tested lines	Ml-genes	Isolate 93-1	Isolate 1352	Isolate JEH37	Isolate 18-75	Isolate 26-78	Isolate 58-74
Pallas	-	4	4	4	4	4	4
Filippa	a13	0	4	0	0	0	4
I-Ml a2	a2	12n	12n	12n	12n	4	12n
Lina	a7,Mu2	4	12n	24n	12n	24n	4
P01	a1	4	0	4	0	0	0
P02	a3	0	0	0	0	4	0
P03	a6,a14	0	4	4	0	0	0
P04A	a4,a7	4	4	4	4	4	4
P07	a4,a9	4	4	0	4	4	4
P10	a12	4	01n	4	0	0	0
P12	a22	4	4	4	0	0	4
P13	a23	12n	1n	1n	4	02n	01n
P19	p	1nc	12n	1n	12n	12n	1nc
P20	At	1nc	23n	12n	4	12n	1nc
P21	gCP	4	4	4	4	4	4
P22	o	0	0	0	0	0	0
P23	La	4	23n	4	4	4	4
Spiti	s	12n	1n	02n	1n	02n	12n
Sv 5751044	a3,Tu2	1n	01n	01n	01	4	01n
Triumph	a7,Ab	4	4	24n	1n	23n	4
93-3135		1n	0	0	0	0	0
93-3145		0	0	01n/i	0	0	0
93-3303		4	12n	12n	12n	4	23n
93-3395		01n	01n	01n	01n	01n/i	0
93-3448		01n	01n	01n	01n	01n	i
93-3499		01n	i	i/01	i	i	i
93-3630		0	01n	01n	01n	0	12n
93-3984		i	i	i	i	i	12n
93-4110		4	1n	1n	1n	01n	23n
93-4716		01n	0	1n	0	i	01n
93-4889		1n	1n	i	1n	01n	1n
93-5433		0	0	1n	0	01n	0
93-5614		01n	0	i	0	01n	0
93-5779		i	i	i	i	i	01n
93-5813		i	0	i	0	i	01n
93-5956		01n	01n	1n/i	01n	01n	01n
93-6019		0	0	0	0	0	i
93-6113		1nc	01n	0	01n	0	i

EFFECT OF NITROGEN LEVELS ON FOLIAR DISEASES OF BARLEY UNDER DRYLAND CONDITIONS. A.G. KARI, Field Crops Section, Agricultural Research Institute 1516 Nicosia, P.O.Box 2016, Cyprus.

INTRODUCTION. The development of foliar diseases of barley depends upon host resistance, environment and agronomic practices. Numerous evidences suggest that nitrogen fertilizer is one of the major agronomic practices for increasing disease severity of many foliar diseases of barley and wheat (Bainbridge, 1974; Couture & Isfan,1986; Jones et al.,1992).

The exhausting barley continuous cropping system employed in Cyprus under dryland conditions forces the farmers to apply excessive levels of nitrogen fertilizer. Therefore, the effect of increased nitrogen fertilizer on disease severity was studied with barley cultivars having different resistance, in conditions of natural infection of *Helminthosporium teres* and *Rhynchosporium secalis*.

MATERIALS AND METHODS. Five field experiments were carried out from 1992 to 1995, three in fallow fields and two in fields where a mixture of barley and vetch was grown in the previous season. The mixture was cut for hay in mid-March at the boot stage of barley. Four levels of nitrogen fertilization, N-O, N-1, N-2 and N-3 corresponding to 0, 50, 100 and 150 Kg ha^{-1}, were applied in two split rates, at sowing and at tillering,GS 25, (Zadoks et al., 1974). In addition, 30 Kg ha^{-1} of P_2O_5 was given during sowing. The cultivars Athenais, Mari/Athenais * 2-02CYB 180 2A-2A-2A-0A-Sel B, Maroc-9-75, 82-RSP-5REG-MSU82-CY-2A-1D-1D-1D-1D-OD and Arizona 5908/Aths//Lignee 640-ICB 81-0210-1AP-9AP-0AP were used. Seed rate was adjusted according to seed size and germinability, based on a seed rate of 105 Kg ha^{-1} of the local cv Athenais. Plots consisted of six 5 m long rows, 0.175 apart. Sowing was done during the second fortnight of November. Nitrogen levels and cultivars were randomized in a split-plot design with five replications, where nitrogen levels were the main-plots and cultivars the sub-plots.

Disease incidence (DI) and disease severity (DS) of net blotch and scald were recorded when the plants were at the early dough stage on the upper three leaves of ten main stems in each plot, at random. To standardise variances data were transformed into angular values.

RESULTS. DI of net blotch increased by increasing the applied nitrogen level from N-0 to N-1, N-0 to N-3 and N-2 to N-3 by 11.6%, 18.4% and 14.3%, respectively. By increasing the applied nitrogen level from N-0 to N-1, N-2 and N-3 DI of scald increased by 12.9%, 17.2% and 19.3% , respectively.

For both diseases, DS increased by increasing the applied nitrogen level. The DS of net blotch was maximum at N-3 and minimum at N-0, N-2 being intermediate. N-1 did not differ significantly from N-0. A similar pattern was also obtained in the case of scald.

Average differences among cultivars of DI and DS values of net blotch and scald were significant. The greatest value of DI of net blotch was recorded on Athenais and the lowest on Arizona. Kantara had the greatest DI value of scald and Athenais the lowest.

Kantara had both the greatest DS of scald and, unexpectedly, of net blotch. The lowest DS value of net blotch was recorded on Arizona, while that of scald

was on Athenais. Intermediate values of DS, for both diseases, were recorded on the other cultivars.

The obtained cultivar x nitrogen interaction, for both diseases, was significant. The DS value of net blotch on Kantara, Athenais and 82-RSP had a tendency to increase with increasing nitrogen level. A similar pattern was followed for Mari/Athenais but only for N-1 and N-3. DS of Maroc and Arizona decreased with N-2 while it increased with N-1 and N-3. The significant cultivar x nitrogen interaction for DS of scald, appears to derive largely from the positive response of Maroc and Athenais to increasing nitrogen level and the negative response of all other cultivars to N-3.

The effect of nitrogen on disease severity was mainly affected by the amount of rainfall, the environment and host resistance. DS, for both diseases, was always lower when barley followed hay than when following fallow.

DI, for both diseases, correlated positively with their corresponding DS, $r=0.91^{***}$ and $r= 0.63^{***}$ (n=120) for net blotch and scald, respectively.

Grain yield was not affected significantly by nitrogen level but differed among the sites. Environments with low rainfall had always lower DS of net blotch and scald but also had low grain yield.

DISCUSSION. Numerous reports published (Couture & Isfan, 1986; Donald & Catherine, 1987; Jones et al., 1992) indicate that the effect of increasing nitrogen fertilization on increasing disease severity of foliar diseases of barley and wheat cannot be doubted.

In the present report also, the nil nitrogen treatment caused the lowest DI and DS of scald and net blotch. The differences among cultivars were expected and presumably reflect on their field resistance. However, the obtained large nitrogen x cultivar interactions, for both diseases, indicate that for some cultivars the nitrogen had little effect on DI and DS. Therefore, it seems that the effect of nitrogen on disease severity may vary to a significant rate depending on genotype (Kari, 1991), the time of nitrogen application (Jordan & Stinchcombe,1986) and the nutritional status of the host (Bainbridge,1974; Jenkyn & Griffiths,1976). However, under rainfed conditions in dryland areas, apart from the genotype it seems that the effect of increasing nitrogen fertilizer on disease severity presumably depends mainly on the prevailing weather conditions (temperature and moisture) and on the availability of natural inoculum.

The non-significant differences among nitrogen levels on grain yield were unexpected. It seems that, under dryland conditions with less than 400 mm rainfall, the response of nitrogen on grain yield depends on the amount and distribution of rainfall during the grain filling period. Therefore, high nitrogen levels are not recommended because even under dryland conditions, such as in Cyprus, the plants are predisposed to foliar diseases by the favourable prevailing temperatures and moisture before and during the anthesis. In addition, high nitrogen levels do not result in higher yield if there is not enough rainfall during the filling period.

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REACTION TO POWDERY MILDEW OF BARLEY LINES CARRYING Ml ALLELES IN THE NORTHEN WEST OF RUSSIA.

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INTRODUCTION

Powdery mildew is the harmfull and wide distributed desease of barley in the northen west of Russia.

The search of the genetic source and exposure of their selecthional value are the basic object of our investigations.

A large number of genes occuring in 12 loci confer resistance to powdery mildew in barley. One of these, Mla consists of 23 alleles or closely linked genes. Of them, alleles Mla 16 through to Mla21 are derived from Hordeum spontaneum collected from Israel (Johoor, Fischbeck 1987). Genes Mla 22 and Mla 23 are closely linked to gene Mla 12 and differ functhionally from 19 designated Mla alleles (J.Helms Jorgensen 1991).Genes Ml(La), Mlp, Mlg are inherited independently from other powdery mildew resestance genes.

In present investigation we have been attempted to reveal effective genes for northen west Russia.

MATERIAL AND METHODS

23 izogenic lines variaty Pallaspossessing genes Mla, Mla2, Mla3, Mla5, Mla6, Mla7,Mla9, Mla10, Mla13, Mlc, Mln, Mlat, Mlg, Mlcp,Mlk, Ml(La), Ml14/145, Ml1402 were used.

Reaction types were grouped in three classes: resistant (reaction type 0-1), intermediate (type 2) and susceptible (type 3 and 4). The study of barley resistance to population of powdery mildew have been conducted in the field in 1991-1993, in Pushkin. Powdery mildew resistance had been tested on provocative fone. The infection resulting on the lines was expressed relatively to the infection occuring on the susceptible control variaty Pirkka.

RESULTS

Meteorolodical conditions were favourable for developmenof the pathogen. The results are represented in the table.

Only 3lines possessed resistance to powdery mildew: P-02 Mla3, P-08 Mla9 P-11 Mla13. Lines with the other genes were susceptile. The line P-11 had complex resistance to stem and leaf rust, powdery mildew and lodging.

lines		vegetative period	yield kg/m^2	resistance pow.mild.	dw.rust
Pallas	Mla_8	81	0.35	3	3
P-01	Mla	79	0.42	1	3
P-02	Mla_3	79	0.48	0	1
P-03	Mla_6 Mla_4	77	0.58	2	3
P-04	Mla_7 Mlk	77	0.52	3	3
P-04b	Mla_7	79	0.46	2	4
P-06	Mla_7 Ml(La)	78	0.47	3	3
P-07	Mla_9 Ml(La)	79	0.47	2	2
P-08a	Mla_9 Mlk	79	0.52	2	1
P-08b	Mla_9	76	0.55	0	2
P-09	Mla_{10}	74	0.5	2	2
P-10	Mla_2	77	0.54	2	3
P-11	Mla_{13}	76	0.4	0	0-1
P-12	Mlc	77	0.35	2	2
P-13	Ml1402	76	0.48	2	2
P-14	Ml41/145	76	0.4	0-1	2
P-15	Ml1192	76	0.49	2	2
P-17	Mlk	76	0.45	3	2
P-18	Mlnn	76	0.45	2	2
P-19	Mlp	76	0.41	1-2	2
P-20	Mlat	76	0.37	2	2
P-21	Mlg Mlsp	75	0.4	2	2
P-22	Mla_5	76	0.4	1	2
P-24	Ml(Lc)	78	0.4	2	0

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Utility of different stem rust resistance sources in barley. W.G. LEGGE[1], D.E. HARDER[2], B.G. ROSSNAGEL[3], M.C. THERRIEN[1], and B.L. HARVEY[3]. [1]Agriculture and Agri-Food Canada, Research Centre, P.O. Box 1000A, R.R.# 3, Brandon, MB R7A 5Y3, [2]Agriculture and Agri-Food Canada, Cereal Research Centre, 195 Dafoe Road, Winnipeg, MB R3T 2M9, and [3]Crop Development Centre, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK S7N 5A8, Canada.

INTRODUCTION. In 1988, race QCC of the stem rust pathogen (*Puccinia graminis* f.sp.*tritici*) was detected in Manitoba and found to be virulent to all barley cultivars (Martens et al. 1989). Race QCC has steadily increased in prevalence in the natural stem rust population and has now become the dominant race (Harder et al. 1994). In other studies, race QCC has been shown to be highly aggressive relative to other races. Thus, race QCC poses a serious threat to barley production should ideal conditions for a stem rust epidemic occur.

From the 1950's to 1988, most barley cultivars grown in Manitoba were resistant to stem rust, with most of the resistance due to a single gene, *Rpg1* (Steffenson 1992). Race QCC is specifically virulent to this gene. The QCC threat has spurred the search for new sources of resistance in barley and the utilization of improved resistance in barley breeding programs. Several genes of interest in addition to *Rpg1* are *Rpg3* (Jedel et al. 1989), *rpg4* (Jin et al. 1994) and *RpgU* (Fox and Harder 1995). Resistance has mainly been evaluated by visual inspection of lines in artificially inoculated nurseries, but there is little information on the level of protection actually conferred in barley by the apparently more resistant lines. This study was undertaken to evaluate the agronomic performance of a selection of barley lines or cultivars with several different resistance sources under severe stem rust epidemic conditions.

MATERIALS AND METHODS. Nine barley lines or cultivars and one susceptible wheat cultivar (AC Reed) were evaluated. Their identity and the stem rust resistance genes that they carry are shown in Table 1. A 4-replicate split plot design was used with the whole plots consisting of eight 5-m rows (30 cm apart) of each line. A spreader row of susceptible wheat and barley lines was planted between adjacent whole plots and also separated each whole plot into two 4-row subplots. One subplot was treated with the fungicide Tilt as required to control stem rust, while the other subplot was not treated. Each subplot was harvested and analyzed separately.

The spreader rows were planted 7-10 days before the plots were planted, and inoculated with race QCC at the early jointing stage.

The tests were planted at Brandon and Winnipeg in 1994 and 1995. In this paper, only the results from Brandon are reported. Data were collected for yield (kg/ha), 1000-kernel weight (g), test weight (kg/hl), kernel plumpness (%), and stem rust severity. The stem rust severity presented is the terminal severity, or last rating before the plants matured. The percent reduction in a trait due to stem rust was calculated as follows: 100 - (untreated subplot/treated subplot x 100).

Table 1. Percent reduction in yield, kernel weight, test weight and plumpness, and final stem rust severity in non-protected plots of nine barley lines and one wheat cultivar combined over 1994 and 1995 in nurseries artificially inoculated with stem rust race QCC at Brandon

			Reduction (%) in:			
Line	Genotype	Stem rust Severity[1]	Yield	Kernel Weight	Test Weight	Plumpness
Q21861	Rpg1+4+?	13MRMS	6.4*a*	4.9*a*	2.9*a*	3.2*a*
Q/SM-041	Rpg1+4	20MRMS	8.4*a*	9.0*ab*	3.9*a*	14.5*b*
BM8923-46	Rpg1+3	30MRMS	9.9*ab*	8.3*ab*	3.6*a*	18.1*b*
Diamond	Rpg1+U	53MRMS	18.7*bc*	12.6*b*	8.3*bc*	41.3*c*
SB90585	Rpg1+?	63MRMS	21.9*cd*	13.6*b*	6.3*ab*	46.8*c*
Robust	Rpg1	88MS-S	22.7*cde*	22.7*c*	10.9*cd*	43.9*c*
Bonanza	Rpg1	85MS-S	29.8*de*	23.8*c*	11.3*cd*	60.1*d*
Harrington	?	65MS-S	31.8*e*	26.9*c*	13.7*d*	42.0*c*
Klages	-	93S	52.7*f*	43.5*d*	25.3*e*	91.7*f*
AC Reed[2]	-	100S	57.2*f*	44.9*d*	14.2*d*	72.6*e*

[1] Severity ratings are interpreted as follows: numerals = mean % level of infection; MR = moderately resistant infection type; MS = moderately susceptible; S = susceptible.
[2] Wheat cultivar.
a-f Means followed by the same letter are not significantly different at P = 0.05 according to Duncan's Multiple Range Test.

RESULTS AND DISCUSSION. There were no significant levels of diseases other than stem rust during either year of the test. The applications of Tilt effectively controlled stem rust in the treated plots, although somewhat higher levels of stem rust developed in the treated plots of Klages and AC Reed, the most suceptible lines in the test. Disease development was good both years as indicated by the high severities in the more susceptible lines (Table 1). Stem rust severities were somewhat higher in 1995 than 1994 due to warmer, drier conditions.

In general, the rankings of the lines or cultivars were consistent over all four traits measured (Table 1). The rankings were also similar both years, but there were some indications of year by genotype effects (data not shown). Although the stem rust severity ratings were generally associated with agronomic performance, they may not fully indicate the potential performance

of a line when stressed by stem rust under varying conditions.

Q21861 consistently had among the lowest reductions in yield, kernel weight, test weight and plumpness over the two years of the test, followed by either Q/SM-041 or BM8923-46 (Table 1). The combination of the genes, *Rpg1* and *rpg4*, as occurs in Q21861 or Q/SM-041, appears to offer excellent protection against race QQC. A probable third recessive gene in Q21861 may contribute additional resistance. The combination of *Rpg1* and *Rpg3*, as in BM8923-46, also appears to be very effective in reducing losses. The *Rpg1* and *RpgU* combination, as in Diamond, is also effective, but less so than for either *rpg4* or *Rpg3*. SB90585, a line carrying *Rpg1* and other unknown genetic factors, has consistently had lower stem rust severities in inoculated nurseries over the years than susceptible cultivars like Bonanza. The losses caused by race QCC in SB90585 are similar to those in Diamond. Gene *Rpg1* by itself is ineffective, as has been shown in other studies (Liu and Harder 1996), and by the relatively higher losses in Robust and Bonanza. However, *Rpg1* should be retained in new cultivars because it still provides resistance to most stem rust races and also may enhance the effectiveness of other genes in combinations. Over a number of years of observation, Harrington has consistently shown somewhat lower stem rust ratings than very susceptible cultivars such as Klages (D.E. Harder, unpublished). The results of the present study bear this out, with Harrington showing significantly lower losses than Klages. Harrington appears to carry a factor contributing a low level of resistance to race QCC.

Historically, suceptible barley has been less affected by stem rust than susceptible wheat. In the present study, losses in Klages approached those in the susceptible wheat cultivar, AC Reed, indicating that the potential for loss to race QCC in susceptible barley may be nearly as high as in susceptible wheat. With appropriate combinations of resistance genes, however, a satisfactory level of resistance is attainable.

In conclusion, the resistance conferred by *rpg4* and *Rpg3* in combination with *Rpg1* appears adequate to protect barley against losses in yield and kernel characteristics due to race QCC. The most resistant lines in this test, however, cannot be advanced directly as potential cultivars. The challenge will be to attain the appropriate combinations of resistance genes in new cutivars that meet all the other agronomic and quality attributes necessary for success.

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Characterisation of the resistance of old winter barley (*Hordeum vulgare* L.) French cultivars to barley mosaic viruses. J. Le Gouis[1] and D. Hariri[2], [1]INRA, Amélioration des plantes, Domaine de Brunehaut, F-80200 Estrées-Mons, France, [2]INRA, Pathologie végétale, Route de Saint-Cyr, F-78026 Versailles, France.

Introduction. Since its first description in the mid-seventies barley yellow mosaic disease became one of the most important diseases of winter barley in Western Europe. Barley mild mosaic virus (BaMMV) and two pathotypes of barley yellow mosaic virus (BaYMV and BaYMV-2) first reported from Japan (Ikata and Kawai 1946) have up to now been found to be responsible for the disease (Huth 1989, Huth and Adams 1990). Due to their transmission by the soil-born fungus *Polymyxa graminis* Led (Toyama and Kusaba 1970) chemical control is either inefficient or unacceptable for ecological or economical reasons. Resistant cultivars have been registered but they likely carry the same gene (ym4) which does not confer any resistance against BaYMV-2. Surveys of germplasm have shown that new sources of resistance were available (Ordon and Friedt 1993) but due to their predominantly exotic origin and poor agronomic performance (Ordon and Friedt 199') these germplams are very difficult to use in a plant breeding programme. A preliminary study (Koenig et al 1991) showed that some old French cultivars were resistant against barley mosaic viruses. The aim of this work was to characterise their resistance and assess the possibility of using them in a plant breeding programme as they are better suited to France agricultural conditions than most exotic resistant germplasms.

Materials and methods. About 70 old French varieties which derived directly from landraces or have such material in their ancestry are kept at INRA of Clermont-Ferrand. Three of them, Marne, Comte de Serre and Superchampenois, all 6-row winter barley cultivars, which were reported to be resistant to BaYMV (Koenig et al 1991) were so further studied. They were crossed with the susceptible 6-row cultivar Plaisant and the resistant 6-row cultivar Express which is supposed to carry the ym4 gene. For each cross, 120 F2 plants were inoculated mechanically with BaMMV at the three leaf stage. The rate of infection was estimated by using 40 plants of the susceptible cultivar Plaisant for each cross. ELISA was carried out three weeks after inoculation to assess resistance or susceptibility. Agreement between observed and theoretical segregations was tested with a chi-square test after correction for the inoculation rate (Ordon and Friedt 1993).

Results and discussion. Table 1 presents the results of the mechanical inoculation with BaMMV of F2 progenies coming from the crosses with old French varieties. Inoculation rate was generally high. For the three crosses involving the susceptible cultivar Plaisant, no good fit of observed segregations was found with the expected 1 resistant : 3 susceptible ratio corresponding to the presence of a single recessive gene. A good fit was then observed with the 7 resistant : 9 susceptible ratio indicating the presence of two recessive independent genes in the three old French varieties.

For the three crosses with the resistant cultivar Express, all the F2 plants were resistant against BaMMV. This result indicates that one of the two genes carried by the old French varieties is identical or closely linked to the ym4 gene.

In different trials, Marne and Superchampenois have been showed to be resistant against all the viruses (BaMMV, BaYMV and BaYMV-2) present in Europe. Results were not so clear for Comte de Serre which was resistant or susceptible depending on the origin of the virus.

Cross	F2 segregation		Infection rate (%)	Probability of chi-square values[1]	
	resistant	susceptible		1r:3s	7r:9s
Comte de Serre X Plaisant	39	37	93	<0.01	>0.05
Marne X Plaisant	55	55	97	<0.01	>0.05
Plaisant X Superchampenois	59	61	100	<0.01	>0.05
Comte de Serre X Express	108	0	93		
Marne X Express	111	0	100		
Superchampenois X Express	111	0	100		

[1] chi-square test corresponding to 1 recessive gene (1 resistant : 3 susceptible) and 2 recessive independant genes (7 resistant : 9 susceptible)

Table 1: results of mechanical inoculation with BaMMV of F2 plants of crosses between the resistant cultivar Express and the susceptible cultivar Plaisant with 3 rsistant Old French varieties.

This experiment showed that a helpful genetic variability exist in old French varieties and that these cultivars may be used in plant breeding programmes as an alternative to ym4 carrying cultivars. Further studies are now needed to know whether the second gene carried by the three cultivars are identical or different and whether this gene is a new one or has already been described.

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The challenge of controlling oat crown rust epidemics in South Brazil through variety mixtures. J. A. MARTINELLI. Departamento de Fitossanidade, Faculdade de Agronomia - UFRGS. Caixa Postal 776, 90012-970 Porto Alegre - RS, Brazil.

Introduction. Many host/pathogen interactions, particularly oat and crown rust, have been controlled at desirable levels by the introduction of new varieties and chemical treatment. However, these methods alone have frequently led to losses in effectiveness and the necessity of replacement of varieties and fungicides, thus increasing costs. Environmental conditions in South Brazil are very favorable for epidemic outbreaks of crown rust on oat. The pathogen frequently overcomes the resistance of new varieties soon after they are released. Consequently, the stability of the crop without the need for chemical sprays may be compromised. The addition of genetic variability in the system by cropping heterogeneous populations of oat, integrated with fungicide treatments on seeds, may provide practical means to reduce the rate of pathogen evolution and increase stability. The pathogen population is unable to become highly adapted to many different methods of control simultaneously. This may be exploited in oat populations in which the neighboring plants are either a different variety or treated with a different fungicide.

Materials and Methods. Several field experiments were done in the last three years by mixing different oat genotypes integrated or not with fungicides on seeds. The number of components per mixture was restricted to three or four proportionally divided to give a total density of 300 seeds per m^2. Plots had 10 rows 17 cm apart and 5 m long. Wheat was sown between oat plots to avoid cross contamination of rust spores. Sowing started in June and harvesting at the beginning of November. The amount of disease on field plots was measured by its severity once or twice a week on a scale from 0 to 100%. The final analysis of oat mixtures considered the area under the disease progress curve (AUDPC), the rate of disease growth and yield.

Results and discussion. In 1993 the overall effect of treating seeds of the three mixtures, as well as their single components, reduced by 38% the amount of disease in relation to the non treated ones (Table 1). However, untreated mixtures were more efficient in reducing the amount of disease in relation to the overall mean of their individual components, than were the treated ones, with less disease. This apparent paradox may be explained by the actions of the mixture, particularly the barrier to spore spread and induced resistance. These increase effectiveness in proportion to disease increase, provided the different genes in the mixture exert a selective pressure on the pathogen population (Wolfe, 1985). This may explain the higher efficiency of mixture 1, with 53 and 14% decrease, in relation to mixture 3, with 24 and 0.5%, respectively for untreated and treated (Table 1). The data presented in Table 2 show the performance of mixtures of three and four components during the 1995 growth season. Mixtures of three components were less consistent in reducing the AUDPC and increasing yield than mixtures of four components. For example, by mixing the three more resistant oat varieties (treatment number 3), the amount of disease was 9% higher than the expected mean of its single components. This, however, did not compromise yield, with 10% more than expected, whereas another mixture (treatment number 1) reduced the amount of disease but gave a

lower yield than expected. On the other hand, all four component mixtures tested in this trial were able to decrease the AUDPC and increase yield, even when added to a highly susceptible variety (UFRGS 7). The data presented here favor the more complex mixtures, as suggested in the literature (Browning, 1974; Parlevliet, 1977; Wolfe, 1985). The number of components, however, should reflect the number of resistance genes available, the proportion of susceptible host population to each pathogen race, the importance of the crop and the disease to be controlled. Additional benefits have been achieved by treating seeds of only one component, as illustrated in Figure 1. Treating one of the mixture components UPF-6 or UFRGS-10 delayed the disease to reach the economic threshold of 5% severity (Martinelli *et al.*, 1994b) by about 15 days in relation to the other component UPF-5. In our conditions, more complex oat mixtures integrated with fungicides on seeds reduced the rate of crown rust epidemics and stabilized yield.

Table 1. Area under disease progress curve (AUDPC) for untreated and treated mixtures of oat varieties in relation to the mean of their single components. Source: Martinelli *et al.*, 1994a.

	Untreated		Treated[1]	
Treatments	AUDPC	P[2]	AUDPC	P[2]
1. UPF3+UPF5+UPF6	340	53	202	24
2. UPF13+UFRGS7+UFRGS10	803	20	584	13
3. UFRGS8+UFRGS9+UFRGS11	725	14	680	0.5
4. Mean single comp. mixture 1	725		265	
5. Mean single comp. mixture 2	1009		675	
6. Mean single comp. mixture 3	1143		683	
Overall mean	834		515	38

[1] = treatment on seeds was done with Triadimenol (40 g of a.i./100 kg seeds).
[2] = Percentage of disease reduction in relation to the mean of single components.

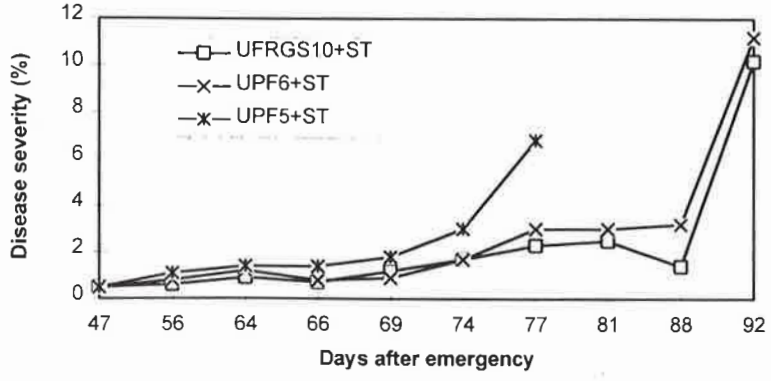

Figure 1. Evolution of crown rust on a mixture of oat cultivars (UPF5 + UPF6 + UFRGS10) by treating the seeds (ST) of one component at a time. The fungicide used was Triadimenol (40 g. of a.i./100 kg seeds).

Table 2. Performance of oat mixtures against crown rust epidemic in South Brazil. Federal University of Rio Grande do Sul State, Brazil. 1995*.

Mixtures	AUDPC	P	Yield (kg/ha)	P
1 UFRGS14+UFRGS15+UFRGS17	277 bcd	(-12)	2462 abcd	(-8)
2 UFRGS14+UFRGS17+Line 905	264 bcd	(-10)	2540 abc	(0)
3 UFRGS15+UFRGS17+Line 905	191 de	(+9)	2679 ab	(+10)
4 UFRGS14+UFRGS15+UFRGS7	949 a	(+7)	2106 bcd	(0)
5 UFRGS14+Line 905+UFRGS7	1039 a	(+21)	1846 d	(-5)
6 UFRGS14+UFRGS15+UFRGS17+UFRGS7	452 bcd	(-38)	2349 abcd	(+2)
7 UFRGS14+Line 905+UFRGS17+UFRGS7	563 b	(-21)	2356 abcd	(+8)
8 UFRGS14+UFRGS15+UFRGS17+Line 905	230 cde	(-11)	2716 ab	(+8)
CV (%)	22		11	

* = This Table do not include the data from all single components.
P = percentage of disease decrease (-) or increase (+) in relation to the mean of the single components.
Disease reaction: UFRGS14=MS; UFRGS15=MR; UFRGS17=MR; Line 905=R ; UFRGS7=S.

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Characterization of Partial Resistance to Oat Crown Rust through the Area Under the Disease Progress Curve. S.C.K. MILACH, G.C.H. THOMÉ, L.C. FEDERIZZI, C.R.A. BOTHONA, C.B. CABRAL, and J.A: MARTINELLI. Departamento Plantas de Lavoura, Faculdade de Agronomia - UFRGS, Av. Bento Gonçalves, 7712, Cx.P. 776, Porto Alegre, RS, 90012-970, Brazil

Introduction. In the last few years, the area cultivated with oat grew substantially in Southern Brazil as a consequence of the superior and adapted genotypes selected by the local breeding programs. The rapid expansion of the oat cultivated area had as consequence an increase in the amount and diversity of the crown rust disease, which is responsible for significant losses in oat grain yield and quality. Because of the rapid changes in the pathogen population, major genes resistance varieties are not lasting more than 3 to 5 years before they become susceptible to new pathogen races. For this reason, breeders can no longer rely only on this type of resistance. Partial rust resistance is being sought to increase the resistance durability and the life span of the oat varieties. In oat, partial resistance has been identified (Luke et al., 1972 and 1975) and suggested as an alternative to increase resistance durability (Rooney et al., 1994). The identification and characterization of partial resistance in oat Brazilian genotypes has not been done. The objective of this study was to characterize crown rust partial resistance in genotypes adapted to the Southern Brazil environments. The first parameter estimated to evaluate partial resistance was the area under the disease progress curve, since it has been used in the literature in similar studies with other cereals (Skovmand et al., 1978). We are now in the process of evaluating other partial resistance traits in the same genotypes to select those which will be important sources of partial resistance to our breeding program.

Materials and Methods. The experiments were conducted at the Federal University of Rio Grande do Sul (UFRGS) experiment station in Eldorado do Sul, RS. Twenty-seven genotypes (the cultivars UFRGS 7 and UPF 10, and 25 breeding lines from the UFRGS breeding program) were sown in two dates (6/2 and 6/21/1995). The experiment was a randomized block design and the plots had four rows 0.17 m apart and 3 m long. Two replicates were sown in each date. Fifteen plants were labeled in each plot and percentage of rust infection evaluated in each plant during six successive weeks. Traces of the disease were identified on the most susceptible line (UFGRS 7) in 8/4/1995 and the evaluations started on 9/1/95. The area under the disease progress curve was estimated for all genotypes for both sown dates using the SAS statistical package.

Results and Discussion. The results for the nine most representative genotypes of all types are shown in Tables 1 and 2. Variability for the area under the disease progress curve was observed among the genotypes and in both sown dates. The UFRGS 7 cultivar had the fastest disease progress in both sown dates and was the most susceptible genotype. UFRGS 93576 was the second most affected genotype and was in the intermediate group with UFRGS 910906 and UFRGS 922003. The other genotypes were the least affected by the disease and did not differ among them for the total area under the disease progress curve. UFRGS 910906 and UFRGS 91906-8 were selected from the same population and had different levels of disease infection, indicating they may have different minor genes for partial rust resistance. The results from both sown dates were similar, the only difference being the amount of total infection which was higher for the first sown date, since the plants stayed in the field two weeks longer. The results of these study indicate that there is variability for the area under the disease progress curve. This information together with other partial resistance traits will be useful in selecting genotypes with more durable rust resistance.

Table 1. Partials and total areas under the oat crown rust progress curve over nine oat genotypes sown in the first date. Evaluations were done at one week intervals.

GENOTYPES	A1†	A2	A3	A4	A5	AT
UFRGS 7	114.1 **A***	146.4 **A**	205.6 **A**	380.9 **A**	1205.1 **A**	2052.1 **A**
UFRGS 93576	4.2 **C**	18.8 **CD**	41.2 **B**	116.1 **C**	640.5 **B**	828.5 **B**
UFRGS 910906	0.7 **C**	2.4 **F**	8.7 **DE**	39.9 **F**	313.0 **C**	365.7 **C**
UFRGS 922003	0.3 **C**	0.7 **F**	0.1 **E**	4.5 **I**	136.2 **E**	142.6 **D**
UFRGS 921186	0.0 **C**	0.4 **F**	0.1 **E**	0.7 **I**	1.4 **F**	3.1 **E**
UFRGS 93598	0.0 **C**	0.0 **F**	0.0 **E**	0.2 **I**	1.1 **F**	1.2 **E**
UFRGS 93641	0.1 **C**	0.1 **F**	0.1 **E**	0.1 **I**	1.4 **F**	4.2 **E**
UFRGS 93611	0.0 **C**	0.0 **F**	0.0 **E**	0.4 **I**	1.4 **F**	1.7 **E**
UFRGS 910906-8	0.0 **C**	0.0 **F**	0.0 **E**	0.0 **I**	0.0 **F**	0.0 **E**

* Means followed by the same letter do not differ significantly at 5% by Duncan´s test.
† A1 to A5 are the partial areas and AT is the total area under the disease progress curve.

Table 2. Partials and total areas under the oat crown rust progress curve over nine oat genotypes sown in the second date. Evaluations were done at one week intervals.

GENOTYPES	A1†	A2	A3	A4	A5	AT
UFRGS 7	69.6 **A***	135.7 **A**	200.8 **A**	386.5 **A**	1147.3 **A**	1922.0 **A**
UFRGS 93576	0.7 **B**	21.0 **B**	67.5 **B**	180.1 **B**	684.4 **B**	950.4 **B**
UFRGS 910906	0.5 **B**	0.7 **E**	0.7 **F**	0.7 **H**	57.7 **E**	60.3 **D**
UFRGS 922003	0.5 **B**	0.7 **E**	0.1 **F**	5.8 **H**	130.1 **C**	143.2 **C**
UFRGS 921186	0.2 **B**	0.5 **E**	0.1 **F**	0.7 **H**	1.4 **F**	3.5 **E**
UFRGS 93598	0.0 **B**	0.0 **E**	0.3 **F**	0.6 **H**	1.3 **F**	2.3 **E**
UFRGS 93641	0.7 **B**	0.7 **E**	0.7 **F**	0.7 **H**	1.4 **F**	4.2 **E**
UFRGS 93611	0.0 **B**	0.0 **E**	0.0 **F**	0.0 **H**	0.0 **F**	0.0 **E**
UFRGS 910906-8	0.0 **B**	0.0 **E**	0.0 **F**	0.0 **H**	0.0 **F**	0.0 **E**

* Means followed by the same letter do not differ significantly at 5% by Duncan´s test.
† A1 to A5 are the partial areas and AT is the total area under the disease progress curve.

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A method for the quantitative proof of mycelium of Puccinia hordei in primary leaves of genotypes of spring barley for the evaluation of resistance

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Introduction

Leaf rust of barley is a very important disease in Germany. This disease occurs every year, the severity of epidemics depends from the infection pressure and the environmental conditions. Today in Europe only the gene Rph 7 is effective to leaf rust and therefore the main interest of the breeders is the quantitative resistance. The assessment of this type of resistance is very difficult because on the one side at least three measurements are necessary to account the area under the disease progress curve (AUDPC) and on the other side the expressivity of the quantitative resistance is influenced very strong by the environmental conditions. Therefore the aim of the work is to find a method to support the breeding work for quantitative resistance.

Material and Methods

In field trials and by means of a leaf segment test in a climatic chamber 220 dh-lines of the cross HOR 1063 x 'Krona' were tested for the level of quantitative resistance. After the infection with the high virulent isolate I 80 (virulent/avirulent - Rph 1,2,3,4,5,6,8,9,'Trumpf', /HOR 1132, Rph 7) the AUDPC was accounted. The determination of the enzymatic activity was carried out with leaf extracts with the intercellular washing fluid (IWF) for the enzymes Cellulase, Xylanase and Protease.

Results and Discussion

By means of an enzyme assay it is possible to measure the enzymatic activities in extracts and IWF from fungus infected leaves of many plants. For a range of host-pathogen combinations the measured activities of different enzymes (e.g. Cellulase, Xylanase) seems to be good correlated with the quantity of the parasitic mycelium in the infected leaves. Investigations with leaf extracts of leaf rust infected primary leaves of barley shows no clear reactions in the enzymatic activities. The IWF was more suitable for such investigations. In the IWF from Puccinia hordei-infected primary leaves of spring barley plants could be found higher protease activities than in the IWF from healthy ones. By the comparison of the measured protease activities it was possible to classify spring barley dh-lines into different classes of Puccinia hordei-susceptibility (Fig. 1). For the most of the as resistant or as very susceptible recognized lines the outcomes from the enzyme assay are good correlated with the results of visual estimations of the intensity of infection by Puccinia hordei in the field trials.

The actual investigations do not allow to say whether the determined proteases are in origin from the plant or from the fungus. The same change in the protease activity was observed under abiotic stress conditions (dry and heat stress). In our trials in a strong regulated climatic chamber the stress was caused only by Puccinia hordei. Therefore this method is useful independent of the origin of protease activity.
Genotypes with the typical hypersensibility reaction of the gene Rph 7 to Puccinia hordei could not be classified with this method. The rate of protease activity was the same as observed for susceptible lines. First tests with the high pressure liquid chromatography shows possibilities for the differentiation.

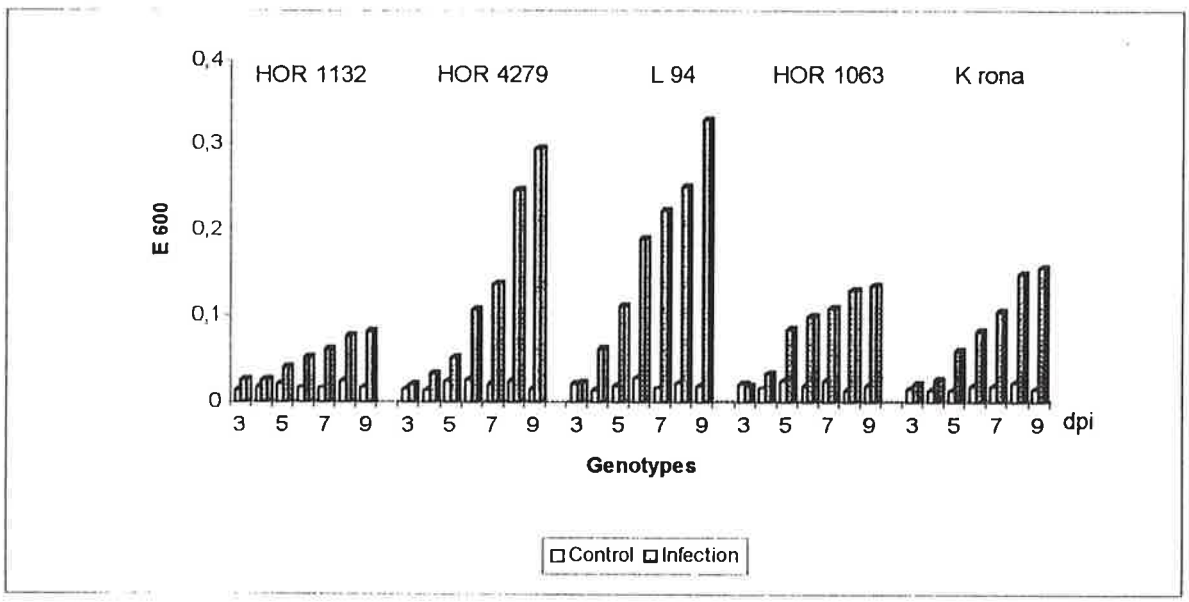

Fig. 1: Differences in the protease activity (extinctions) in the primary leaves of five spring barley genotypes in dependence of the level of susceptibility to the leaf rust after inoculation with isolate I 80

The studies of effectiveness barley resistance genes to loose smut (Ustilago nuda) and use it in the breeding program.

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Introduction. Loose smut (Ustilago nuda Kell.et Swing.) is one of the widespread barley disease.It often occurs all area of barley cultivating.Now this problem is solved with success by chemical treament of seeds.However the more optimal control to loose smut -breeding of the resistance varieties.It's the most important for Russia were 15 mln.ga of crop area.

The biology and genetic of resistance to loose smut (U.nuda) had been studied good enough (V.I.Krivchenko, 1984). As far back as the 1930-es Dg. Levingston determined dominant gene which control resistance of the cultivars Trebi and Missuri. D.B.Robertson et al. named these genes by symbol Un and Un2. New genes had been discovered (including var. Jet) as the sourse for breeding varieties with resistance to loose smut in Canada, Russia and the other countries. Now are known about 20 different genes of resistance to loose smut. We studied the effectiveness of 15 genes Run including Run 15 discowered in Ethiopia"s sample Hiproly.

Materials and methods. The varieties and samples with indentificationed genes Run had been used for studing of effectiveness barley resistance genes to loose smut. Ustilago nuda spores were picked from cultivated varieties and breeding fild of the central Russia. Ears were infected by vacuum method and instrument. A sussceptible barley cultivar Zazerskyi 85 was used as a check of effectiveness of infection. The same time barley cultivars of Russia and the other countries, doubled-haploid lines and collection samples had been estimated to resistance to Ustilago nuda.In artificial inoculations had been checked to 2000 samples every year in 1990-1995.

Results and discussion. Had been establisned that Run 3+Run6, Run 8, Run 15 genes are provided effective protection from loose smut infection. Run 3, Run 6, Run 12 were effective too. The indicated genes meintained effectiveness in the different varieties and samples where it had been introgressed. The influence of the genetic and environmental factors to disease development had been determined. Genes Run

1,2,4,7,9,10,11,13,14 were not effective against loose smut. Barley samples with these genes were affectioned by 40-90%.

It should be remarked that genes Run3+6, Run8, Run15 keeped there resistance to loose smut on the different regions of Russia guite long time. The investigations showed that loose smoot rase composition changet insignificant by last 15-20 yars in the some regions of Russia in spite of 3 or 5 of changing of spring barley varieties. The races 1,3,6,10,12,13 and et al. are predominant in the pathogene population as before in the central Russia. The relative stability of the rase composition of U.nuda depend on spring barley susceptible to varieties to loose smut were cultivated on 1-3 mln ga area. New varieties with resistance to loose smut can chandge this process.12 of 98 spring barley varieties, cultivated in Russia in 1995, had been defendet by genes Run3,6,8,15. The number of resistant to loose smut varieties of spring barley in Russia begin to grouth since breeders gave more attention to this problem.

It is known several methods the breeding of resistant varieties to loose smut. Our experience and research of the others show that the result of the breeding to resistance of barley to loose smut depend on productiving level of using donors of genes Run and the methods of it introgression. One short way to achieve this aim - breeding doubled-haploid lines from F1 hybrid by crossing the best barley cultivars with high productive genes Run donors.

A single crossing betwin doubled-haploid lines with introgressed effective genes Run shows a good results. The barley cultivars resistant to loose smut - Bios-1, Elf, Suzdalec, Ramos, Rachet bred by these methods.

Genes Run8 and Run15 were put into spring barley varieties Moskowskiy 2, Moskowskiy 3, Risk bred before by backcross method. During the infection by loose smut the cultivars keeping resistance many yars. In spite of simple genetic control of the barley resistance to loose smut as domininante and monofactor genetics, this problem not enough alaborated. Not all known Run genes are localised. It is make difficult genes identification and testing and also reveal new one.

Well-known that world genepool contain many spring barley samples with resistance to loose smut and other smut species. However genetic analysis is limited by test of resistance to pathogene population and some races. Take limitation of effective genes Run into consideration necessary to continue

to search nev one and its localization at the same time. More wide knowledge about genetic of barley resistance to different species of Ustilago make it possible to solve problem breeding of new highly productive and resistant varieties.

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Barley Yellow Dwarf Virus and Oat Breeding in Australia

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Introduction. BYDV is a major limiting factor in the successful cultivation of oats in Australia. Field selection for tolerance / resistance has been limited by the unpredictability of BYDV infection from season to season. An attempt has been made to develop a reliable, repeatable field and greenhouse method of screening breeder's line for BYDV.

Materials. A collection of 192 lines from various Australian breeders was assembled as representing a wide range of tolerances for BYDV. The collection was infected in the seedling stage with PAV over two seasons in the field, and a subset of these a number of times in the greenhouse. The material was scored, on a 0-9 (poor-excellent) scale, for height, colour and plant vigour at maturity in the field, and for height and colour four weeks after inoculation in the greenhouse. The data was analysed for correlation between sites and years with a view to establishing a reliable method for routine scoring of breeder's lines.

Results. Results from the field and greenhouse were analysed using a 'Spearman's Rank Correlation'. 1994 and 1995 field results were reasonably correlated. There was similarity in the rank order of the oat lines both in the field and in the greenhouse, and there was a relationship between the total score of a line in the field and in the greenhouse.

Discussion and Conclusions. The significant correlations between field and greenhouse results indicates that scores from greenhouse trials will successfully reflect field results. Preliminary greenhouse testing will be used to select the more tolerant / resistant lines or eliminate the susceptibles, thus enabling more efficient field trials. The PBI, Cobbitty has facilities to field test up to 10,000 lines for BYDV each year. Further testing to distinguish between tolerant and resistant lines will be done with ELISA.

Virulence of net blotch in Australia. G.J. PLATZ[1], R.G. REES[1] and V.J. GALEA[2], [1]Queensland Wheat Research Institute, Department of Primary Industries, PO Box 2282, Toowoomba, Queensland 4350, Australia. [2]University of Queensland Gatton College, Lawes, Queensland 4343, Australia.

Introduction. Net blotch *(Drechslera teres* f. sp. *teres*) is an important disease of barley in Australia and is destined to become increasingly important as conservation tillage is more widely adopted. In the winter rainfall areas of Western and southern Australia it is regularly present while in the northern production areas, where winter rainfall is erratic, incidence and severity of the disease correspond to the frequency of rainfall during crop growth.

Physiologic specialisation in the net blotch population in Western Australia was demonstrated by Khan and Boyd (1969) and Khan (1982). Variability in the net blotch fungus has also been detected in Queensland using a set of local varieties (G.J.Platz and R.G.Rees, unpublished); however the findings of both groups lack international comparability because of the differential lines employed. A study is being conducted using an extended set of differentials to determine the range of virulence in the net blotch population in Australia.

Methods. Samples of net blotch from around the continent have been obtained and single conidial cultures developed on V8 agar. Spores from these cultures were harvested and inoculated onto barley seedlings. The resultant diseased leaves were then dried and stored at -80C. Leaf pieces were later surface sterilised and plated onto peanut oatmeal agar. After 9 days conidia were washed from the plates, made up to a standard suspension and sprayed onto seedlings of the differential set. Plants were incubated and infection types determined after 9 days under controlled conditions, using the scale of Tekauz (1985).

The differential set was composed of lines used in similar work in North America by Tekauz (1990) and Steffenson and Webster (1992). These included the lines previously used in Australia by Khan (1982). A further 12 local and 3 European varieties gave a total of 44 differential lines.

Results. Preliminary results on 18 isolates screened to date, indicate a wide range of pathotypes of *D. teres* f. sp. *teres* is present in Australia yet few are virulent on the North American differentials. The percentage of isolates virulent on the individual lines is shown in Table 1.

Discussion. Australian populations of *D. teres* f. sp. *teres* vary considerably in virulence making breeding for resistance more challenging. The virulence spectrum detected in our initial studies probably reflects the reliance on two-row European barleys as parents in Australian breeding programs and the comparative neglect of North American six-row germplasm. The lack of virulence on many North American lines indicates that these, and other varieties from there, may offer valuable sources of resistance to the disease.

Table 1. Percentage of isolates of *D. teres* f.sp. *teres* virulent on differential barley lines.

Origin/line	% virulent	Origin/line	% virulent
Canada		***USA***	
Betzes	29.4	Tifang	0
Herta	29.4	Canadian Lake Shore	0
Norbert	0	Atlas	5.9
Bonanza	0	Kojo	0
Heartland	0	Coast	0
Steptoe	0	Manchurian	0
CI 9214	0	Ming	0
CI 9776	0	CI 9819	0
Australia		Algerian	11.8
Algerian	11.8	Kombar	11.8
CI 7584	0	CI 11458	5.9
CI 5791	0	CI 5791	0
Beecher	5.9	Harbin	0
Atlas	5.9	CI 7584	0
Clipper	23.5	Prato	0
Dampier	29.4	Manchuria	0
Grimmett	58.8	CI 5822	0
Corvette	41.2	CI 4922	0
Tallon	52.9	Hazera	5.9
Skiff	11.8	Cape	41.2
Gilbert	64.7	Beecher	5.9
Franklin	35.3	Rika	23.5
Golf	64.7		
Patty	11.8		
Prior	35.3		
Yerong	35.3		
Cameo	70.6		
Kaputar	11.8		

The relative lack of virulence on North American varieties not only reflects the avoidance of this material in breeding programs to date but also indicates the effectiveness of Australian quarantine procedures in limiting imports of North American barley into Australia. This policy is currently being challenged and if relaxed could lead to virulences from North America becoming established in the Australian net blotch population. Should this occur the potential usefulness of sources of resistance from North America would be limited.

Net blotch in Australia also occurs as the spot form (*D. teres* f. sp. *maculata*), and a closely related species *D. hordei* (Wallwork *et al.* 1992) causes Arno Bay blotch. A study of the variability within these pathogens is being undertaken using a similar set of differential varieties.

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Evaluation of selected barley accessions for resistance to Fusarium head blight and deoxynivalenol concentration. L. K. PROM[1], B. J. STEFFENSON[1], B. SALAS[1], T. G. FETCH, JR.[1], AND H. H. CASPER[2]. [1]Department of Plant Pathology and [2]Department of Veterinary and Microbiological Sciences, North Dakota State University, Fargo, ND 58105, USA.

Introduction. Severe epidemics of Fusarium head blight (FHB) occurred on barley in 1993-95 in the upper midwest region of the USA. Precise yield loss estimates caused by this disease are not available; however, the reduction in crop quality was devastating due to the widespread contamination of grain with deoxynivalenol (DON) (Schwarz et al. 1995), a mycotoxin produced by the primary causal organism *Fusarium graminearum* (teleomorph: *Gibberella zeae*). *Fusarium poae* and several other *Fusarium* species also have been isolated from infected barley kernels during these epidemics (Salas et al. 1995; unpublished). All of the common barley cultivars grown in the midwest are susceptible to FHB. Therefore, a program was initiated to identify and transfer FHB resistance into midwestern barley germplasm. Resistance to FHB in barley has previously been reported (e.g. Shands 1939; Takeda and Heta 1989). The objective of this study was to evaluate several of these selected barley accessions for resistance to FHB and deoxynivalenol concentration under midwestern conditions.

Materials and Methods. Seven barley accessions and the susceptible check Steptoe were planted in FHB nurseries at Fargo and Langdon, North Dakota in 1995. Inoculum was prepared using a method modified from Dodman and Wildermuth (1987). Four group 2 (Francis and Burgess 1977) isolates of *F. graminearum* were grown on acidified potato dextrose agar (APDA) at 25°C for 7 days in complete darkness and 2 days under fluorescent light. Agar plugs containing the fungal cultures were added to sterilized barley and corn grains and incubated at 25°C for 7-14 days under complete darkness. These colonized seeds were then spread over the nurseries (5 g per accession) at weekly intervals for seven consecutive weeks to ensure that sufficient inoculum was available for accessions with different maturation periods. Abundant perithecia and subsequently ascospores were produced from the colonized seed within 5-10 days. To increase disease development, nurseries were irrigated with overhead misters for 20 minutes during the evening when rain did not fall for 3-4 days. Disease assessments were made on ten randomly selected spikes of each accession at the mid-dough stage of development. The severity of FHB was determined by dividing the total number of infected kernels by the total number of kernels per spike. To determine the percentage of kernels infected with the respective *Fusarium* species, 50 randomly selected kernels from each accession were plated onto APDA and incubated at 25°C with a 12 h photoperiod. Species determinations were made according to the descriptions given by Burgess et al. (1988) after 5 days of incubation. DON assays were made on random 6 gram samples of each accession (Tacke and Casper 1996).

Table 1. Percent severity of Fusarium head blight, deoxynivalenol concentration, and percentage of *Fusarium* species isolated from kernels of selected barley accessions grown at Fargo and Langdon, North Dakota in 1995

Accession	Location	FHB Severity[1]	DON (ppm)	Percentage of kernels with *F. graminearum*	*F. poae*
Chevron	Fargo	1.0 a	<0.5	0	6
	Langdon	2.0 a	5.2	46	2
Fuji Nijo	Fargo	6.0 a	0.6	--[2]	--[2]
	Langdon	6.0 a	8.5	74	0
Imperial	Fargo	8.0 a	<0.5	8	6
	Langdon	5.0 a	11.7	64	0
Svanhals	Fargo	--[2]	--[2]	--[2]	--[2]
	Langdon	7.0 a	18.6	70	0
Horni Peseky	Fargo	8.0 a	<0.5	2	28
	Langdon	7.0 a	9.0	82	0
Svansota	Fargo	8.0 a	--[2]	--[2]	--[2]
	Langdon	3.0 a	4.1	82	2
Kanto Nijo 2	Fargo	11.0 bc	1.7	24	2
	Langdon	20.0 b	14.0	96	0
Steptoe	Fargo	19.0 c	1.1	12	24
	Langdon	39.0 c	21.2	86	4

[1]LSmeans followed by the same letter within a location are not significantly different at the (P<0.05) probability level.

[2]Missing data.

Results. Chevron exhibited the lowest FHB severity (1 and 2%), whereas the susceptible check Steptoe exhibited the highest FHB severity (19 and 39%) at both locations (Table 1). The other accessions exhibited FHB severities ranging from 6-11% and 5-20% at Fargo and Langdon, respectively. The concentration of DON was lower on all accessions at the Fargo location where data were available. Chevron, Imperial, and Horni Peseky all had DON levels less than 0.5 ppm. At Langdon, Svansota (4.1 ppm) and Chevron (5.2 ppm) had the lowest level of DON, whereas Kanto Nijo (14.0 ppm) and Steptoe (21.2) had the highest. Natural inoculum of *F. poae* was present in both nurseries. Based on the mycoflora analyses of kernels, the level of *F. poae* inoculum was much higher at Fargo than at Langdon. At Fargo, marked differences were observed among the selected accessions for the percentage of

the respective *Fusarium* species isolated from kernels. A higher percentage of *F. graminearum* was isolated from Kanto Nijo 2, whereas a higher percentage of *F. poae* was isolated from Horni Peseky, Steptoe, and Chevron. *Fusarium graminearum* was the predominant species isolated from kernels at Langdon.

Discussion. Chevron was the most resistant genotype identified from this group of select accessions. This result confirms the work of Shands (1939) who first reported the resistance of this genotype to FHB. Several accessions reported to possess resistance to FHB in Japan by Takeda and Heta (1989) also exhibited low to moderate levels of disease under midwestern conditions. It is likely that some of these accessions possess different genes for resistance to FHB. The introgression of resistance genes from one or more of these accessions into midwestern germplasm should provide adequate levels of protection against FHB during most years.

The higher DON levels detected in accessions at the Langdon nursery was expected due to the higher incidence of *F. graminearum.* At Fargo, the overall percentage of kernels infected with *F. graminearum* and *F. poae* was similar (mean=9.2 and 11.2%, respectively). Some accessions with moderate to high levels of FHB (Imperial, Horni Peseky, and Steptoe) exhibited low levels of DON. This may be due to higher levels of *F. poae* infection on these accessions as this species is not known to produce DON. The percentage of *F. graminearum* and *F. poae* isolated from kernels was markedly different for some accessions (e.g. Imperial and Horni Peseky). This result suggests a possible host genotype by pathogen species interaction. An important implication of this finding is that it may be necessary to evaluate the reaction of barley accessions to the two *Fusarium* species separately. Additionally, assays should be made for all of the possible mycotoxins produced by *Fusarium* species known to infect barley.

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Study of possibility to use Hiproly for obtaining barley forms with complex phytopathogen resistance. V.A. PUKHALSKIY[1], M.I. RUDENKO[2], D.A. SOLOMATIN[3], T.M. KOLOMIETS[3], M.I. KISILEVA[3], and E.D. KOVALENKO[3], [1] Vavilov Institute of General Genetics, Russian Academy of Sciences, Gubkin st., 3, Moscow 117809, Russia, [2] Agricultural Research Institute of Non-Chernozem Zone, Nemchinovka, Moscow region 143013, Russia, [3] All-Russian Research Institute of Phytopathology, Golitsino, Moscow region 143050, Russia.

Introduction. In recent years, such barley pathogens as Erysiphe graminis D.c.f. sp. hordei March, Ustilago nuda (Jens) Kell. et Sm., Drechslera teres (Sacc.) Shoem., and Bipolaris sorokiniana (Sacc.) Shoem. became common in the central non-chernozem region of Russia. New spring barley cultivars created by Russian breeders are usually resistant only to one infection. That is why barley cultivars resistant to two or more phytopathogens has considerable promise. This approach allows us to reduce crop loss and to avoid fungicide contamination of environment. In this work, we studied possibility to create barley forms (lines) with resistance to several phytopathogens. We considered different combination of resistant genes in a genotype and possible effect of cytoplasm on expression of these genes.

Materials and Methods. In the crosses, we used the following spring barley cultivars and lines: Nosovskii 9, Nutans 518, KM A-10, Imula, DGS 1325, Moskovskii 3, Pallidum, Trebi, L-2401, Temp, KM 1192, and Hiproly (CI 3947). Two breeding schemes were used. The first one was the topcross with Hiproly as maternal form, the second - incomplete diallel scheme (7 x 7). F_5-F_6 hybrid populations were obtained by SSD-method. F_5-F_6 plants produced F_6-F_7 lines, which were estimated by artificial infection. As inoculates, we used aggressive strains Drechslera teros and Bipolaris sorokiniana isolated from local populations of fungi in Moscow region, a population of barley powdery mildew represented by several clones from four virulent groups (a, b, c, and d), and a local population of loose smut. The artificial infection and estimation were performed according to [1-3].

Results and Discussion. Analysis of lines obtained according to the both schemes showed that 78% of F_6 lines were homozygous for the traits studied, if the resistance in parental cultivars or lines was determined by a single gene. However, results of SSD-methods depended on the genotype of the hybrid population analyzed. For example, studying 190 F_6 lines, we obtained 15.8% of lines resistant to net blotch and 72.6% of lines resistant to powdery mildew. These data may be explained by frequencies of the genes for resistance and possible linkage between the genes and genes for plant viability. In total, using the first breeding scheme, we selected four lines with genes Pt and Run. We put three of them at the disposal of the All-Russian Institute of Plant Industry, St. Petersburg, where the national gene bank collection is maintained. In the collection, the lines have numbers k-29625, k-29626 (Hiproly/Nosovskii 9), and k-29627 (Hiproly/Nutans 518). The fourth line GC-173/86 (Hiproly/L2401) is of particular interest. It was resistant not only to loose smut and net blotch, but to powdery mildew and partially to B. sorokiniana. The resistance to B. sorokiniana was determined by elongation of the latent period: only 11-25% of mature plants were infected, whereas the control was completely infected, spots were less in size, and sporulation was reduced. All breeding cultivars and lines studied in our experiments were sensitive to B. sorokiniana (infection more than 75%). We showed that the line GC-173/86 has inherited resistance to loose smut (Run 15), net blotch and B. sorokiniana from Hiproly and resistance to powdery mildew - from KM 1192. We employed this line in the second scheme of crosses (diallelic crosses) to study it as a donor and to analyze possibility to create spring barley with resistance

to several phytopathogens. Using the second scheme, we obtained 260 spring barley lines resistant to different diseases. Thirteen lines had complex resistance to loose smut (0% of infected plants), powdery mildew (0-10% of infected plants), net blotch (0-25% of infected plants), and B. sorokiniana (11-25% of infected plants). All the resistant lines were obtained from the line GC-173/86:

breeding combination	number of lines
GC-173/86 x Moskovskii 3	5
GC-173/86 x Trebi	4
GC-173/86 x KM-A10	3
GC-173/86 x DGS 1325	1

In these crosses, we also selected lines resistant to one, two, and three phytopathogens. Pedigrees of most lines included the line GC-173/86. We demonstrated that genotypes of the parental forms affected frequency of occurring lines with specific resistance. At present, we identify genes for resistance and analyze their number.

Thus, our experiments demonstrated that the Ethiopian barley Hiproly can be used in intervarietal crosses to create barley forms with complex resistance to phytopathogens.

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Identification and management of genes for scald and net blotch resistance in Finland. J. ROBINSON and M. JALLI, Agricultural Research Centre of Finland, Departments of Plant Breeding Research and Crop Protection, 31600 Jokioinen, Finland

Introduction. Net blotch (*Pyrenophora teres* Drechs. f. *teres* Smedeg.) and scald [*Rhynchosporium secalis* (Oud.) J.J. Davis] are the two most serious diseases of barley (*Hordeum vulgare* L.) in Finland. Currently Finnish barley varieties lack adequate resistance to these pathogens. Major gene resistance to both has been located in barleys of two differential series, and differences in symptom expression of Nordic barleys in the field indicate that resistance controlled by minor genes exists, although to a greater extent for net blotch than for scald. Neither pathogen has been exposed to major gene resistance in Finland and there is no strong evidence of race differentiation in either pathogen - the resistance genes were effective against all isolates, although for net blotch the resistance was not complete in all cases. The challenge now is to incorporate resistance to both diseases into good genetic backgrounds and deploy the germplasm to provide durable resistance to the diseases in Finland.

Materials and Methods. During 1994 and 1995 a series of trials were conducted in the field, under artificially initiated epidemics, to assess the degree of quantitative resistance which existed to both net blotch and scald in selected Nordic barleys. Assessment of resistance was made on the basis of disease progress measurements. Seedlings of genotypes, including the same Nordic cultivars and differential series, containing many documented sources of resistance to both diseases, were also screened in the greenhouse following inoculation with conidial suspensions of a range of isolates of *P. teres* and *R. secalis*. Infection responses were monitored over time.

Results and Discussion. Sources of both major and minor gene resistance to both diseases (see Figure 1 for details of resistance to *P. teres*) were identified, and now the genes should be managed to provide durable resistance in Finnish barleys. Two principal approaches are possible, a) exploitation and development of minor gene resistance, b) introgression of major gene resistance into good genetic backgrounds, possibly pyramiding several resistance genes. For net blotch resistance it appears that quantitative resistance is considerable in some genotypes (Figure 1), and if minor genes could be accumulated in good agronomic types, by repeated crossing of parental germplasm with this resistance, they would serve to reduce the effects of net blotch infection significantly. Genes of major effect were also shown to function against numerous isolates of the pathogen, although there was a range of reaction from a complete to an incomplete effect, but the latter was much greater than the best quantitative resistance of the Nordic genotypes. For scald there was a contrasting situation in that all major genes and major gene combinations were completely effective against all isolates of the pathogen. However, differences

in quantitative resistance levels between the Nordic genotypes, which were all highly susceptible to all isolates of the pathogen under the stringent conditions of inoculation in the greenhouse, were small, and maybe not exploitable. Both scald and net blotch caused significant yield losses in Nordic barleys in the field, mainly through a reduction in seed size, but there were no obvious correlations between degrees of symptom severity and grain yield loss.

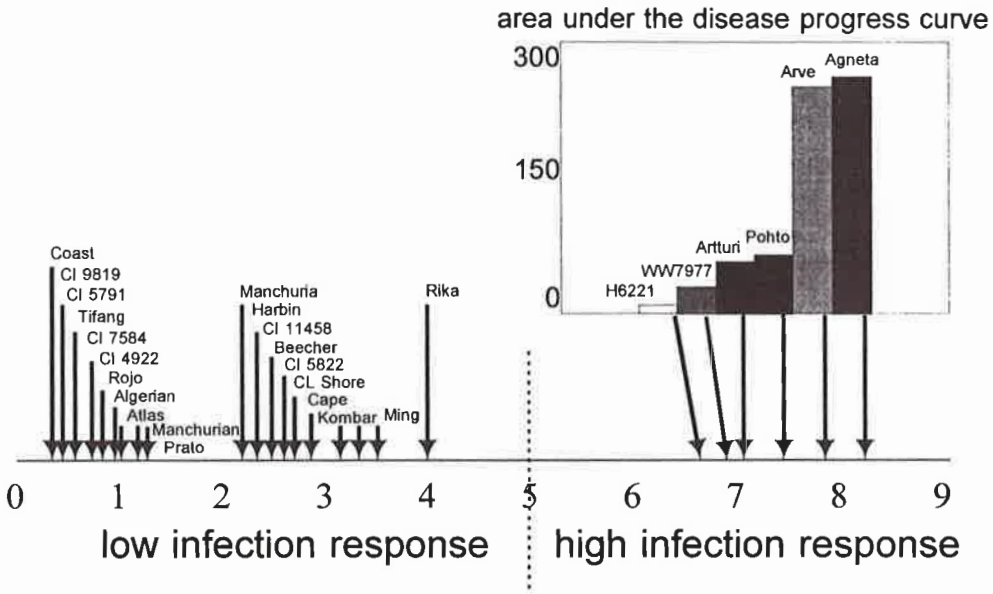

Figure 1. Mean infection response of a differential series of barleys and six Nordic genotypes to artificial infection with *P. teres*. Infection responses were scored on the third seedling leaf of plants inoculated with 20 *P. teres* isolates and grown in the greenhouse. The inset graph depicts the areas under the disease progress curves for plants of the six Nordic genotypes, grown and artificially infected with a mixture of *P. teres* isolates in the field. Each column represents a mean of measurements taken from the four uppermost leaves.

Being able to pyramid major resistance genes for both diseases into a quantitatively resistant background would represent an optimum gene management strategy, but it is generally recognised that phenotypic effects of major genes can only be measured against differential populations of the pathogen, and that incorporation of major genes for resistance is generally accompanied by loss of minor genes (Vertifolia effect). The situation is complicated by the requirement to improve resistance to two diseases simultaneously. With development and use of maker-assisted-selection (MAS) and QTL it may be possible to obviate the major obstacles to combining both types of resistance, but it is not possible to predict epistatic effects arising from various gene combinations, nor of pleiotropic effects.

Resistance of oat to fungal diseases in Europe. J. SEBESTA[1], B. SWATZ[2], D.E. HARDER[3], L. CORAZZA[4], H.W. RODERICK[5], and S. STOJANOVIC[6]. [1]Research Institute of Crop Production, 161-06, Ruzyne 507, Prague, Czech Republic; [2]Bundesamt und Forschungszentrum für Landwirtschaft, Vienna, Austria; [3]Cereal Research Centre, Agriculture and Agri-Food Canada, 195 Dafoe Road, Winnipeg, MB R3T 2M9; [4]Instituto Sperimental per la Patologia Vegetale, Rome, Italy; [5]Institute of Grassland and Environmental Research, Aberystwyth, UK; [6]Institute for Small Grains, Kragujevac, Yugoslavia.

INTRODUCTION. The European Oat Disease Nursery project (EODN) has been included in the FAO European System of Cooperative Research Networks in Agriculture. This project was set up to monitor important oat diseases in Europe and to assess sources of resistance (Sebesta et al. 1995b). The project is closely associated with research programs in various countries, and is coordinated through the Institute of Crop Production in Prague, Czech Republic. Disease resistance indexes were calculated, based on the number of observations of resistant to moderately resistant reactions throughout the test area (Austria, Bulgaria, Czech Republic, France, Germany, United Kingdom, Greece, Italy, Poland, Russia, Slovakia, Spain, Sweden, and Yugoslavia (Sebesta et al. 1995a). This report summarizes the most important oat diseases in Europe from 1988 to 1994, and the availability of resistance. The disease resistance index values are not comparable between diseases, but indicate only relative resistances within each disease.

1. **Crown rust** (*Puccinia coronata* Cda. f. sp. *avenae* Eriks.). Crown rust was widespread over most of Europe, with the highest levels recorded in Austria, France, Greece, Italy, Poland, Russia, and Slovakia, and at moderate levels in Bulgaria and the Czech Republic. The widest range in pathogen virulence phenotypes and the highest virulence indexes tended to occur in southern Europe, and the lowest in the United Kingdom.

In field tests, the following lines showed the highest levels of resistance, in descending order (disease indexes in brackets): Pc68 (185), Pc58 (183), Rodney ABDH (183), Rodney E (179), Pc50-2 (175), Pc59 (172), Pc39 (170), Garland (168), Pc50 (167), Pc63 (167), IL 85-2069 (167), Pc55 (165), Pendek//CAV 1376 (a Canadian *Avena sterilis* accession) (163), Rodney H (163), Pc62 (162), CAV 2648 (a Canadian *Avena sterilis* accession) (161), IL 86-5698 (157), OA 504-5 (156), Pc56 (155), KR 288/73L/569 (155), CA 503-1 (154), Cc 4761 (149), IL 86-1158 (145), Pg16 (142), KR 3813/73 (141), and IL 86-6404 (141). In seedling tests, lines with genes *Pc39*, *Pc55*, *Pc58* and *Pc68* were effective against all European isolates, and lines genes *Pc48*, *Pc50-2*, *Pc50-4*, *Pc54-1*, and *Pc59* also were potentially effective donors of resistance.

2. **Stem rust** (*Puccinia graminis* Pers. f. sp. *avenae* Eriks. & Henn.). Stem rust is less widespread than crown rust, occurring at more severe levels only sporadically. The highest incidences were recorded in Austria, Bulgaria, Czech Republic, Italy, Poland, Spain, and Slovakia.

The nursery results indicated disease resistance indexes for lines in the following

order: Garland (93), Rodney M (*Pg13*) (88), Pga (81), Pg16 (74), Pc58 (68), KR 3813/73 (63), Rodney B (*Pg 4*) (61), Pc54-1 (60), Pg15 (60), Rodney ABDH (*Pg2,4,1,9*), Pc50-2 (57), Pc62 (55), Rodney H (*Pg9*) (53), Pc59 (53), IL 85-1158 (52), Roxton (50), and IL 85-2069 (49). In seedling tests, the percentages of virulence to individual resistance genes were as follows: *Pga* - 0.0%, *Pg13* - 7.4%, *Pg16* - 8.1%, *Pg4* - 11.9%, *Pg9* - 11.9%, *Pg15%* - 23.7%, *Pg8* - 55.3%, *Pg1* - 57.3%, *Pg3* - 64.2%, and *Pg2* - 70.9%. An accession of *A. strigosa* (var. Saia) was effective against all isolates from Austria, Czech Republic, Slovakia, and Yugoslavia.

3. **Powdery mildew** (*Erysiphe graminis* DC f. sp. *avenae* Em. Marchal). Generally the incidence of powdery mildew has been high at all nursery locations, and has been increasing over the past several decades.

Disease resistance indexes for lines tested for powdery mildew resistance, from most effective to least effective are: APR 166 (190), APR 122 (173), Cc 6490 (171), Cc 4761 (165), CM 1621 (160), Maelor (150), Roxton (150), Cc 4146 (149), Melys (140), Orlando (134), Cc 3678 (131), Pg15 (131), OM 1387 (129), *A. sterilis* accession CAV 2648 (128), Pg16 (127), Kasadra (125), Mostym (125), Pc39 (122), Pga (118), Rodney E (113), Adam (107), KR 8122 (107), Manod (106), Rodney M (106), Maldwyn (102), and Garland (101). From seedling tests using currently prevalent races, the *Pc54* line (Cc 7422), with a single incompletely dominant gene (Sebesta et al. 1993), and lines APR 122 and APR 122, with resistance derived from *A. pilosa* (Kummer et al. 1991; Sebesta et al. 1991) appear to offer the most effective resistance. The *A. pilosa* - derived resistance also occurs in the lines OM 1387 and OM1621 (Roderick and Jones 1991). A number of lines have shown common resistance to crown rust, stem rust, powdery mildew, and tolerance to barley yellow dwarf virus (BYDV) (Sebesta and Zwatz 1995).

4. **Septoria blight and black stem** (*Stagonospra avenae* f. sp. *avenae*). High to moderate incidences of septoria blight occurred in several localities in Austria and Poland and moderate incidences in Germany in 1993 and in Italy in 1990 and 1992.

The highest diesease resistance index was calculated for *A. sterilis* accession CAV 2648 (48), followed by Cc4761 (47), Pc55 (46), Pc67 (45), Pc50-2 (43), Pc60 (43), Pc50-4 (42), Pc54 (40), IL 86-6404 (40), Garland (38), Pc58 (38), Pc48 (38), Cc 6490 (37), Pc56 (36), Cc 3678 (35), IL 86-4189 (34), IL 86-1158 (33), Pg15 (33), Pc68 (32), Pendek//CAV 1376 (32), IL 85-2069 (300, and IL 85-6467 (30).

5. **Pyrenophora leaf blotch** (*Pyrenophora avenae* Ito et Kurib.). Moderate to high levels occurred 1990-93 in Poland, Austria, Italy, Finland, Russia, Czech Republic, Sweden, and Germany.

Resistance to *P. avenae* is not yet widely known. Differences between lines, however, were observed, with the following diseases resistance indexes: IL 86-1158 (64), IL 85-6467 (61), IL 86-4189 (57), Maldwyn (56), Manod (56), Cc 3678 (54), Pc61 (52), Pc60 (52), IL 85-2069 (51), IL 86-6404 (51), Cc 4761 (50), Pc67 (50), Pc58 (49),

IL 86-5698 (49), Orlando (48), Pg15 (47), Pc59 (46), Pc50 (46), Rodney A (45), Pg16 (45), Cc 6490 (45), Jostrain (43), Garland (42), Pc50-2 (42), Pc55 (42), Roxton (42), KR 3813/73 (41), and Pc39 (41). The IL lines in this group also had higher levels of tolerance to BYDV.

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Severity of Fusarium head blight and concentrations of deoxynivalenol in near-isogenic lines of barley differing for several agronomic characters. B. J. STEFFENSON[1], L. K. PROM[1], B. SALAS[1], T. G. FETCH, JR.[1], D. M. WESENBERG[2], AND H. E. BOCKELMAN[2]. [1]Department of Plant Pathology, North Dakota State University, Fargo, ND 58105 USA and [2]USDA National Small Grains Germplasm Research Facility, Aberdeen, ID 83210 USA.

Introduction. From the evaluation of barley germplasm to *Fusarium graminearum* (a Fusarium head blight [FHB] pathogen), several investigators have reported that the level of disease is generally lower in two-rowed barley genotypes than in six-rowed barley genotypes (Takeda and Heta 1989; Xikang et al. 1991). This finding raises an important question as to whether the reduced level of FHB observed in the two-rowed types is due to an active resistance response or perhaps a factor related to spike morphology. Morphological differences between two- and six-rowed spikes could affect the efficiency of trapping pathogen propagules in the infection court, the duration of wetness on the surface of developing kernels, and perhaps kernel to kernel spread of the pathogen within the spike. It is also possible that other agronomic traits (e.g. normal vs. erect spikes, dense vs. lax spikes, hulled vs. hulless caryopsis, and early vs. late maturity) could affect the level of FHB observed and therefore mycotoxin concentrations such as deoxynivalenol (DON) in barley genotypes. Thus, the objective of this study was to compare the severity of FHB and concentration of DON in near-isogenic lines (NILs) of barley differing for several agronomic traits.

Materials and Methods. Lines near-isogenic for the traits of early/late maturity, normal/erect spike, normal/club spike, two-rowed/six-rowed spike, lax/dense spike, and hulled/hulless caryopsis (Table 1) were obtained from the USDA National Small Grains Collection at Aberdeen, Idaho. These lines were increased in the greenhouse and then planted in a FHB nursery at Langdon, North Dakota in 1995. Inoculations were made by scattering *F. graminearum* infected barley kernels over the nursery (5 grams/plot) for seven consecutive weeks beginning 10 days prior to spike emergence in the NILs. The severity of FHB (number of FHB infected kernels/total number of kernels per spike) was assessed on ten randomly selected spikes in each replicate at the mid-dough stage of development. After harvest, the concentration of DON was assessed in random 3 gram grain samples of each replicate by GC/ECD (Tacke and Casper 1996). Mycotoxin analyses were provided courtesy of Dr. H. H. Casper at North Dakota State University. Because the inoculum density of the pathogen can differ markedly within a field, the pairs of NILs were planted adjacent to each other within the nursery. The experimental design was a randomized complete block with four replicates. Differences between the means of NILs for levels of FHB and DON were tested for statistical significance using the paired t-test.

Table 1. Fusarium head blight severity and DON concentration in near-isogenic lines of barley differing for several agronomic traits.

Line	Trait	% Severity of Fusarium head blight Rep 1	Rep 2	Rep 3	Rep 4	Mean	DON ppm
CI 16127	Early maturity	10.4*	13.1*	21.2*	20.7*	16.4	5.5
CI 16128	Late maturity	1.6*	2.5*	4.8*	2.2*	2.8	5.9
CI 16532	Erect spike	29.9*	32.5	66.2*	20.9	37.4	21.0
CI 16533	Normal spike	14.1*	32.2	39.4*	19.1	26.2	21.7
CI 16635	Club spike	15.9*	9.6	5.8	6.2	9.4	10.3
CI 16636	Normal spike	5.1*	4.5	2.8	3.2	3.9	10.8
CI 16059	Two-rowed spike	8.3	20.6	27.5	10.4	16.8	17.9
CI 16060	Six-rowed spike	7.8	21.6	22.5	20.2	18.0	21.5
CI 16077	Two-rowed spike	10.8	14.9	8.8	11.2	11.4	6.9
CI 16078	Six-rowed spike	21.9	11.8	12.3	11.7	14.4	11.9
CI 16049	Lax spike	32.3	25.1	29.5	31.8	29.6	32.8
CI 16050	Dense spike	33.1	31.5	26.7	41.5	33.2	37.4
CI 16051	Lax spike	29.1	27.7	15.9	19.4	23.0	24.9
CI 16052	Dense spike	34.9	27.3	26.2	29.5	29.5	27.8
CI 16023	Hulled	10.9	24.7	28.0	13.1*	19.2	43.7
CI 16024	Hulless	10.2	20.5	18.7	6.9*	14.1	22.4
CI 16025	Hulled	32.2	19.8	15.1	31.1	24.6	29.9
CI 16026	Hulless	22.1	22.3	14.1	31.4	22.5	19.2
CI 16493	Hulled	2.7	5.9	11.3	15.3	8.8	3.9
CI 16494	Hulless	2.0	7.5	16.1	16.3	10.2	3.6
CI 16027	Hulled	7.7	4.0	20.1	15.3*	11.8	10.0
CI 16028	Hulless	7.8	4.9	16.1	6.6*	8.9	8.9
CI 16184	Hulled	1.8*	10.9	16.4	16.5	11.4	8.4
CI 16183	Hulless	14.5*	12.5	12.4	19.2	14.7	9.1

*Indicates statistical significance (at P=0.05) between the near-isogenic lines within a replicate according to the paired t-test.

Results and Discussion. The level of FHB was high throughout the nursery based on the disease severity (range of 20 to 33%) of the susceptible check 'Steptoe'. The severity of FHB in the early maturing NIL was significantly greater than the late maturing NIL in all four replicates (Table 1) even though

sufficient inoculum was present in the nursery throughout heading period. This result may be due to differences in the environmental conditions (possibly affecting the number and duration of infection periods for the pathogen) during the kernel development period in the NILs and underscores the importance of comparing disease levels on genotypes with similar heading dates and maturities. Surprisingly, the levels of DON were very similar in these NILs. FHB severity was significantly higher in the erect spike NIL in two of the four replicates; however, the DON concentrations were nearly identical. Similar results were observed for the normal/club spike NILs, with the latter exhibiting the higher FHB severity. No significant differences were detected between the two-rowed/six-rowed spike NILs or the lax/dense spike NILs for FHB or DON. The overall means for FHB and DON were, however, higher in the six-rowed and dense spike components of the NIL pairs, respectively. Significant differences for FHB severity were detected in one replicate in three of the five hulled/hulless NIL pairs tested. In two of the three cases of statistical significance, the hulled component exhibited higher levels of FHB than the hulless component. The concentration of DON was higher in the hulled component line in all cases, except the CI 16183/CI 16184 NIL pair, whose mean DON levels were very similar. The lower DON concentrations found in most hulless components was likely due to the loss of *F. graminearum* biomass in the kernel hull during threshing. However, the relatively high concentrations of DON that were detected in the hulless components indicate that the fungus is ramifying beyond the lemma and palea and into the caryopsis.

Near-isogenic line components with a higher overall kernel density within the spike (i.e. six-rowed, dense, and club) exhibited higher levels of FHB and, in most cases, DON. Although the differences were not often statistically significant, they may be biologically significant. Spikes with higher kernel densities may retain moisture for a longer period of time, a condition that favors infection by the FHB pathogen. Additionally, the closer proximity of kernels in the six-rowed, dense, and club type spikes may facilitate the spread of the pathogen from an initial infection locus. From this study, one cannot state with certainty whether the differences observed in these NILs were due to the factors stated above as no attempt was made to quantitatively estimate dew retention or FHB spread. However, when evaluating barley accessions for FHB, it is important to determine whether low levels of disease are indeed due to resistance because morphological and agronomic characters may have an unexpected affect on disease development.

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Evaluation of screening techniques for loose smut of barley. T.K. TURKINGTON[1], R.I. WOLFE[2], J.H. HELM[1], P.A. BURNETT[3], and P. JEDEL[1]. [1]Alberta Agriculture, Food and Rural Development, 5040 50 St. Lacombe, AB, T4L 1W8, Canada, [2]Agriculture and Agri-Food Canada, 6000 C&E Trail, Lacombe, AB, T4L 1W7, Canada, [3]Agriculture and Agri-Food Canada, P.O Box 3000 Main, Lethbridge, AB, T1J 4B1, Canada.

Introduction

The evaluation of germplasm and advanced lines of barley for resistance to loose smut is an important component of a barley breeding program. Various methods have been used to inoculate barley with loose smut. Moore (1936) used a partial vacuum technique to screen barley and wheat for resistance to loose smut. Injection of an aqueous spore suspension, where the lemma of an individual floret is pierced, has been used to screen barley germplasm for loose smut resistance (Poehlman 1945). However, these screening methods can be laborious, which may preclude the inoculation of large amounts of breeding material. The development and refinement of early generation screening techniques may help to permit large scale evaluation of breeding material for loose smut resistance.

Recently, scientists from the Alberta/Canada Barley Development Group have been investigating the potential of an aqueous air brush inoculation technique. Results tend to indicate that this technique may be suitable for the evaluation of large amounts of breeding material (Wolfe et al. 1993). Preliminary experiments have shown a higher percentage of smutted plants with needle versus air brush inoculation (Wolfe et al. 1993 & 1994). However, the needle technique was more time consuming and damaged florets during inoculation, resulting in decreased seed set and germination. Metcalfe (1962) successfully used a jet-nozzle method to inoculate barley florets, where a spore suspension of *Ustilago nuda* (Jens.) Rostr. was directed under pressure at each floret. The objective of the current study was to compare the efficiency of several techniques for inoculating barley with *U. nuda*.

Materials and Methods

In 1995, a field inoculation experiment was set up at Lacombe, Alberta using a four-replicate randomized complete block design with a factorial arrangement of treatments. Plots were seeded on May 10, 1995 using *Hordeum vulgare* L. cultivar Harrington, a susceptible 2-row malting variety. Individual plots consisted of single rows 6 m in length. Each single row plot was separated by two 6 m rows of triticale cv. Wapiti with individual rows spaced 20 cm apart. The first factor consisted of five inoculation treatments including: air brush inoculation (Wolfe et al. 1993 & 1994); high pressure jet inoculation (Metcalfe 1962, Moore & Munnecke 1949); needle inoculation (Poehlman 1945); boot injection (Fuentes-Davila 1995); and no inoculation. The second factor consisted of either bagging or not bagging after inoculation. Wax paper bags were placed over each inoculated head, secured and left on for 96 hours. Five individual spikes per plot were used for each treatment combination.

Air brush inoculation was accomplished using a commercial air brush at 482 kPa (70 psi) with pressure provided by a portable gas driven pump. Inoculated heads were gently bent and a fine inoculum spray directed towards the top of each floret to spread the

lemma and palea apart and allow access to the developing ovary. For the high pressure jet technique the barrel of a 3 ml plastic syringe was attached to an pneumatic air nozzle. A 22 gauge luer lock needle, with the needle removed, was attached to the plastic syringe. A fine stream of aqueous inoculum at 620 kPa (90 psi) was directed at the florets of each head in a manner similar to that described for the air brush technique. Needle inoculation was accomplished using procedures described by Poehlman (1945). The boot injection technique used similar procedures to that described for karnal bunt by Fuentes-Davila (1995). Approximately 2 ml of a spore suspension was injected into each boot as awns were beginning to emerge. On July 6, 1995 plants at G.S. 49 (Zadoks et al. 1974) were boot inoculated. All other inoculation treatments were applied on July 10, 1995 when plants were at G.S. 61-65.

At maturity the five inoculated heads from each plot were harvested separately. Three of the five heads were used to assess percentage smutted plants from each inoculated head, seeds per head, seed weight, and seed germination (%). The remaining two heads per plot were used as spares. During the winter of 1996, greenhouse facilities at Olds, Alberta were used to grow out the seed from inoculated heads. Mean number of seeds per head, mean thousand kernel weight, arcsine-transformed mean percentage smutted plants and arcsine-transformed mean percentage germination per plot were used for the analysis of variance.

Results and Discussion

Method of inoculation had a significant effect ($P<0.01$) on the number of seeds per head, thousand kernel weight, percentage smutted plants, and percentage seed germination (Table 1). The interaction of inoculation method and bagging was not significant ($P>0.05$) for any of the variables tested.

Table 1. The influence of inoculation method on mean thousand kernel weight, number of seeds per head, percentage smutted plants, and percentage seed germination per plot, loose smut inoculation experiment, 1995.

Variable[†]	Air brush	High pressure	Needle	Boot injection	Uninoculated
Thousand kernel wt. (g)	50.1a	44.5b	37.0c	49.3a	51.5a
# seeds per head	23.7ab	24.1ab	21.5c	23.0bc	24.6a
% smutted plants[‡]	15.8c	42.3b	85.9a	0.1d	0d
% seed germination[‡]	95.9a	84.0b	75.6c	96.4a	98.0a

[†] Means in the same row followed by a different letter are significantly different according to the least significant difference test (LSD) ($P=0.05$).

[‡] Back-transformed from arcsine-transformed values.

Bagging of heads had a significant effect on thousand kernel weight ($P<0.01$), percentage smutted plants ($P<0.05$), and percentage seed germination ($P<0.05$) (Table 2).

Table 2. The influence of head bagging on mean thousand kernel weight, number of seeds per head, percentage smutted plants, and percentage seed germination per plot, loose smut inoculation experiment, 1995.

Variable[†]	Head bagged	Head not bagged
Thousand kernel wt. (g)	45.1b	47.8a
# seeds per head	23.3a	23.4a
% smutted plants[‡]	22.8a	17.8b
% seed germination[‡]	89.7b	93.4a

[†] Means in the same row followed by a different letter are significantly different according to the ANOVA ($P=0.05$).

[‡] Back-transformed from arcsine-transformed values.

The needle and high pressure techniques were the most effective methods of loose smut inoculation. However, both methods resulted in a significant reduction in seed weight and germination. Although bagging of inoculated heads, to create a humid microenvironment, did result in a slight increase in percentage smutted plants, it would not be practical as part of a large scale loose smut nursery. Air brush inoculation was not as efficient as the needle or high pressure techniques, but still produced a significantly higher percentage of smutted plants compared with the control. In addition, air brush inoculation did not result in a significant reduction in seed set, weight, or germination. High pressure and air brush inoculation were less time consuming than needle inoculation. Modifications in spray pattern and pressure may increase efficiency of the air brush technique. Successful inoculation appears to depend on the placement of sufficient inoculum in the space between the lemma and palea. This space would then act as a humid "micro-chamber" promoting infection of the developing seed.

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Molecular analysis of barley-*Pyrenophora graminea* interaction. G. VALE', H. TOUBIA-RAHME, E. TORRIGIANI and G. DELOGU, Experimental Institute for Cereal Research, Section of Fiorenzuola d'Arda, I-29017, Via S.Protaso, 302, Fiorenzuola d'Arda (PC), Italy

Molecular response of barley to leaf stripe infection has been investigated by using several cultivars with different levels of resistance/susceptibility against two highly virulent *P. graminea* isolates (Valè et al., 1995).
The histological studies describing the infection process have shown that the coleorhiza and the root tip have a key role in plant response reaction. Therefore the analysis of the changes in gene expression induced by pathogen infection have been carried out on mRNAs isolated from root tips few hours after the contact between fungus and rootlets. Analyzing the mRNAs population by *in vitro* translation we found that the majority of the detected changes in gene expression occurred within 24h after inoculation and that for a longer exposure times a smaller number of variations in the mRNAs accumulation was noticed.
Northern analysis experiments allow us to find that the accumulation of several defence-related transcripts occurs in barley rootlets infected with *P. graminea*. Plant cells challenged with the isolates I-2 and I-5 (described in Gatti et al., 1992) respond by the induction of genes coding for peroxidases, thionins, thaumatin-like proteins (Valè et al., 1994).
Northern blots probed with *pCD1311*, a barley cDNA clone coding for peroxidases, showed that the corresponding transcript was accumulated as early as 6h after inoculation of barley rootlets with *P. graminea*. This was the most immediate molecular response observed. Within 18h after inoculation an accumulation of transcripts homologous to the wheat cDNA clone *pWIR232* coding for thaumatin-like proteins and of transcripts coding for thionins have been also detected (Valè et al., 1994). A cDNA clone corresponding to the latter class of genes has been isolated in our laboratory via RT-PCR and named *Th26*. The mRNAs homologous to the *pWIR232* are accumulated in both resistant (cv Thibaut/I-2) and susceptible (cv Thibaut/I-5, cv Gerbel/I-2 and cv Gerbel/I-5) interactions, although the transcript amount was greater in presence of isolate I-2 than of isolate I-5 regardless the tested cultivars. This particular behaviour has also been confirmed by other Northern experiments run with mRNAs isolated from different barley cultivars following inoculation with the same isolates I-2 and I-5; in all the tested genotypes the mRNAs coding for thaumatin like proteins were accumulated in a higher amount in the presence of the isolate I-2. A similar behaviour has also been reported for the accumulation of mRNAs corresponding to clone *pCD1311* (Valè et al., 1994). Since the condition of the infection, such as the density and the conditions of the mycelium, were exactly the same in all the experiments, an isolate-specific factor(s) controlling the accumulation of peroxidase and particularly that of thaumatin like protein mRNAs is probably involved in the regulation of the plant molecular response.
Transcripts corresponding to the cDNA clone *Th26* coding for thionins are also accumulated in response to inoculation with the isolates I-2 and I-5 of *P. graminea*. These transcripts are accumulated at a high level only in the rootlets of the cv Thibaut while in those of the cv Gerbel only a weak signal is detectable 72h after the infection with isolate I-2. The analysis of a larger number of cultivars confirms that barley molecular response to *P. graminea* infection, depending from the cultivar tested, may include or may not include

the accumulation of thionin mRNAs (Valè et al., 1995). So the accumulation of the pathogen induced transcript *Th26* depends more from the cultivar than from the isolate used. Transcripts corresponding to the thionin gene class were already found in leaf tissues and in barley endosperm; in our experiments we have shown their expression even in roots as a response to fungus infection. Because the accumulation of these defence related mRNAs seems to depend more from isolate specific factor(s) (for transcripts homologous to "thaumatin like" and peroxidase genes) or from the cultivar tested (for transcripts homologous to *Th26* gene) regardless of the compatible or incompatible interaction, a genetic role in host response to *P. graminea* it has been postulated for these genes.

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Resistance to corn leaf aphid in barley germplasm

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Barley is predominantly cultivated in the Indo-Gangatic plains in India, where the corn leaf aphid (Rhopalosiphum maidis Fitch) is a serious pest, attacking the crop from tillering stage to flowering. The cold and humid agroclimatic conditions during the months of January and February promotes the aphid infestation, which attains the peak during second fortnight of February, and starts declining after first week of March due to rise in temperature. Heavy losses in grain yield (67.5%) (Bhatia and Singh, 1977) alongwith effect on forage yield and quality as well as on malting/brewing quality (Rantappa 1968), are caused by severe aphid infestation. Also the aphid acts as vector to barley yellow dwarf virus (Orlab, 1962) which, off course is not a problem in India.

Though the Chemical controls for aphid have been recommended, but for a low input crop like barley in India, it is not practiced, therefore the development of resistant cultivars becomes more important. The diverse and stable sources of resistance are the important component of any insect resistance breeding programme, contrary of which only EB 921 or its derivative sources have been utilised in India. To meet the challange of breakdown of resistance, due to new biotypes development, it is essential to identify new and diverse sources of resistance from the available genetic resources.

MATERIALS AND METHODS

The material consisted of 4191 barley accessions of indigenous and exotic origin (table 1). The accessions were grown during winter of 1992-93 at Directorate of Wheat Research, Karnal, which was incidently the epidemic season for corn leaf aphid infestation. The observations on aphid infestation were recorded at boot leaf stage (second week of February 1993), on number of aphids per shoot and the lines were classified in to the following categories as per scale of Sinha et al. 1979 with some modifications (Verma, 1993).

Class	Score	Description
Resistant	1	0-10 aphids per shoot
Moderately Resistant	2	11-20 aphids per shoot, (with no visible loss of growth & development of plant).
Susceptible	3	21-50 aphids pershoot (with colony formation on individual leaf and loss in growth & vigour of plant).
Highly susceptible	4	More than 50 aphids per shoot (with profused colonies of aphid on leaves and stunted plant with no or very small spike formation).

The resistant or moderately resistant accessions were inspected twice more at 10 days interval, to confirm their reaction.

Further the 75 accessions (15 resistant and 60 moderately resistant) were evaluated for aphid resistance at 5 locations (Karnal, Ambala, Hisar, Kanpur and Durgapura) in 1993-94 and at 4 locations (minus Ambala) during 1994-95 crop season, alongwith the susceptible check under field conditions.

RESULTS AND DISCUSSION

The observations from different locations were compiled and figure 1 indicates the number of accessions in different categories. Out of 15 accessions classified as resistant, 8 (including EB 921) were resistant to aphid at all the locations. These accessions seems to be diverse looking into their origin, row-type and maturity groups. (Table 1)

Interestingly it was observed that out of the remaining 7 accessions, 4 were found moderately resistant at Hisar and 3 accessions were recorded as moderately resistant/ susceptible at Kanpur (Table 1). However, all these 7 accessions behaved as resistant at the remaining locations in both the years. It is possible that the different environment at these location might have resulted in changed reaction of these accessions to aphid. But the possibility of the existance of different biotypes of the aphid can not be ruled out at Hisar and Kanpur. In that case, there may be atlease 3 biotypes of aphid existing-

Biotype I, prevalent at Karnal, Ambala and Durgapura to which all the 15 accessions are resistant.

Biotype II, prevalent at Hisar, where 11 accessions were resistant and 4 were moderately resistant.

Biotype III, prevalent at kanpur, to which 12 accessions were resistant, 1 moderately resistant and 2 susceptible.

Since the accessions are behaving differently at Hisar and Kanpur, the biotypes at Kanpur and Hisar seems to be different. However, since these observations are based on natural incidence (with field inoculation of aphid followed at Karnal during 1993-94 and 1994-95), further confirmation is required through controlled conditions studies, though we feel that these lines posses adequate levels of resistance to aphid.

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Table 1 : Barley accession screened for resistance to corn leaf aphid.

Type of Accessions	Origin	Number
1. Barley germplasm at DWR Karnal	Indigenous /exotics	3454
2. Accessions from ICARDA Syria	Exotic	679
3. Accessions from Thialand	Exoitc	58
		4191

Table 2: Reaction of barley accessions to aphid.

Name	2/6 Row	Origin	Reaction to aphid(1-4)	Maturity
A. Accessions resistant at all locations (8)				
EB 921	2	E	1	Medium
DL 532	6	I	1	Medium
DL 534	6	I	1	Medium
BCU424	6	E	1	Medium
BCU2030	2	E	1	Medium
BCU 2164	2	E	I	Medium
K492	6	I	1	Medium
IB 571	6	I	1	Medium
B. Accession resistant at all locations except Hisar (4)				
BCU 310	2	E	1(2*)	Medium
BCU 311	2	E	1(2*)	Medium
BCU 313	2	E	1(2*)	Early
BCU 392	6		1(2*)	Medium
C. Accession resistant at all locations except Kanpur (3)				
EB 2507	6	E	1(3*)	Early
BCU 390	6	E	1(2*)	Early
BCU 2137	2	E	1(3*)	Late
D. Moderately resistant accessions (60)				
E. Susceptible accessions (3224)				
F. Highly susceptible accessions (892)				

* - Maximum aphid score recorded.

Selecting For Resistance To Oat Mosaic Virus And Oat Golden Stripe Virus In Oats

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INTRODUCTION. Oat mosaic virus (OMV) and oat golden stripe virus (OGSV) are both pathogens which exclusively infect winter oats (*Avena* sp.). The disease is most frequently reported in the southeastern USA, but OMV and OGSV have both been reported to occur in Great Britain (MacFarlane et al., 1968; Catherall and Hayes, 1970), the northwestern USA (Bruehl and Damsteegt, 1961), and France (Hariri and Lapierre, 1985).

Symptoms of infection include chlorotic streaks and spots on leaves with stunting and reduced yield. Symptomatic tissue may be infected with either OMV or OGSV or both (Elliott et al., 1991) and yield loss of 100% due to OMV infection has been reported (McKinney, 1946).

Both OMV and OGSV are vectored by the soilborne fungus *Polymyxa graminis* (Adams et al., 1988). The fungal zoospores are released in the fall whenever free water is available in the soil. The zoospores penetrate the roots of oat plants and release the viruses. Both viruses can persist in the resting spore stages of *P. graminis* allowing the viruses to survive in unfavorable environmental conditions (Kendall and Lommel, 1988). Once a field becomes infested with OMV and OGSV, the disease reoccurs each season and the planting of resistant cultivars is the only means of control. Resistance to the viruses is a polygenic trait and no species of *Avena* is immune.

The purpose of this study was to correlate disease ratings of OMV and OGSV infection with yield loss to make recommendations for selecting oat lines which are able to produce acceptable yields under high disease pressure from OMV and OGSV.

MATERIALS AND METHODS. Coker 716, a hexaploid oat cultivar resistant to OMV and OGSV was crossed to three moderately susceptible oat cultivars: Brooks, Madison, and Tech. The three resulting populations were advanced to the F_2 generation and individual lines in the F_3 generation were derived from each F_2 plant. The C716 X Brooks population consisted of 46 lines, with 67 and 77 lines in the C716 X Madison and C716 X Tech populations respectively.

The experimental design was a split-split plot with two treatments (infested versus non-infested soil) as the main plot factor. The three populations functioned as the split plot factor; the split-split plot factor consisted of the $F_{2:3}$ lines and $F_{2:4}$ lines tested in 1994 and 1995 respectively. Each line was replicated three times within each main plot treatment for a total of twelve replications per line for each year of testing. Each line was tested in hillplots that were spaced 24 cm apart in all directions and totaled 4680 over the two year testing period. The study was conducted in Johnston County, NC representing the coastal plain with sandy soils and high spring temperatures, and in Rowan County, NC location with a heavy clay soil and cooler temperatures associated with the piedmont region of North Carolina.

Each hillplot was rated twice during the 1994 season and three times during the

1995 season on a five point scale for symptoms of OMV and OGSV infection. Yield and harvest index also were recorded. Yield loss was calculated as the percent difference between mean yield of each line in the infested and non-infested treatments at each location. Regression analyses were performed on yield loss plotted against rating, year, and location using the SAS procedure Proc Reg with the maxr and the Mallow's CP functions. Separate slopes and intercepts were calculated for each cross.

RESULTS AND DISCUSSION. Symptoms in foliar tissue associated with OMV and OGSV infection were observed at each location and year. Little or no disease occurred in the non-infested treatments. Mean rating values for individual lines in the infested treatment were normally distributed in all three crosses for both years.

Yield loss estimates ranged from 2-86% depending on rating, population and environment. The Rowan location had consistently higher disease ratings for all three populations, indicating heavier soil types and cooler spring temperatures may influence the disease. In all three populations, yield loss could only be minimized by selecting lines with an average disease rating of 1 or less. Therefore, selection of plants with 10% or less of the tissue symptomatic should keep yield loss below 40%. The C716 X Brooks population had significantly lower yield loss, even though the three susceptible parents are not significantly different in susceptibility to OMV and OGSV. (Elliott et al. 1991). This may indicate the existence of specific combining ability among different cultivars for disease resistance. Some lines with low disease ratings suffered high yield loss, while other lines with much higher mean ratings suffered lower yield loss, indicating tolerance.

The data collected from this study suggested yield loss due to OMV and OGSV infection can be severe even at low sympton expression.. Lines displaying little or no symptoms must be selected to ensure acceptable yields. There is also evidence that tolerance may be important in this disease and further study in this area is needed. The high levels of yield loss due to OMV and OGSV indicate a need to find and utilize more effective sources of resistance.

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Table 1. Multiple regression analysis for yield loss due to infection by OMV and OGSV in each cross based on Mallow's CP and Maxr.

	PARAMETERS					
Cross[a]	b_0	$b_{1\ (rating)}$	$b_{2\ (year)}$	$b_{3\ (location)}$	R^2	R^2
B X C	3.1 ±4.1	11.7 ±1.7	12.0 ±3.3	24.4 ±3.4	0.52[b]	0.52[c]
M X C	-4.9 ±4.8	3.9 ±1.8	14.9 ±2.5	20.2 ±3.7	0.52	0.52
T X C	33.4 ±4.0	5.3 ±1.4	-12.4 ±2.5	27.4 ±2.7	0.38	0.38

[a]B X C = Brooks X C716, M X C = Madison X C716, T X C = Tech X C716.
[b]Based upon Mallow's CP statistic.
[c]Based upon maximun r^2

Table 2. Predicted yield loss (in percent) due to infection by OMV and OGSV associated with various rating values for each year, and location in Coker 716 X Brooks lines using the formulas described in Table 1.

	1994 Yield Loss		1995 Yield Loss	
RATING	ROWAN	JOHNSTON	ROWAN	JOHNSTON
0.5	33.3	9.0	45.0	21.0
1.0	39.2	14.8	51.2	26.8
1.5	45.1	20.7	57.0	45.1
2.0	50.9	26.5	62.9	38.5
3.0	62.6	38.2	74.6	50.2
4.0	74.3	49.9	86.3	81.9

A new leaf blotch disease on barley. H.WALLWORK, South Australian Research and Development Institute, Hartley Grove, Urrbrae, South Australia, 5064, Australia.

In 1990 an unrecognised leaf blotching disease was identified in barley crops at Arno Bay on the Eyre Peninsula in South Australia. Although the symptoms were similar to the spot form of net blotch (*Pyrenophora teres* f. *maculata*), a previously undescribed *Pyrenophora* species was found to be responsible. The pathogen was formally described as *P. hordei* (Wallwork et al, 1992) and the disease is now commonly known in Australia as Arno Bay blotch. Neither the net or spot forms of net blotch have been recorded to occur in the region and so confusion with these diseases has been avoided.

Symptoms of Arno Bay blotch range from small dark brown spots to larger dark brown blotches with yellow chlorotic margins. The larger blotches often have straight edges where the fungus is contained along leaf veins. Older or more severe infections often result in chlorosis and withering of leaves.Infection is also observed on leaf sheathes, glumes and awns.

In the regions where Arno Bay blotch has been most prevalent, many farms have been growing barley in two year rotations with the intervening year being left to naturally regenerated pasture. Barley stubbles remain on the soil surface and provide a large reservoir for pathogen inoculum to survive and disperse to neighbouring paddocks. Combined with early sowing, now possible with the use of modern herbicides and tillage equipment, the opportunity for species of stubble-borne pathogens to survive and infect cereals has been greatly increased in recent years. This is seen in an increased incidence of yellow leaf spot (*P. tritici-repentis*) in wheat and scald (*Rhynchosporium secalis*) in barley.

Distribution and origins Arno Bay blotch had been observed by farmers on the Eyre Peninsula at low levels for two or more years prior to its scientific identification in 1990. In 1992 most barley crops on the southern and eastern Eyre Peninsula, covering some 84,000 ha, were infected by Arno Bay blotch, many quite severely. The season was characterised by early seeding, a high frequency of days recording rain and high humidity; factors which favoured fungal infection. The extent and severity of the disease in 1992 was unprecedented and it is unlikely that similar levels of the pathogen, had they occurred in previous years, would have gone undetected.

The disease has subsequently been found more widely across southern Australia although mostly at lower levels. Dispersal of the pathogen can be as ascospores which are produced early in the season from large numbers of perithecia within the barley straw. Mature perithecia have also been observed on infected green leaves. Wind-borne conidia are produced within plant lesions and these also allow the pathogen to be widely dispersed later in the season.

P. hordei has also been identified in South Africa from barley stubble and as a minor leaf blotching disease (Scott, 1995).

It is possible that the natural niche of *P. hordei* has been as a straw saprophyte and weak parasite and that it has only recently acquired increased aggressiveness as a parasite. A shift in aggressiveness may have arisen from stronger selection pressures arising as a consequence of much larger quantities of inoculum occurring.

Relationship to other species. The anamorph of *P. hordei* bears a close resemblance to the barley pathogens *P. teres* f. *teres* and *P. teres* f. *maculata*, causal agents of net form and spot form of net blotch respectively, and *P. japonica*, causal agent of a spot form of net blotch in South Africa (Scott, 1991). The anamorph is indistinguishable from that of *P. chaetomioides* and falls within the range for *P. teres* as found in Israel (Kenneth, 1962). However these species can be readily distinguished on the basis of their teleomorphs which, in the case of *P. hordei*, are much smaller, lack setae and are mostly submerged in the straw, not erumpent.

Smedergård-Petersen (1977) reported that both forms of *P. teres* are intercrossable and also with *P. graminea*. It is probable that *P. hordei* is also a member of this group of inter-related species. Kenneth (1962) suggested that *P. teres* originated in close relation to barley in the Middle-East, where both the host and fungus evolved to form genetically diverse populations. It is likely that as barley seed was carried around the world, the fungus was carried with it but that only one or more isolates arrived in each of the different regions. This may have resulted in the fungus in each new region being different from each other and less diverse than the original population. This may be the basis for the differential abilities for the fungus to form pycnidia or perithecia in different regions. Similarly, it is likely that populations similar to *P. hordei* should also be found near to the centre of diversity of barley. The close similarity of *P. hordei* to *P. teres* may have disguised the presence of this species in areas where either form of net blotch has been recorded. It is postulated therefore that the occurrence of *P. hordei* is more widespread than reported here.

Disease control. A return to stubble burial or burning would greatly reduce inoculum levels but is not an acceptable control method as soil conservation is a high priority. Grazing of stubbles would also have a partial effect in reducing disease. The most effective method of cultural control would be the widening of crop rotations, provided that alternative and economically viable options were available.

Some populations of barley grass (*Hordeum leporinum*) have been observed to be infected with fungal isolates very similar to *P. hordei*. Inoculation trials in the glasshouse have shown that isolates from both barley and barley grass will affect both hosts (Wallwork, unpublished). Control of barley grass would therefore assist in controlling the disease.

The most effective means of control will be the use of resistant varieties. Differences in field resistance have been observed in Australian material. Whilst most barley varieties and breeding lines were susceptible, varying degrees of partial resistance were evident. Resistance data appeared similar to results obtained for spot form of net blotch in Western Australia. No lines showed complete resistance although cv.

Galleon appeared sufficiently resistant to avoid significant yield loss. Diversity in the fungal population may reduce the effectiveness of some resistances and this should be taken into consideration in planning breeding strategies.

Seed treatment trials have shown no significant (P>0.5) effect of triadimenol, flutriafol, imazalil, or carboxin on the number or size of lesions in seedlings 40 and 69 days after sowing (Wallwork unpublished). In this respect Arno Bay blotch is similar to the spot form of net blotch which responds less to these compounds than the net form.

No yield loss data is available, but it is likely that disease-yield loss relations would be similar to those recorded by Khan (1987,89) for net-type and spot-type net blotch in Western Australia.

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Interaction between *Pyrenophora teres* and *Rhynchosporium secalis* on spring barley under field conditions. K. XI[1], A.G. XUE[2], and P.A. BURNETT[3], [1]Agriculture and Agri-Food Canada, Lacombe Research Centre, 6000 C & E Trail, Lacombe, Alberta, Canada T4L 1W1, [2]Morden Research Centre, Unit 100-101 Route 100, Morden, Manitoba, Canada R6M 1Y5, [3]Lethbridge Research Centre, Highway 3 East, P.O. Box 3000, Main, Lethbridge, Alberta, Canada T1J 4B1.

Introduction. *Pyrenophora teres*, the causal agent of net blotch, and *Rhynchosporium secalis* (Oudem.) J.J. Davis, the causal agent of scald, are two foliar pathogens of barley in central Alberta. They are commonly present on the same plant and in the same field (Xue et al 1994). Numerous reports indicate that either disease may reduce yield (Martin 1985, Orr & Burnett 1993). However, there are few reports in the literature concerning the interaction between two pathogens and how this influences disease development and crop yield. Xue and Burnett (1995) showed in greenhouse studies that symptom development on barley leaves was altered after both inocula were applied together, and the effect was considered to be antagonistic. A combined infection of barley with net blotch, scald and *C. sativus* caused severe defoliation in Kenya (Anon 1960). Shaw and Royle (1978) indicated that the effect of both pathogens was additive on yield loss of winter barley in field trials. This study was undertaken to evaluate the interaction of two pathogens on disease progress in relation to crop yield of spring barley under field conditions.

Materials and Methods. *Pyrenophora teres* and *R. secalis* were isolated from naturally infected barley cv. Harrington at the Agriculture and Agri-Food Canada (AAFC), Lacombe Research Centre, Lacombe, Alberta. *Pyrenophora teres* was grown on 10% V8 juice agar for 10-14 days at 17-20 C with a 14 h photoperiod under cool white fluorescent light. The petri dish cultures were flooded with distilled water containing 0.01% Tween 20 and scraped using a glass rod. The resulting suspension was filtered through two layers of cheesecloth and the concentration was determined using a haemacytometer. *Rhynchosporium secalis* was grown on wheat germ agar (WGA) amended with 100 ppm of streptomycin or in yeast malt extract broth (YMB) for approximately three weeks at 20 C. Conidial suspensions from WGA and YMB were obtained using similar procedures to that described for *P. teres*.

Field trials were conducted during the growing seasons of 1993 and 1995 at the AAFC, Lacombe Research Centre. The experiments were set up as completely randomized block designs with four replicates. Plots consisted of 4 rows, 3 m long, with 0.23 m row spacing. Cultivar Harrington was seeded with a small-plot tractor drawn seeder. Treatments included inoculation of *P. teres* alone, *R. secalis* alone or a mixture of the two pathogens. In 1993 individual spore suspensions were adjusted to 5×10^3 spores/mL and the combined inocula were prepared by mixing a half portion of each suspension (v/v). In 1995 the concentrations of individual suspensions or the mixed were adjusted to 5×10^4 spores/mL. In the evening inocula were sprayed onto plants until run-off using a compressed air sprayer with a single nozzle. Inoculations were made at the growth stage (GS) 2.3-2.6 (Zadoks et al 1974).

In 1993, symptoms due to both pathogens were assessed on the three upmost leaves (flag leaf, flag-1, and flag-2) of 10 randomly selected tillers in each plot based on a 0-7 scale. Scores were then converted to leaf area with symptoms (LAS) using the equation: LAS= Σ(median value in a category x number of leaves in the category)/total

number of leaves. In 1995, percentage area diseased due to both pathogens was assessed on all leaves of 10 randomly selected tillers per plot. Scores were then converted to Mean LAS using the equation: Mean LAS= total percentage of diseased area/number of leaves. Disease assessments were made from GS 30 to 80, at one week intervals throughout the growing season. Area under disease progress curve (AUDPC) was calculated for each plot using the formula described by Shaner and Finney (1977). Plots were harvested to determine thousand kernel weight and grain weight. Kernel size was determined by measuring the percentage kernel weight of a 200 g sample per plot that passed through a 0.397 cm sieve. Number of heads per two rows per plot were counted.

Results and Discussion. Contrasts among the inoculation treatments for AUDPC in 1993 were significantly different (Table 1), this was due to high level of scald caused by inoculation with *R. secalis* (Table 2). The mixed inocula resulted in a significantly higher AUDPC than either inoculum, and *P. teres* inoculation gave a higher AUDPC level than *R. secalis* in 1995 (Tables 1 & 2). The mixed inocula caused significant yield reductions compared with the average of either inoculum alone in both trials (Table 1). Losses in yield expressed as kg/ha were approximately 13% in 1993 and 7% in 1995 (Table 2). No significant differences in yield between single inoculations of *P. teres* and *R. secalis* were found for either year. Inoculation treatments had no significant effect on thousand kernel weight (Table 2), kernel size or number of heads (data not shown).

In 1995 AUDPC for either inoculum alone consisted mainly of symptoms for the corresponding inoculum, whereas AUDPC for the mixed inocula consisted mainly of symptoms caused by *P. teres* (data not shown). The predominance of symptoms due to *P. teres* over *R. secalis* in the field trial agreed with previous observations from greenhouse tests (Xue & Burnett 1995). Higher AUDPC levels from the mixed inocula may have resulted from rapid development of *P. teres* with the aid of infection by *R. secalis*. There was a trend in 1995 where as mean AUDPC increased, yield decreased, but there was no such trend in 1993 (Table 2). Results from the present study suggest that when the two pathogens were applied together they were not antagonistic towards each other under field conditions. This test will be repeated in 1996.

Table 1. ANOVA and specific comparisons of AUDPC and yield of Harrington barley in relation to inoculation with *Pyrenophora teres* or *Rhynchosporium secalis*, or a mixture of both pathogens, 1993 and 1995

		1993		1995	
		AUDPC	kg/ha	AUDPC	kg/ha
Source	DF	MS	MS	MS	MS
Block	3	17.6	1150935.8	31789.8	94451.5
Treatment	2	1025.7**	478616.8*	49085.5**	128157.6
P.t.& R.s. vs mixture	1	328.6**	844060.4*	76148.6**	202642.4*
*P.t.*vs *R.s.*	1	1722.8**	113173.1	21534.1*	53672.7
Error	6	18.1	87471.3	2866.8	31294.8

*, ** significant at $p<0.05$ and 0.01, respectively.

Table 2. Mean AUDPC, yield and thousand kernel weight of Harrington barley in relation to inoculation with *Pyrenophora teres* or *Rhynchosporium secalis*, or a mixture of both pathogens, 1993 and 1995

Treatment	1993			1995		
	AUDPC	kg/ha	tkw(g)	AUDPC	kg/ha	tkw(g)
P.t.	50.9	4311.8	41.2	744.7	3943.4	45.1
R.s.	80.3	4073.9	41.3	641.0	4107.2	44.4
P.t.+ *R.s.*	54.5	3630.3	40.5	861.8	3749.6	44.8

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Characterisation of *Puccinia coronata var. avenae* (crown rust) populations in Moscow Region. YUKHNINA,E.K., LYZLOV,E.V. and MAGUROV.P.F., Scientific Agricultural Research Institute, Nemchinovka-1, Moscow Region, Russia.

The success of selection in immunology is closely connected to, and is based on, an understanding of the relation between host plant and pathogen. A knowledge of the virulence of the population and of the dynamics of the rust composition is necessary planning selection investigations and the timely replacement of resistance genes.

In 1991-93 an analysis of crown rust populations collected from the fields of Moscow Region has been carried out by the Laboratory of Oat Breeding.

The investigation of the composition of *P.coronata* populations was carried out using standard methods with selection of monopustal isolates based on monogenic set of differentiated varieties of Pendek (*1). Monogenic lines of Pendek with resistance gene: Pc-14, Pc-35, Pc-38, Pc-39, Pc-40, Pc45, Pc-46, Pc-47, Pc-48, Pc-50, Pc50-2, Pc-50-4, Pc-55, Pc-56, Pc58, Pc-59, Pc-60, Pc-61, Pc-62, Pc-63, Pc64, Pc-67, Pc-68 were used.

In the course of the investigation a total of 450 monopustal isolates were analysed.

From the results of the investigation we can remark that the virulence genes Pc-14, Pc-50, Pc-50-2, Pc-50-4, Pc-59, Pc-60 were not observed in the Moscow population of crown rust. Highly effective were lines with resistance genes Pc-39, Pc-40, Pc-46, Pc-48, Pc-55, Pc-58, Pc-61, Pc-68, where the level of their susceptibility ranges from 1.3% (Pc-61) to 8% (Pc-55).

Each year, the samples revealed from 10 to 17 genes which are virulent to different degrees to the resistance genes of the host plant. The virulence of the population in general is shown by isolates with the genes: Pc-35, Pc-38, Pc-45, Pc-47, Pc-56, Pc-58, Pc-62, Pc-64, Pc-67. The level of virulence in this group fluctuates from year to year, which can be deduced from different factors.

In total 41 pathotypes of pathogen were described. The majority of these were demonstrated by isolates with one gene, and by isolates with combinations of 2-3 virulence genes. The dominant positions are occupied by lines with virulence gene Pc-56, the remainder of the isolates with single virulence genes are detected rarely (Pc-35, Pc-45, Pc-61, Pc-62). Isolates of pathogens with 2-4 virulence genes comprise 40% of the population and only a few lines have 5-6 genes.

Our data partly correspond to those from *P. coronata* populations determined from 13 economic regions of the FSU (*2,*3). According to those results high concentrations of virulence genes (Pc-35, Pc-47, Pc-38, Pc-45) were observed, but genes Pc-39 and Pc-48 did not make up a significant part of the population.

Although the general structure of the population remains stable through time, there is a continuous process of replacement of virulence genes. This replacement is reflected in an annual diversity of phenotypes.

During the period of investigation from 1991-1993, from 40 to 70% of the pathotypes from the Moscow population remain common.

The general structure of the Moscow crown rust population is distinguished from the European pathogen population by a having a different frequency of gene virulency distribution. Genes Pc-50-2, Pc-50-4, Pc-58 and Pc-60 were not observed in the Moscow population, whereas they are present in the European population. However, genes Pc-39, Pc-55 and Pc-68 are present in the Moscow population but are absent in the European population (*4).

The studied Pendek lines were examined against the natural infection background and in the conditions of artificial epiphytoty. Lines Pc-14, Pc-50, Pc50-2, Pc-50-4, Pc-55, Pc-59, Pc-60, Pc-61 and Pc-68 were not infected in the local natural crown rust population nor in the synthetic population of pathogene.

The results from field investigations of Pc-14, Pc-50, Pc-50-2, Pc-50-4, Pc-59 and Pc-60 correspond to the results of analysis of the Moscow *P. coronata* population. During these studies, no virulence genes were discovered which could infect these samples.

The introduction of these resistance genes as a donor resistant to crown rust in the breeding process would be favourable.

However, the genes Pc-55, Pc-58, Pc-61 and Pc-68 are not recommended as resistant donors since some pathogen isolates are capable of infecting them were found in the regional population. Varieties based on these genes may lose their resistance quickly.

Lines Pc-35, Pc-45, Pc-47, Pc-56, Pc-62, Pc-62, Pc-64 and Pc-67 are poor for breeding as they are infected by most pathotypes.

In conclusion, the results of our three-year investigations into the genotypic composition of the Moscow population of the pathogen revealed that the structure of this population has relative stability, but at the same time there is a constant replacement of virulence genes, manifesting itself in the diversity of the pathogen population. The investigation of the Pendek set in field conditions allowed us to distinguish some resistant lines which can be recommended as resistant sources with adoptable agronomical characteristics. Of these, the resistant line Pc-59 is the best.

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TABLE
Effectiveness of the Pendek lines against the Moscow population of crown rust

Pendek lines with resistance genes	% severity of crown rust (1993)		concentration of Puccinia coronata virulence genes	
	natural inoculation	artificial inoculation	Moscow population	European 92-93 popln. (*4)
Pc-14	0	0	0	no data
Pc-38	5	10	++	++
Pc-39	5	0	+	0
Pc-45	no data	100	++	no data
Pc-48	0	5	0	++
Pc-50-2	0	0	0	+
Pc-50-4	0	0	0	++
Pc-55	0	0	+	0
Pc-56	10	100	++	++
Pc-58	0	0	no data	0
Pc-59	0	0	0	+
Pc-60	0	0	0	++
Pc-61	0	0	+	++
Pc-62	5	50	++	++
Pc-63	5	0	+	++
Pc-64	0	100	++	++
Pc-67	10	50	++	++
Pc-68	0	0	+	0

0 = virulence gene absent
\+ = concentration of virulence genes 1-10%
++ = concentration of virulence genes over 10%

Genetic characterization of powdery mildew resistance in common oat cultivars grown in Europe and North America. F.J. ZELLER and S.L.K. Hsam, Technische Universität München, Institut für Pflanzenbau und Pflanzenzüchtung, D-85350 Freising-Weihenstephan, Germany

Introduction. Powdery mildew caused by the fungus *Erysiphe graminis* f. sp. *avenae* is a deleterious leaf disease of common oat, *Avena sativa* L. in maritime regions (CLIFFORD 1995). In Europe and North America damage by this fungus is increasing. This study reports the characterization of mildew resistance in common oat cultivars and advanced breeding lines grown in Europe and North America.

Materials and Methods. Oat materials were provided by European and North American germplasm banks and private breeders. The tests for mildew resistance were carried out on segments of primary leaves cultured on 6 g/l agar and 35 mg/l benzimidazole. The powdery mildew isolates were collected in Germany and Denmark. Twelve plants per line were tested.

Results and Discussion. A total of 120 populations of *Erysiphe graminis avenae (Ega)* were tested for their disease responses to differentiate standard oat cultivars and lines possessing documented resistance to oat powdery mildew. Twelve *Ega* isolates derived from single spore pustules which enables the differentiation of the known mildew resistance (OMR) groups were established (Table 1). Cultivar Manod, lines Cc 4146, 9065Cn 6/3/74, Cc 6490 represent the OMR groups 1, 2, 3 and 4, respectively. Cultivar Milford (OMR 0) shows susceptible response to all the isolates, whereas OMR 1, OMR 2, OMR 3 and OMR 4 are characterized by isolate-specific virulence and avirulence responses.

Among the 129 cultivars and lines grown in Europe 26 of them showed resistance response at the seedling stage. Disease responses characteristic for OMR groups 2 and 3 occurred in cultivars derived from France, Germany, Great Britain and Ireland. Only OMR 3 was found in a cultivar grown in Belgium, and OMR 4 in two cultivars grown in The Netherlands.

Table 1. Reactions of five differential oat cultivars/lines after inocultaion with 12 isolates of *Erysiphe graminis avenae*

Cultivar/ Line	*Erysiphe graminis avenae* isolates												OMR[1]
	F9	F10	F22	L4	L7	L8	L20	L42	D5	D19	D20	D32	
Milford	s[2]	s	s	s	s	s	s	s	s	s	s	s	0
Manod	r,i	r,i	s,i	i	i	r,i	s	s	s	i	i	i	1
Cc 4146[3]	s	s	r	r,i	r	i,s	r	s	s	s	s	r	2
9065 Cn 6/3/74[4]	r	r	s	s	s	s	s	s	s	s	s	s	3
Cc 6490[5]	r,i	r,i	s	i	i,r	r,i	s	s	s	r	r	r,i	4

[1] Oat Mildew Resistance Group

[2] r = resistant, s = susceptible, i = intermediate

[3] Cc 4146 = *Avena sterilis* L. var. *ludoviciana* derivative

[4] 9065 Cn 6/3774 = *A. sterilis* L. var. *ludoviciana* derivative

[5] Cc 6490 = *Avena sativa/A. barbata* derivative

In addition, 17 cultivars showed intermediate and susceptible reactions to the isolates used. From the 118 North American oat cultivars and lines tested, two of them showed resistance patterns corresponding to OMR 3. Three cultivars and lines displayed a resistance response not characterized by the *Ega* isolates employed. 30 cultivars and lines exhibited intermediate and susceptible responses to specific isolates.

FINKNER et al. (1953) have found 59 resistant oat accessions from about 4000 cultivars and breeding strains grown in the USA. The present study revealed that most of the North American oat cultivars and lines lack seedling resistance. However, it could

not be ruled out that the cultivars and lines showing intermediate response in seedling stage possess genes for quantitative resistance. LEATH et al. (1991) had earlier reported that resistance of American oat cultivars and lines in the field is expressed quantitatively and may not correspond with seedling resistance.

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