

V International Oat Conference

&

VII International Barley Genetics Symposium

Proceedings

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INVITED PAPERS



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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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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

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Biochemistry of malting – The way forward

A.W. MacGregor

*Canadian Grain Commission, Grain Research
Laboratory, Winnipeg, Canada*

Introduction

Significant progress has been made in our understanding, in biochemical terms, of some of the important changes that must occur within barley kernels during malting. The origin, biosynthesis and action pattern of many of the hydrolytic enzymes responsible for endosperm modification are now known. Many of the pertinent functional properties of major endosperm macromolecules such as starch and beta-glucans are well understood, and progress is being made in identifying the role of specific proteins in the malting and brewing process. There is a perception that most of the important malt biochemistry is now understood, and it is up to molecular biologists and barley breeders to exploit this knowledge to develop new and improved malting barley cultivars.

However, full exploitation of genetic engineering technologies requires detailed knowledge of the factors that affect malt quality, followed by identification of the genes that control these factors. Barley and malt contain mixtures of complex components that do not function in isolation during malting and brewing. Possible interactions amongst the components or products formed from the components during malting and brewing must be considered if trouble-free processing of barley and malt is to be achieved. The effect of growing environment on functional properties of barley components must also be taken into consideration if a complete understanding of what constitutes “malting quality” is to be attained.

The following discussion will address not only specific quality-related components in barley and malt that require further research but will also identify some component interactions that could cause processing problems during malting and brewing.

Barley aleurone-GA₃ interactions

Modification of barley endosperm during malting is carried out, mainly, by hydrolytic enzymes synthesized in and secreted by the aleurone layer. Numerous studies have shown that isolated aleurones treated with GA₃ synthesize up to 30 enzymes, several of which are required to mobilize endosperm reserves during malting (Jones and Jacobsen, 1991). It is now generally accepted that, in the germinating seed, aleurone cells respond in a similar way to gibberellins that are produced in the embryo. Recent results indicate that the gibberellins do not enter

the aleurone cells but react with the aleurone cell wall (Gubler et al., 1995). It is this interaction that induces a series of biochemical events within the cell that results in the synthesis of an array of hydrolytic enzymes. These biochemical reactions are now undergoing fairly intensive study. Apart from this aspect of GA_3 interaction, some other questions remain about aleurone-endosperm interactions.

Before hydrolytic enzymes can be synthesized in aleurone cells, extensive breakdown of aleurone protein reserves must occur to provide amino acids for protein synthesis. Very little is known about the initiation, sequence of events and control mechanisms of this process, although some information is now available on the type of protein present in aleurone cells (Yupsansis et al., 1990). Presumably, rapid synthesis of high levels of enzymes within aleurone cells requires a large pool of available substrate. The aleurone appears to be self-sufficient in that there is no evidence that it requires protein supplies from the endosperm to sustain its metabolism. If this is so, it might be possible to have high protein levels in the aleurone to provide adequate reserves for enzyme synthesis, but low levels in the endosperm to maximize the starch content.

There is some evidence that enzymes formed in isolated aleurones treated with GA_3 are not identical to those formed in intact kernels (Skadsen, 1993). For example, alpha-amylase 2 represents a high proportion (90–95%) of the total alpha-amylase synthesized in germinating barley kernels but a much smaller proportion of the alpha-amylase formed in isolated aleurones treated with GA_3 . There may be some feedback mechanism between the endosperm and aleurone that controls protein synthesis in the aleurone.

It is not only the rate of enzyme synthesis in the aleurone that is important for malt quality but also the rapidity and efficiency of enzyme secretion into the endosperm. Rapid and extensive degradation of the endosperm cell wall polysaccharides, beta-glucan and arabinoxylan, must occur throughout the endosperm during malting (Palmer et al., 1985). It would be desirable, therefore, to have the corresponding hydrolytic enzymes secreted rapidly into the endosperm so as to have maximum time to complete cell wall degradation. A similar argument can be made for the proteases required to solubilize hordein proteins and to degrade the protein matrix in which the starch granules are embedded. In this case, care must be taken to ensure that protein degradation is not excessive or other quality parameters in the malt may be at risk. It is not important that a high proportion of alpha-amylase is secreted into the endosperm because the major action of this enzyme occurs during the mashing stage of brewing. Differential control over the synthesis and secretion of these major groups of hydrolytic enzymes would be advantageous for malting, so long as this did not weaken the germination ability of the seeds.

Endosperm cell walls

The major component of barley endosperm cell walls is (1–3)(1–4)-beta-glucan (beta-glucan). This material hinders enzyme movement in the endosperm during malting, causes filtration problems during brewing and may participate in the formation of hazes during beer storage. It is imperative that this polysaccharide be degraded extensively during malting to avoid these processing problems. Much

is now known about the general structure of the polysaccharide and the properties and action patterns of the beta-glucanases (Fincher, 1992). However, details of the fine structure of beta-glucan should be clarified because the functionality of the polysaccharide and of its hydrolytic products may depend as much on their chemical structure as on their molecular size. Barley beta-glucan does contain stretches of beta(1-4)-linked glucose residues that could have the potential to form aggregates, precipitates and gels when released from beta-glucan through the action of beta-glucosidase. Such chains would be resistant to hydrolysis by beta-glucanase but could be reduced to small harmless dextrans through the action of enzymes such as beta-glucosidase. These enzymes are present in malt and their properties are now being studied (Leah et al., 1995; Hrmova et al., 1996). Nevertheless, further research is required to clarify the potential role of beta-glucan hydrolytic products in filtration and stability problems in brewing.

Very little information is available on the structure and functionality of the other cell wall polysaccharide, arabinoxylan (Fincher, 1992). This polysaccharide also has the potential to cause processing problems because it produces viscous solutions and may also form gels through interchain cross-linking with ferulic acid. The fate of arabinoxylans during malting and mashing needs to be clarified, and the structure and functionality of arabinoxylans present in wort should be determined to assess their potential for causing filtration problems during brewing or stability problems during beer storage.

Endosperm proteins

Proteins play a role in many aspects of malting and brewing, including forming a matrix around starch granules that may restrict hydrolysis of the granules during mashing, forming aggregates or gels that cause filtration problems, formation and retention of beer foam, formation of colour and hazes in wort and beer, provision of amino acids for yeast nutrition during fermentation, and, of course, hydrolytic functions during malting and brewing. Controlled hydrolysis and solubilization of storage proteins such as hordein must be accomplished during malting and early stages of mashing to prevent protein-associated processing problems and to enhance protein-related quality parameters (Enari and Sopanen, 1986). Progress is being made on understanding protein functionality during malting and brewing but more details are required on the effect of specific proteins or groups of proteins on endosperm texture (vitreous or floury), rate of endosperm modification, wort filterability and beer quality. Numerous proteases have been identified in malt but their specificities and, hence, their possible roles in malting and brewing are only beginning to be understood (Zhang and Jones, 1995). The presence of endogenous protease inhibitors in barley (Jones and Marinac, 1995) adds even more complexity to the protein system, and their possible role in modulating protein modification during malting has yet to be determined.

Starch

The starch content of malting barley and the gelatinization temperature of the starch are the two most important aspects of barley starch for malting and brewing

quality. Starch is a major contributor to malt extract, so it is desirable to have as high a starch level as possible in malting barley. Two-rowed cultivars tend to have higher starch contents than six-rowed cultivars, and there is evidence of genetic variation in the starch content of barley cultivars (Kong et al., 1995). This should be explored and exploited, if proved correct, to maximize starch levels in malting barley. Growing conditions have a significant effect on barley starch content, as is the case with other cereal grains (Tester et al., 1991). Hot weather during kernel development lowers the level of one of the starch synthases in wheat (Jenner et al., 1993), and a similar effect is likely in barley, resulting in thin, low starch but high protein barley that would be undesirable for malting. Increased heat stability of critical starch-synthesizing enzymes would be advantageous and would lead to the development of more widely adapted cultivars. There is evidence that some cultivars may be better adapted than others to high temperatures as far as starch synthesis is concerned (Allan et al., 1995). This finding deserves further exploration.

High temperatures during kernel development also affect starch functionality. In particular, they increase the starch gelatinization temperature (Tester et al., 1991) and this could slow down or even limit the conversion of starch to fermentable carbohydrates during mashing. The reason for this effect of environmental temperature is not well understood but, presumably, is caused by an imbalance in the starch synthesis system. Again, more detailed knowledge of the enzymes involved in starch synthesis and of possible environmental effects on these enzymes could provide ideas on how to develop barley starch with a low gelatinization temperature that would be relatively resistant to growing temperatures. Such barley would offer a quality advantage to the malting and brewing industries.

Hydrolysis of starch to fermentable carbohydrates during mashing is carried out by a group of starch-degrading enzymes (MacGregor, 1996). High levels of these enzymes are required in malts used for starch-based adjunct brewing as practised in North America. In all-malt brews, or when sugar adjuncts are used such as in Australia, then lower levels of starch-degrading enzymes are required. Alpha- and beta-amylases are major starch-hydrolyzing enzymes but it is unlikely that these enzymes are limiting in most high quality malts. However, wort and beer contain significant levels of branched dextrins (Enevoldsen and Schmidt, 1973). This indicates that the starch debranching enzyme, limit dextrinase, is largely ineffective during mashing. Recently, the presence of two low molecular weight proteins in barley that complex with and inhibit limit dextrinase has been reported (MacGregor et al., 1994). It is probable that these proteins are responsible for the apparent lack of hydrolysis of the alpha-(1-6) linkages in amylopectin during mashing. Possible approaches to reducing the level of these inhibitors in malting barley and malt should be explored. If successful, then the development of barley with the potential to provide malt of high fermentable extract would be possible.

Beer flavour and flavour stability

The undesirable "cardboard" or stale flavour sometimes found in beer has been traced to the aldehyde trans-2-nonenal. The mechanism of formation of this

compound from barley lipids via a complex series of reactions involving lipases and lipoxygenase is now well understood (Cantrell and Griggs, 1996). Possible biochemical and processing approaches to reducing the potential levels of trans-2-nonenal in beer are being explored. However, the whole area of the possible influence of barley components on beer flavour has been somewhat neglected. Are there cultivar differences? Do soil and environmental factors have an effect? What impact do hull components have on flavour and would malt from hull-less barley affect the flavour profile of beer?

Endosperm texture

Floury, mealy endosperms are a desirable characteristic of malting barley because this characteristic facilitates water uptake and distribution during steeping and endosperm modification during germination (Palmer, 1989). Vitreous kernels tend to have higher levels of protein and beta-glucan and more small starch granules than floury kernels, but the precise differences between floury and vitreous kernels have not been explained in biochemical terms. It is possible to have floury and vitreous areas within the same kernel! Although genetics play a role in determining endosperm structure, the growing environment is very important. Long, cool growing conditions tend to produce floury endosperms whereas hot, dry conditions tend to produce low starch barley that is more vitreous. There is a perceived need to determine the effect of growing environment not only on the quantity and characteristics of the major endosperm components, protein, starch and non-starch polysaccharides but also on the way in which these components interact with one another during kernel desiccation. It is perhaps the environment prevailing during desiccation (hot or cool, wet or dry) that may have the greatest influence on endosperm texture.

There will continue to be a demand for increased "quality" in malting barley and malt. Changes in malting and brewing technology may require different types of quality in barley and malt, so an increased understanding of some of the areas outlined in this paper will help in the development of more versatile malts of high quality.

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Protein engineering of enzymes for improved malting quality of barley

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Introduction

Polysaccharides from endosperm cell walls, together with the enzymes that depolymerize them in germinated grain, are central determinants of malting quality in barley. Cell wall degradation is required before alpha-amylases, endo- and exopeptidases, and other hydrolytic enzymes that are secreted from surrounding aleurone layers and scutellar tissues can gain free access to the storage polymers that are packaged within the cell walls of the starchy endosperm (Fincher and Stone, 1993). Thus, sub-optimal levels of cell wall degradation can result in malts with lower extracts (Bamforth and Barclay, 1993). Furthermore, the major polysaccharide constituents of endosperm cell walls typically produce solutions of high viscosity, which can lead to filtration difficulties in the brewery. The polysaccharides can also precipitate from solution, particularly in high ethanol concentrations, at low temperatures, or after treatment with certain industrial centrifuges (Letters, 1977; Yamashita et al., 1989; MacGregor and Fincher, 1993).

It can be concluded, therefore, that cell walls and their polysaccharide components cause problems in the malting and brewing processes, and that rapid enzymic degradation of wall polysaccharides in germinated grain is a highly desirable property of malting barleys. Here, we describe how fundamental molecular information on enzymes that hydrolyse cell wall polysaccharides can be applied for the solution of important practical problems in the malting and brewing industries. In particular, we will focus on the barley (1-3,1-4)-beta-glucanases and the enhancement of their thermostability through protein engineering.

Degradation of cell walls in germinated grain

The two major constituents of the cell walls of starchy endosperm in barley are (1-3,1-4)-beta-glucans and the arabinoxylans (Table 1). Cellulose constitutes a very small percentage of the walls; this probably reflects the evolutionary advantage of endosperm cell walls that can be rapidly degraded during endosperm mobilization in germinated grain (Fincher, 1992).

Properties of arabinoxylans and their hydrolysis

Arabinoxylans consist of a family of polysaccharides that have a (1-4)-beta-xylopyranosyl backbone and single alpha-L-arabinofuranosyl constituents linked mostly through C(O)3 but also through C(O)2 of backbone xylosyl residues (Vieter et al., 1992). Phenolic acids, mainly ferulic acid but also *p*-coumaric acid, are covalently linked via ester linkages to C(O)5 atoms of arabinosyl residues. Cereal arabinoxylans generally form viscous solutions, because of their molecular asymmetry (Andrewartha et al., 1979).

Hydrolysis of arabinoxylans in germinated grain is effected by the concerted action of endoxylanases, arabinofuranosidases and possibly xylosidases (Preece and MacDougall, 1958). There is relatively little information on the individual barley enzymes that participate in this hydrolytic process, although three (1-4)-beta-xylan endohydrolases (EC 3.2.1.8) have been purified and characterized (Slade et al., 1989).

Properties of (1-3,1-4)-beta-glucans

Up to 75% by weight of walls of the starchy endosperm is composed of (1-3, 1-4)-beta-glucans. These polysaccharides consist of linear chains of beta-glucosyl residues polymerized through (1-3)- (approximately 30%) and (1-4)- (approximately 70%) glucosidic linkages (Woodward and Fincher, 1983). Blocks of two or three contiguous (1-4)-beta-glucosyl residues, separated by single (1-3)-linkages, account for nearly 90% by weight of the polysaccharide; longer blocks of up to 14 adjacent (1-4)-beta-glucosyl residues are also present (Woodward and Fincher, 1983).

The problems associated with barley (1-3,1-4)-beta-glucans in the brewing process can generally be attributed to their tendency to form solutions of high viscosity. The molecules consist of up to 1200 unbranched glucosyl residues and remain soluble in water because the irregular distribution of (1-3)-linkages along the chain prevents aggregation and precipitation (Woodward and Fincher, 1983). The polysaccharide chains are highly asymmetrical, with axial ratios of up to 100, and it is this property that accounts for the very high viscosity of (1-3, 1-4)-beta-glucan solutions.

Hydrolysis of (1-3,1-4)-beta-glucans

Several enzymes are involved in the complete depolymerization of (1-3, 1-4)-beta-glucans to glucose. Perhaps the most important are the (1-3, 1-4)-beta-glucan endohydrolases (EC 3.2.1.73), which hydrolyse (1-4)-beta-linkages of

Table 1. Composition of cell walls from barley

Tissue	Neutral Monosaccharide Composition (%)				Protein (%)	Phenolic Acids (%)	Major Polysaccharide Components*	References	
	Ara	Xyl	Glc	Man Gal					
Aleurone (mature grain)	24	47	26	2	2	16	1.2	71% arabinoxylan 26% (1-3,1-4)- β -glucan 2% cellulose 2% glucomannan 1% (1-3)- β -glucan	Bacic and Stone (1981)
Starchy endosperm (mature grain)	11	11	75	3	0	5	0.05	75% (1-3,1-4)- β -glucan 20% arabinoxylan 2% cellulose 2% glucomannan	Fincher (1975)

* Values for polysaccharide components are estimated from compositional analyses presented in the references cited.

4)-beta-glucan endohydrolases been unequivocally identified, although beta-glucan exohydrolases (Hrmova et al., 1996) and beta-glucosidases (Leah et al., 1995) can hydrolyse these oligosaccharides to glucose *in vitro*, and are present in extracts of germinated barley grain (Hrmova et al., 1996).

(1-3,1-4)-beta-glucanases in malting quality

The clear relationship between (1-3,1-4)-beta-glucanase levels and malting quality was demonstrated by Stuart et al. (1988), who showed that the ability of the grain to rapidly produce high levels of the enzyme was a particularly important indicator of malt extract values (Table 3). Edmunds et al. (1994), using a

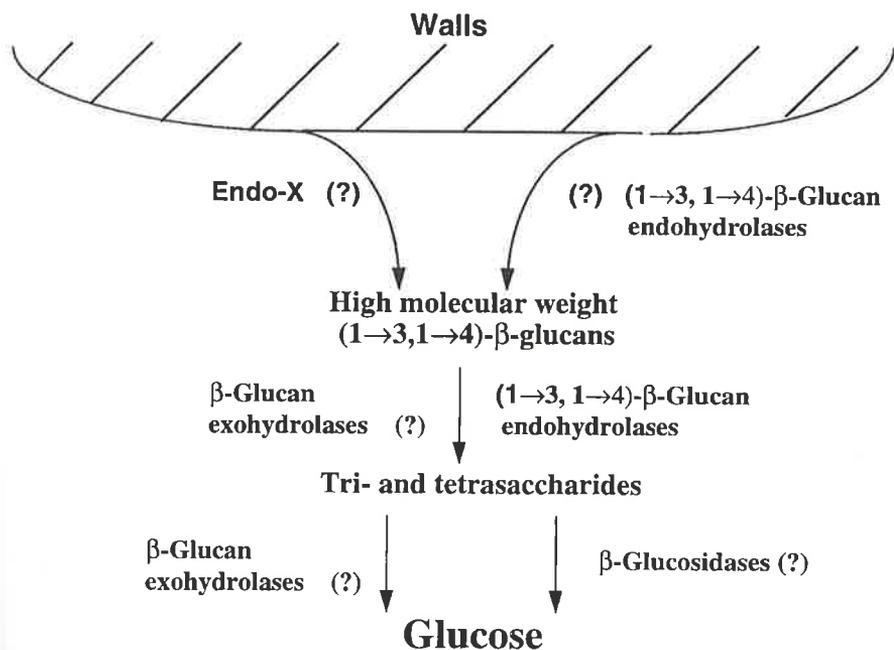


Figure 1. Hydrolysis of cell wall (1-3,1-4)-beta-glucans in germinated barley

Table 3. Linear correlations (*r*) between barley quality parameters

	Total β-glucan	Malt β-glucan	% loss β-glucan	beta- glucanase	Malt extract
Total beta-glucan	1.00	0.18	0.22	0.27	0.15
Malt beta-glucan	—	1.00	-0.92*	-0.61	-0.74*
% loss beta-glucan	—	—	1.00	0.72*	0.81*
beta-glucanase	—	—	—	1.00	0.85*
Malt extract	—	—	—	—	1.00

* Significant to $P < 0.01$. Source: Stuart et al. (1988).

monoclonal antibody-based, enzyme-linked immunosorbent assay (ELISA), extended these results to show that levels of isoenzyme EI 24 hours after the initiation of germination were significantly correlated with malt extract, and concluded that this presented opportunities as a rapid screening assay for barley vigour and malt extract values. In any case, it can be concluded that (1-3,1-4)-beta-glucanases are of central importance in the removal of endosperm cell walls in germinated barley, and that this is correlated with extract values achieved with the malt.

Genetics of barley (1-3,1-4)-beta-glucanases

Both cDNAs and genes encoding the two barley (1-3,1-4)-beta-glucanases have been cloned and sequenced (Slakeski et al., 1990; Wolf, 1991). The chromosomal locations of the genes have been identified (Slakeski et al., 1990; Table 2) and their positions on the barley genome have been mapped and incorporated into a high density, consensus linkage map of barley (Langridge et al., 1995). In addition, molecular markers have been used to locate individual loci that control levels of (1-3,1-4)-beta-glucan and (1-3,1-4)-beta-glucanases (Han et al., 1995). These quantitative trait loci (QTLs) for (1-3,1-4)-beta-glucanase levels have been mapped on chromosomes 2H, 5H and 7H, and indicate that genes at these positions are important determinants of malt extract.

Instability of (1-3,1-4)-beta-glucanases at elevated temperatures

Problems encountered with the incomplete hydrolysis of (1-3,1-4)-beta-glucans in the brewery can be attributed, in part at least, to the instability of (1-3,1-4)-beta-glucanases at elevated temperatures. Thus, enzyme levels in green malt may be adequate to hydrolyse any residual (1-3,1-4)-beta-glucans extracted from the malt, but the activities of both isoenzyme EI and EII decrease precipitously at temperatures above 45-50°C (Doan and Fincher, 1992; Figure 2). As a result, isoenzyme EI is almost completely destroyed during kilning, and less than 40% of isoenzyme EII survives the kilning process (Loi et al., 1987). Furthermore, (1-3,1-4)-beta-glucanase isoenzyme EII in the kiln-dried malt is rapidly inactivated during simulated mashing at 55°C or 65°C (Loi et al., 1987). This rapid loss of activity at temperatures used during normal malting and brewing procedures results in a reduced capacity to degrade (1-3,1-4)-beta-glucans that are extracted from the malt. This, in turn, gives rise to the filtration difficulties and the contributions to beer haze formation that are commonly associated with high (1-3,1-4)-beta-glucan levels.

Engineering barley (1-3,1-4)-beta-glucanases

Several approaches might be taken in attempts to enhance (1-3,1-4)-beta-glucanase activity. Firstly, if (1-3,1-4)-beta-glucanase activity in green malt could be dramatically increased, the absolute amount of the enzyme that survived the kilning and mashing protocols would also be expected to increase. Enhanced levels of (1-3,1-4)-beta-glucanases might be achieved by inserting multiple

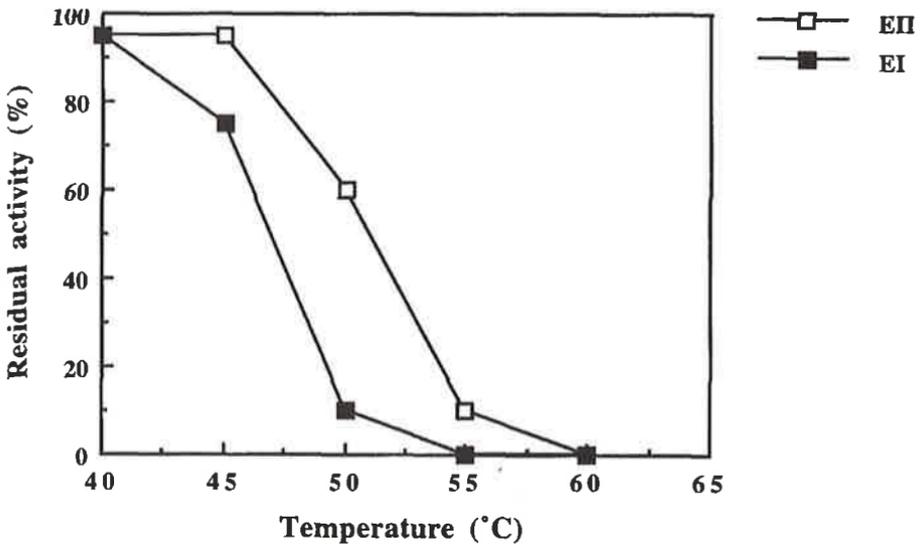


Figure 2. Thermostability of barley (1-3,1-4)-beta-glucanase isoenzymes EI and EII. Residual exzyme activity was measured following incubation for 15 minutes at temperatures in the range 40°C to 60°C.

copies of the two genes into the barley genome. Alternatively, a barley (1-3, 1-4)-beta-glucanase gene driven by a more powerful promoter could be constructed. A barley alpha-amylase promoter could well direct increased transcription of a barley (1-3, 1-4)-beta-glucanase gene, and expression would be expected to be physiologically appropriate with respect to tissue location and timing. Provided the inserted genes were expressed in a spatially and temporally correct way, (1-3,1-4)-beta-glucanase levels in the germinated grain should increase. Both these approaches are underway (J.V. Jacobsen, R. Kalla, R. Singh and G.B. Fincher, unpublished). A potential limitation to this approach is that greatly increased transcription and translation of (1-3,1-4)-beta-glucanase genes would lead to a metabolic imbalance in aleurone and/or scutellar cells, such that energy or amino acids available for the synthesis of other key enzymes would become limiting. Thus, high levels of (1-3,1-4)-beta-glucanase might be achieved at the expense of other enzymes and lead to other problems, such as reduced levels of alpha-amylase, that would adversely affect malt extract or brewing performance.

Another possibility that would avoid the need for increased expression of (1-3, 1-4)-beta-glucanase genes, would be to engineer increased thermostability into the enzyme itself without any manipulation of expression levels of the corresponding genes. Similar levels of (1-3, 1-4)-beta-glucanases would be synthesized, and there would be no attendant perturbation of metabolic balances in the germinated grain, but a higher proportion of the enzyme would survive the kilning and mashing procedures. The practicalities of such an approach are summarized in the sections below.

Finally, it might be possible to find naturally occurring thermostable (1-3, 1-

4)-beta-glucanases from other sources. Indeed, (1-3,1-4)-beta-glucanases from the bacterium *Bacillus* spp. are relatively stable, and a number of thermostable enzymes have been engineered through the construction of hybrid genes (Olsen et al., 1991). Similarly, Aspegren et al. (1995) have transformed barley with a heat-stable beta-glucanase from *Trichoderma reesei*.

Requirements for protein engineering

The underlying imperative in engineering increased thermostability into any protein is to generate amino acid changes that will lead to enhanced intramolecular bonding within the three-dimensional conformation of the protein, such that more heat energy is necessary to disrupt or denature that stabilized conformation. The amino acid changes must be effected at the DNA level, either by random or site-specific mutagenesis, and it must then be possible to express the mutant DNA for the rapid evaluation of any changes in thermostability that have been achieved.

Random mutagenesis. In this procedure, DNA encoding a particular protein is subjected to non-specific or random changes in individual nucleotides that can result in amino acid changes in the expressed protein. The mutations can be generated *in vitro* by the polymerase chain reaction (PCR), in which random nucleotide substitutions are produced as a result of errors in DNA replication by the DNA polymerase. The random mutants so generated must subsequently be expressed, preferably in a micro-organism that is easily manipulated in the laboratory, and a rapid method for measuring stability of the expressed protein must also be available.

Okada et al. (1995) have used random mutagenesis to generate barley beta-amylase mutants with increased thermostability. Mutant DNA was produced by PCR and crude enzyme extracts from *E. coli* colonies that were expressing the enzyme were assayed for stability. The thermostabilities of the mutant beta-amylases were measured as the temperature at which 50% activity is lost after incubation at that temperature for 30 minutes. The mutation rate during the PCR reaction was 0.12%, and two mutants with increased thermostabilities of 1.0°C and 2.3°C were selected from 10,000 colonies screened (Okada et al., 1995).

Site-directed mutagenesis. This procedure relies on the use of specific oligonucleotide primers to generate specific changes in a nucleotide sequence. Thus, an oligonucleotide of say 30 nucleotides, incorporating a single nucleotide mismatch, is synthesized (Figure 3). The mutagenic oligonucleotide is used as a primer from which a DNA polymerase will synthesize the remainder of the gene or cDNA. The mutant DNA, with the single nucleotide substitution, is cloned and expressed, and the thermostability of the protein product is measured.

In contrast to random mutagenesis, protein engineering through rational, site-specific changes in particular amino acids requires a detailed knowledge of the molecular environment of individual amino acids in the overall three-dimensional structure of the protein. Thus, site-directed mutagenesis depends on the availability of the complete amino acid sequence of the protein and a

precise knowledge of its three-dimensional conformation. The latter is usually provided by X-ray crystallography and, given the relatively small number of plant proteins that have been successfully crystallized, this approach to increased thermostability through rational protein design is seldom possible. However, the three-dimensional structure of the barley (1-3,1-4)-beta-glucanase isoenzyme EII has now been solved to 2.3-Å resolution by X-ray crystallography (Varghese et al., 1994). The enzyme has an (alpha/beta)₈ barrel structure and the catalytic

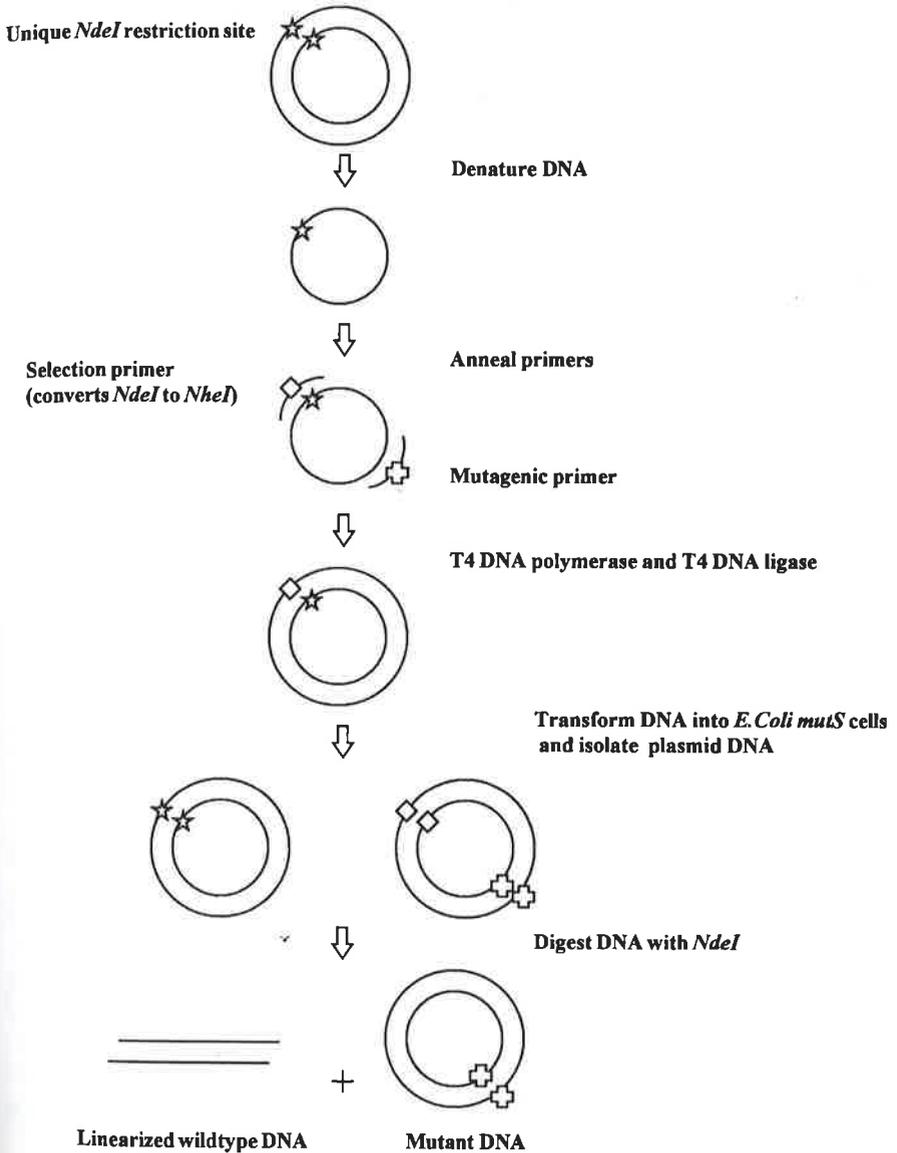


Figure 3. Unique Site Elimination method for site-directed mutagenesis

amino acids have been located within a deep substrate-binding groove that extends across one face of the enzyme (Varghese et al., 1994). Full-length cDNAs encoding the enzyme are also available (Slakeski et al., 1990), together with *E. coli* and insect cell expression systems (Doan and Fincher, 1992; Chen et al., 1995).

Target sites for mutagenesis

Armed with these expression systems and detailed information on the three-dimensional structure of the enzyme, it is now possible to design and test changes in specific amino acids that could lead to increased thermostability of barley (1–3,1–4)-beta-glucanases. It should be emphasised, however, that while the effects of amino acid substitutions can be predicted to some extent, conformational and other changes can complicate the prediction. In practical terms this means that, although the thermodynamic consequences of amino acid substitutions can be investigated by computer-based molecular modelling programs, the thermostabilities of mutants must ultimately be tested at the enzyme level.

The selection of specific site-directed mutations that might increase the thermostability of proteins is based on existing knowledge of molecular interactions that stabilize proteins (Vriend and Eijsink, 1993). However, clear rules have not yet emerged and there remains an element of trial-and-error in the overall exercise. Hydrophobic interactions in the protein core are major stabilizing factors, and attempts have been made to fill internal cavities with amino acids that carry larger hydrophobic side chains. The incorporation of charged, helix-capping residues to neutralize alpha-helix dipoles has confirmed the importance of interactions between charged residues and the helix dipole (Vriend and Eijsink, 1993). The generation of salt bridges can sometimes improve protein stability, as can mutations aimed at completing unsatisfied hydrogen-bond donors and acceptors in the interior regions of the protein. The incorporation of disulphide bridges, which might be expected to yield large increases in stability, has met with only limited success (Vriend and Eijsink, 1993). Site-specific substitutions of Arg for Lys residues have also been shown to impart significant increases in thermostability, probably as a consequence of the increased hydrogen-bonding potential of the guanidinium group of Arg residues and because the Arg sidechain reaches out further into the solvent (Mrabet et al., 1992).

Finally, mutations that increase the rigidity of specific regions of a protein, and thereby reduce the entropy increase during unfolding, have resulted in significant increases in stability (Matthews et al., 1987). Gly–Ala mutations, particularly in alpha-helices, and the incorporation of Pro residues can have dramatic stabilizing effects (Vriend and Eijsink, 1993). Because unfolding processes involve solvent-exposed regions of the protein, mutations at surface-located positions have relatively large effects on stability. Furthermore, crucial mutations are often clustered in the region of the protein which unfolds first during heating (Eijsink et al., 1995).

In the case of the barley (1–3,1–4)-beta-glucanase, several of these types of

mutations have been evaluated, and those which reduce the entropy increase of unfolding in specific areas of the protein have been shown to increase stability (unpublished data). Furthermore, the addition of an N-glycosylation site in (1-3,1-4)-beta-glucanase isoenzyme EI through the mutation (Thr¹⁹⁰-Ala-Ser) (Asn¹⁹⁰-Ala-Ser) caused a small but significant increase in thermostability (Doan and Fincher, 1992).

Summary

Barley (1-3,1-4)-beta-glucanase levels in green malt are correlated with malt extract values and also contribute to malt quality through their ability to hydrolyse troublesome (1-3, 1-4)-beta-glucans that are extracted from starchy endosperm cell walls during the mashing procedure. However, in kiln-dried malt (1-3,1-4)-beta-glucanase levels can be severely reduced because of the inherent instability of the enzymes at temperatures above 45-50°C. If more (1-3, 1-4)-beta-glucanase activity were to survive the kilning and mashing steps, the enzyme could hydrolyse residual cell wall (1-3,1-4)-beta-glucans during brewing and overcome processing and quality difficulties imposed by these polysaccharides. The availability of cDNAs and genes for the barley (1-3,1-4)-beta-glucanases, together with the complete amino acid sequences of the enzymes and the X-ray crystal structure for isoenzyme EII, provide opportunities for engineering increased thermostability into the enzymes through rational protein design. Site-specific mutagenesis has been used to successfully produce mutant forms of the enzyme that have a single amino acid substitution and dramatically enhanced thermostability.

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Developments in the brewing fermentation

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Brewing is a multidisciplinary science based on the chemistry, biochemistry and microbiology of the raw materials used, and the processing technologies of malting, mashing and fermentation. Fermentation technology is one of the least understood parts of the brewing process and is often referred to as the "black box." How far our understanding of fermentation has come can be seen by examining the 1839 report by von Liebig and Wöhler on alcohol production (said to be a purely chemical process in which dead decaying yeast participate), which described small animals (yeasts) with a snout who sipped sugar, and who metabolized and excreted ethanol from the gut in spurts and carbonic acid from urinary organs. The chemical view of alcohol production persisted with some scientists, in spite of the reports by Pasteur and others, until about 1883, when Hansen at the Carlsberg Laboratory described and perfected pure culture yeast technologies that, for lager brewing, soon became accepted technology worldwide.

The last one hundred years has seen an intense period of study to improve and understand brewing fermentation technology, and the brewing literature describes a number of advances in:

- identification of bacteria/wild yeast responsible for wort/beer spoilage
- characterization of the physiology of the wort/beer spoilage process
- characterization of brewing yeasts, leading to knowledge about:
 - sugar uptake and fermentation
 - flocculation of yeast at end-fermentation
 - ethanol tolerance of yeasts
 - osmotolerance of yeasts
 - influence of microbial end products on flavor
 - yeast nutrition/yeast growth
 - requirements for oxygen (sterols and unsaturated fatty acids)
 - nitrogenous nutrient requirements
 - vitamin requirements
 - pure culture propagation technology
 - genetic stability
 - genetic manipulation

Reports on these topics continue to fill the brewing literature. However, I wish to address some of the newer developments – many still not totally off the lab bench –

that are beginning to lead breweries away from brewing methods that are steeped in tradition and are now almost totally explained by the science of the last 5–10 decades.

Continuous fermentation

One of the many interesting innovations has been with respect to continuous fermentation. A desire to move from a batch process to one that could reduce capital investment, labor costs and cost of product were not successful in the past – probably due to the increased technical knowledge requirements, and the increased requirements of trouble-free equipment and lab monitoring. Moreover, the more complex and demanding technology required has been said to create more problems than it solves (Portno, 1978). In fact, it reduces brewery flexibility in operations – a phenomenon dictated by multi-brand marketing philosophies and seasonal beer consumption patterns. The failure, however, of continuous fermentation in engineered vessels, such as tower fermentors that made use of a “plug flow” of yeast through which sterile aerated wort passed and was converted to beer, was not lost on brewing scientists. Today’s innovation is a variation on the same theme and does allow for some flexibility with brands through changeover of wort streams or production of a “base beer.” This process, called “immobilized yeast technology,” has been worked on for many years, but since 1992 has been in limited commercial production (Saipan Island, Japan, by the Kirin Brewing Company), and warrants some discussion due to the two-fold productivity increase claimed, and the fact that the flavor attributes of such beer are conventional (Onaka et al., 1985).

Tower fermentors depended on the flocculation of the cells to keep the yeast “plug” intact, but when yeast is immobilized it is entrapped at a high cell concentration in a matrix of calcium alginate or carrageenan, for example. Small bead size is imperative due to the fact that CO₂ released during fermentation can disrupt larger beads. Mass transfer of sugar into the beads and carbon dioxide out is crucial due to the high yeast cell numbers in the gel. Immobilization normally relies on the use of a bioreactor into which the beads are packed. Wort flows through the bed. Some yeast growth (and cell sloughing) is seen as wort is converted to beer. The Kirin system has been diagrammed and described (Onaka et al., 1985; Gopal and Hammond, 1993).

In the first vessel, which is agitated and aerated, the yeasts quickly take up free amino nitrogen from the wort. Uptake of amino acids is difficult in packed bed (immobilized yeast) bioreactors (Masschelein, 1990). Yeast is centrifuged from the wort, which is then passed through a carbonator/deaerator and then to the immobilized yeast column, where beer is made while the partially aerated wort at 8°C passes through the bioreactor. In this vessel, sugar is fermented to alcohol and CO₂. Primary fermentation is complete within 24 hours, but lagering time was found to be extensive, until an immobilized maturation stage was added. This led to a beer process that produced palatable product in 24–72 hours. The Kirin scientists deaerated the wort to minimize the diacetyl levels in the beer. The Kirin system allows uptake and a nearly normal flavor and aroma profile

(Onaka et al., 1985).

Finnish workers have developed and extensively used immobilization for rapid maturation of green beer (Pajunen et al., 1987). The process involves centrifugation to remove yeast, an extensive heat process (90–95°C for 7 minutes) to convert diacetyl precursors to diacetyl (and to pasteurize), and passage through a DEAE cellulose-immobilized yeast column. In 2–3 hours, diacetyl is reduced and beer is ready for final filtration and packaging.

The major impediments to full-scale operation appear to include bacterial contamination of the bioreactor bed (requiring shutdown, sanitation and establishment of the catalytic potential of the new immobilized yeast), the proper choice of bioreactor size and flow rate to allow variable throughput during the year, and flavor matching of the new beers with traditionally produced brands.

High-gravity/Very-high-gravity brewing

Traditional brewing is carried out with worts of 11–12° Plato (P) producing beers of 4.5–5% v/v ethanol. When worts are increased from 11 or 12 to approximately 15°P (high-gravity brewing), then fermented and aged in this more concentrated form, the brewer is able to increase plant efficiencies and capacity, reduce labor and energy inputs, and lower capital costs. Beer flavor and stability improve and a slightly higher yield of alcohol per degree Plato of extract is obtained. The beer is then diluted in fermentation or after aging, with specially treated water, to a specified alcohol content prior to bottling. This amount of water would traditionally have been added during mashing. However, at gravities near or above 15°P, a number of breweries have experienced fermentation difficulties called *stuck* or *sluggish* fermentations, where the rate of fermentation became protracted or stopped – leaving residual sugar in the beer. As a result, a 15–16° Plato maximum limit in wort original gravity was self-imposed by most brewers. This was blamed on the alcohol intolerance of brewers' yeasts and led to the dogma that brewers' yeasts were only tolerant to 7–8% alcohol.

Over the last few years, we have described what we now call very-high-gravity (VHG) brewing, where worts up to 32°P in lab experiments (Casey et al., 1984) and up to 24°P in pilot plant work have been fermented, aged and tasted (McCaig et al., 1992). Further improvements in economics are evident in VHG brewing, and the beers produced appear to exhibit even better flavor, and both biological and physical stability. They are lighter, sweeter, and smoother than regularly produced lagers, and bacterial contaminants in the process are suppressed. The work done by our lab provided confidence and incentives to brewers to raise the OG of beer wort to levels higher than 16° Plato. Beer is now being produced commercially under VHG conditions (18–19° Plato), and most major brewers not yet into VHG fermentation are examining the possible implementation of this technology.

In fuel alcohol work using wheat mashes, alcohol concentrations of 23.8% v/v ethanol can be reached (Thomas et al., 1993). This proves that the genetic tolerance of commercial yeasts is well over 20% v/v, and that fermentations can

proceed at high initial substrate concentrations in a batch mode without using specially selected yeasts or preconditioning. Nutrition is the key to VHG technology (Ingledew, 1993, 1995), and the technology is useful for brewing, enology, and industrial alcohol production.

Genetic engineering

For many years, it has been recognized that alteration in certain biochemical pathways mediated by yeast would be beneficial to the brewing process. Now that genetic engineering is possible and that specific genes might be movable from one species (or genus) to another in a safe and controllable fashion, without alteration of desirable metabolic properties, brewing scientists have begun to create microbes with such abilities. Desirable characteristics identified include the ability of yeast to degrade dextrans, the degradation of beta-glucans, rapid diacetyl reduction, predictable flocculation, and resistance to bacterial (and wild yeast) contamination.

Dextrans are present in beer wort at 20–25% of total carbohydrate and make a major contribution only to mouthfeel and body of the product. Normal brewer's yeasts are unable to utilize dextrans – only low-molecular-weight sugars such as glucose, sucrose, fructose, maltose, and maltotriose are assimilated. Scientists at the BFRI in England have now produced the first beer made with gene technology (Nutfield Iyte), which was created by transferring the *STA2* gene (coding for glucoamylase activity) from a baker's yeast strain to a lager yeast (Coghlan, 1995). Glucoamylase degrades linear and branched chains of dextrin to glucose and thus aids the yeast to utilize the dextrin levels created by the malt amylase enzymes during mashing. Higher alcohol levels result, but, if diluted, a low-alcohol, low-calorie beer can be prepared. BFRI has also taken a leading role on behalf of its member companies in the UK, in cooperation with the Ministry of Agriculture, Fisheries and Food, to submit suitable yeast strains for government approval. These strains and some described below hold promise for improving the efficiency of beer production and the quality of the product.

Brewers have always been concerned about beta-glucan content in barleys and the effect that beta-glucan has on wort runoff and its influence in haze and viscosity of worts. Beta-glucanase activity has been introduced into yeast both via marked plasmids as well as through cotransformation, where a stable integration of the beta-glucanase gene into yeast chromosomal DNA takes place. Expression of the protein leads to reduction of beta-glucan and elimination of hazes and gels (Enari, 1995). The transformed yeast is stable and, in this latter case, contains no marker genes or extraneous DNA. Such modified yeast, however, would have no influence in reduction of viscosity prior to lauter tun or mash-filter runoff.

Yeast-produced diacetyl, a potent butterscotch flavor note that is detrimental to beer, is a major factor in the lagering process and in the length of time required for aging of beer prior to filtration and packaging. A genetic approach can and has been made with respect to reduction in diacetyl. Japanese scientists from

Kirin Brewery have taken a bacterial gene encoding for alpha-acetolactate decarboxylase and incorporated it into brewing yeast, where it converts alpha-acetolactate to acetoin, eliminating a spontaneous non-enzymatic conversion of alpha-acetolactate to diacetyl. In normal brewing, diacetyl is slowly reduced enzymatically during lagering – a process that takes nearly a month.

It is not likely that antibiotics will ever be broadly used in brewing (although heat-sensitive antibiotics may attain extensive use in the distillation industry, where penicillin and virginiamycin are already used worldwide to control contaminant lactic acid bacteria). However, the use of proteinaceous molecules called bacteriocins – such as nisin produced by *Streptococcus lactis* – have widespread approval in foods in most countries except Canada. Nisin is active against Gram-positive lactic acid bacteria, *Bacillus* spp., *Pediococcus* spp., and *Clostridium* spp. It has been suggested that this gene be cloned into yeast, but little or no success has yet been reported. Similar proteins or glycoproteins produced by yeast (called zymocins) have received attention in recent years (Jacobson, 1985), and Lallemand Inc., a yeast company based in Canada, already sells a yeast with a killer factor for wine fermentations.

In recent years, the normal spectrum of bacteria capable of beer spoilage has broadened to include obligately anaerobic bacteria such as *Megaspaera* and *Pectinatus*, in addition to previously identified spoilage agents such as lactic acid bacteria, acetic acid bacteria, coliforms including *Obesumbacterium* (*Hafnia*), and *Zymomonas* spp. At times, the brewer requires a sensitive and highly specific test to quantify or identify the wide range of bacteria. Monoclonal and polyclonal antibodies may be the answer (Whiting et al., 1992; Gares et al., 1993; Whiting, 1995) and are under test for this purpose. Antibodies may also be useful to detect wild yeasts.

Other areas where genetic engineering may aid the brewer include control of flocculation, respiratory-deficient mutants or fermentation of pentose sugars. These have been discussed in detail by Gopal and Hammond (1993) and Stewart and Russell (1986).

The last decade has been an exciting time for research directed towards the brewing industry. It is somewhat ironic, then, that this industry has retrenched itself in large degree because of the recent recession. Many research departments have been closed or significantly reduced in size at the very time when agricultural biotechnologists are poised to provide definitive answers to century-old problems that this industry has experienced.

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North American brewing industry requirements

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This presentation will outline the current situation, examine some trends and speculate on the future of the North American brewing scene, with special reference to malting barley. Brewing is a mature industry with little or no prospect for growth. Numerous reasons have been advanced for this senescence, including, an aging population, excessive taxes, an unjustified association of the product with its irresponsible use and the idea that even modest beer consumption is somehow incompatible with a healthy lifestyle.

Beer and malt in North America

Table 1 gives some recent figures for malt usage and beer production. Only in Mexico does there seem to be any growth (3–4%). This information was taken from trade journals. According to unpublished reports, there is no reason to expect dramatic differences in total volume in 1995 or 1996 for Canada or the U.S.

In both Canada and the U.S., there is ample capacity for malt exports. It is these exports that will drive the development of the malting industry. All those involved are now paying heed to that market. The scope of this presentation is,

Table 1. Beer production, malt usage and malting capacity in North America

Beer Production ('000 hL)				
	<u>World Rank</u>	<u>1992</u>	<u>1993</u>	<u>1994</u>
U.S.	1	237,174	237,306	237,144
Mexico	7	42,168	43,794	45,168
Canada	11	21,570	22,999	22,991
Malt Usage in Breweries (tonnes)				
		<u>1992</u>	<u>1993</u>	<u>1994</u>
U.S.		2,189,411	2,187,517	2,224,489
Mexico				450,000
Canada		285,155	256,256	n/a
1995 Malting Capacity (tonnes)				
U.S.		2,771,229		
Canada		732,000		
Mexico		350,000		

however, limited to North America. Mexico grows about 395,000 tonnes of barley and imports both barley and malt.

In Canada, per capita beer consumption in the period 1982–1992 fell from 83.1 litres to 71.7 litres. Per capita consumption in the U.S. peaked at 23.8 U.S. gallons in 1981 and has declined to an estimated 21.5 gallons in 1994. These decreases have been offset by population gains to give the appearance of a static volume. Vigorous and occasionally vicious competition is generating enormous turmoil in both markets. There has been an explosive growth in the U.S. of mini, micro and regional brewers, i.e., companies who do not have national distribution. In Canada, numerous smaller breweries have opened for business. In both countries there have been as many failures as successes.

In the U.S. in 1994, there were 193 brewing companies whose volumes ranged from 88,500,000 bbls (Anheuser-Busch) to 65 bbls (Treaty Grounds Brewpub).

Effect of market on malt usage

Large companies seek to maintain existing brands whose image has been entrenched in the public mind by prodigious marketing efforts. Changes in the product (malt : adjunct ratio) are unlikely. Most of the major brand names were established several years ago, and are now associated with Light, Dry, Ice and Draft versions.

Small brewers are more adventurous. They favour all malt brews and exploit the whole gamut of malt products. Because their volume is increasing, they are not so inhibited by malt costs as their larger counterparts, most of whom budget on a cost per unit of volume basis.

Malting and brewing may be reverting to craft industries, fostering local loyalty through limited distribution. The success of this marketing strategy has not been lost on national brewers.

Malting barley characteristics

In this intensely competitive business, any edge will be promoted; any weakness will be exploited. This attitude will touch breeders and producers through vertical integration, private breeding and contract growing.

“Barley must do it all.” These are some of the features considered desirable in malting barley:

- genetic resistance to pathogens, predators and parasites
- ready and rapid modification
- low protein, low soluble protein
- trouble free wort formation, separation and fermentation
- flavour.

However, note the following:

- Chemical residues from pesticides, herbicides or desiccants are unacceptable. The issue here is not safety but perception. Beer is a tempting target to the politically correct.

- Maltsters expect barley to germinate at 100% two weeks after harvest and maintain this vigour for the next eighteen months, no matter what the storage conditions.
- Protein is expected to provide enzymes, yeast food and foam. Too much soluble protein, however, gives beer a satiating taste, inflates extracts and makes colour and flavour control in the kiln more difficult.
- Friability is related to cell wall degradation, as is wort viscosity. Excessive levels of glucans (and pentosans) hamper wort filtration and can be devastating both to diatomaceous earth and sterile filters. Wort fermentability is controlled by mash temperature in the presence of sufficient DP.
- The greatest frustration for any breeder must be to see a potential variety overcome all hurdles until his creation fails in the inner sanctum of a brewery taste panel room.

Malt brews do not allow malt faults to be diluted or blended away by adjunct. It is worthy of note that some craft beers in the United Kingdom have acquired a mystique associated with a barley variety.

These factors are less critical, but may well serve to give a variety an edge over its rivals.

Low DMS	– low soluble protein
Low anthocyanogen	– physiology ?
Low linoleic acid	– physiology ?
Foam formation	– mixed gas, hop acids

Note:

- DMS (Dimethyl Sulfide) removal is energy-intensive. Any trait that diminishes the formation of DMS and especially its precursor DMSP offers a potential energy savings. Low anthocyanogen barleys malt and brew well, but have performed poorly in the field. Linoleic acid is connected with the oxidation off-flavour in beer, but like anthocyanogens its precise role in barley physiology is unclear.
- Foam formation is irreversible. Most brewing methods and equipment now seek to limit turbulence. Collapsed foam forms “bits” in beer. Hop beta-acids and mixed gas dispense both contribute to lacing and cling, key features of foam.

The future

In Figure 1, the increase in area in Canada sown to U.S. bred six-rowed white aleurone varieties is clearly shown, as is the ongoing decrease in six-rowed blue aleurone types. To what extent this is due to the *Fusarium* outbreak in the Red River Valley states is difficult to judge. Many U.S. maltsters, some of whom already own Canadian malting companies, have come to rely on Canada as a source for barley.

Spurred by export orders, the area under two-rowed malting barleys has increased, while that sown to feed types has decreased, even though the largest use for barley in Canada is still animal rations. Hulless types offer obvious

advantages for livestock feed. Interest in their potential as high extract malts in a mash filter, or as an extract supplement to regular malts, is just emerging.

No dramatic changes to either the area or types of barley grown in the U.S. are clearly evident. The movement of the corn and soybean economy to the north and west must be disquieting to promoters of U.S. barley.

As the genome map becomes better understood and exploited, more varieties can be expected. New techniques to screen and test more early generation lines are overdue.

Imagination, inoculation, innovation and insertion are all vital to our futures, but let no one forget:

“Physiology comes first”

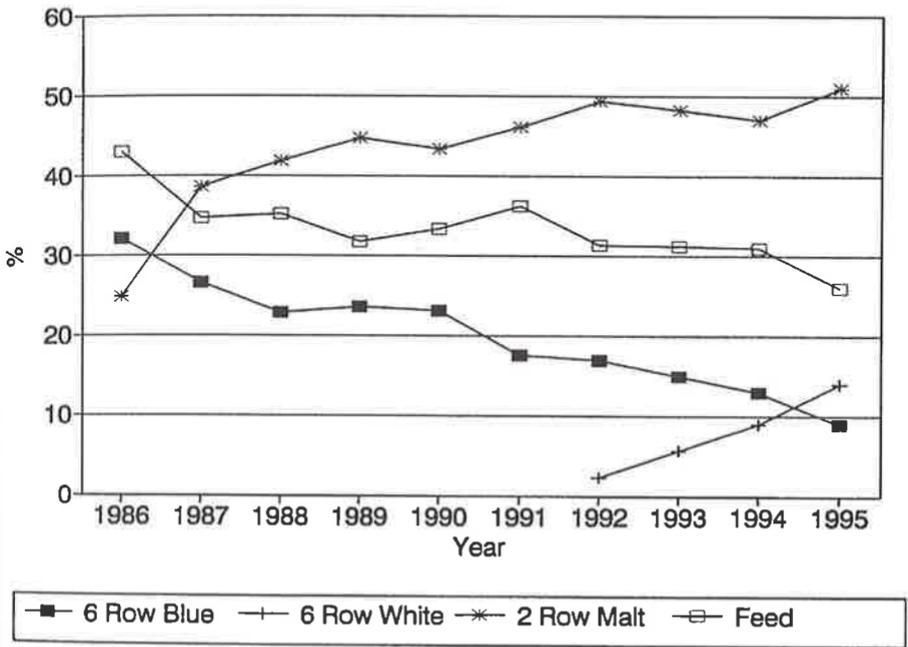


Figure 1: Barley type – Percentage of area

Brewing industry requirements for malting barley in Japan

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History of the brewing industry and the breeding of malting barley in Japan

Beer is not domestic to Japan. Beer brewing started in the 1870s just after the introduction of the "open door" policy by the new government. The government actively introduced Western industries to modernize the country, and thus the brewing industry grew rapidly. Breweries were mostly built by Sake brewers, the traditional rice wine makers. The government itself also built up and managed a brewery in the northern island of Hokkaido for the development of the industry. Selection of suitable barley varieties from the Western varieties introduced to Japan was carried out mainly by the national laboratories.

As a result, Chevalier was selected for cultivation in Hokkaido, where the conditions for cultivation are almost identical to Europe. On the main land of Honshu and the southern island, barley is cultivated as a second crop after the rice harvest in the winter season. Golden Melon (the original name appears to be Golden Soap) was selected in 1890, but several improvements were required because of the difference in cultivation conditions. The brewing industry expanded until 1939, but the per capita consumption of beer was still very low, at 3 litres per year. After the Second World War, another large wave of Westernization started, and in line with the remarkable economic growth, beer consumption increased until the 1970s, when the per capita consumption reached 35 litres per year. Barley breeding trials also resumed and new technologies were introduced. Until the late 1950s, the four major brewing companies reactivated their own breeding stations, which had been unified during the war. Their researchers carried out breeding experiments based on the needs of the users. Two national research stations specifically for the breeding of malting barley cooperated with them, but their interests were more agricultural. Good communication and cooperation of these two kinds of research stations are said to account for the remarkable successes achieved within a short period of time.

The major target for the improvement of malting barleys, mostly derived from the Golden Melon variety cultivated in Honshu and the south, was to shorten the height of the plants and bring about early maturity. Because barley is sown just after the rice harvest in autumn, it is harvested between late May and early June, which is just before the start of the rainy season in those areas. Tall barley

plants tend to be flattened by the rain and become moldy in the warm, humid weather of the season. This sometimes caused the beer to gush, due to the hydrophobic cyclic peptides produced in the contaminated barley (Kitabatake and Amaha, 1977). Varieties with a short culm height were targeted as a solution to this problem, as were early maturing varieties. Even one or two days earlier maturity would be very effective in preventing damage by the rain. As a result of thirty years of collaborative research, the height of the new varieties was reduced to three-quarters of the height of Golden Melon, and the maturing time was reduced by almost ten days, as shown in Figures 1 and 2, respectively. Values shown in the figures are weighted averages of the cultivated barley varieties. In addition to these remarkable achievements, a variety resistant to barley yellow mosaic virus and a variety suitable for snowy regions were bred, and the reason for sterility in Hokkaido was determined to be due to the infection by the stripe mosaic virus.

Economic growth in the 1960s promoted a remarkable development of the beer market, but also promoted a drastic reduction in the number of farmers. As can be seen in Figure 3, the percentage of domestic malting barley used by the Japanese brewing industry decreased dramatically around 1970. The brewing

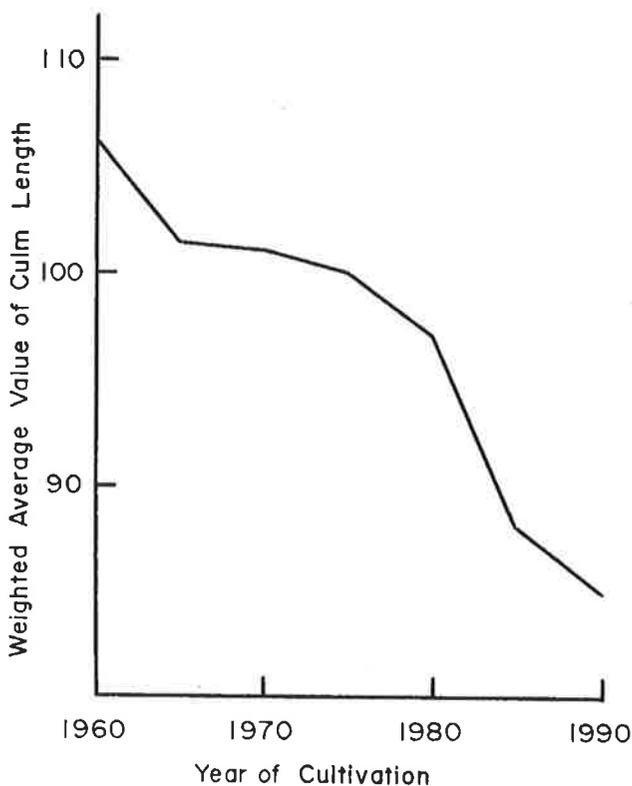


Figure 1. Improvement in culm length

industry was able to import barley and malt that was cheaper and of higher quality, and this shifted the breeding target to producing malting barley of higher quality. Each breeding station was equipped with a multi-sample pilot malting plant and an auto-analyzer system for the rapid evaluation of the quality of numerous barley samples. Due to the good collaboration between the stations in Japan, a unified evaluation system was used, and the average extract yield of the newly bred varieties was increased by 5% to the present values.

Brewing industry requirements for malting barley in Japan

Table 1 compares the characteristics of the brewing method and beer quality in Japan with those of North American. No liquid adjunct is used for mashing, which is the reason why a high diastatic power is required in the malt. Some malt samples produce wort that induces a stuck fermentation, which is caused by the early flocculation of yeast when the fermentation temperature is low, as in the case of traditional lager fermentation. Enormous efforts were made to determine the key substance in this type of malt, since at the time only low

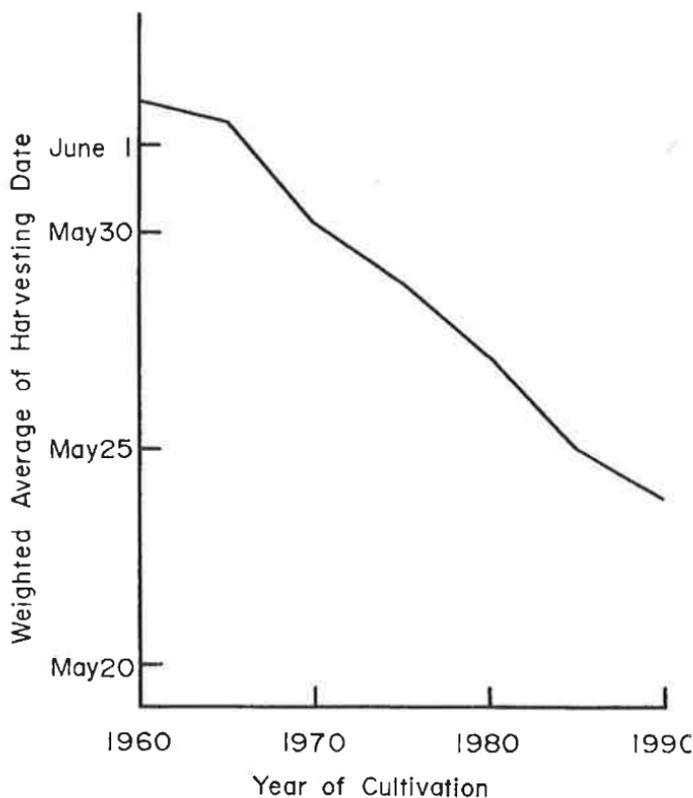


Figure 2. Improvement in harvesting date.

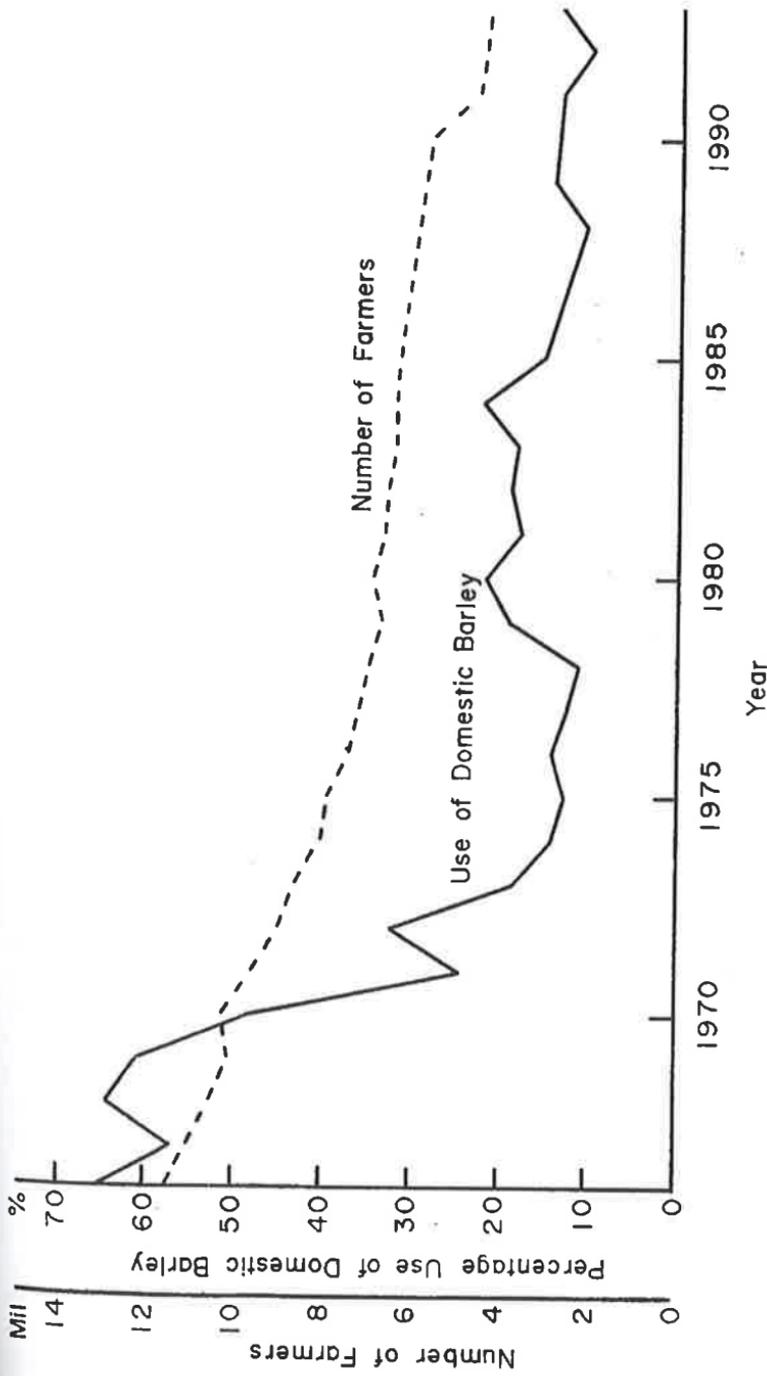


Figure 3. Changes in use of domestic barley for brewing, and number of farmers

quality domestic barley could be used due to the shortage of hard currency in Japan, but these efforts have been unsuccessful so far. The recent isolation of a flocculation gene specifically for brewer's yeast could be the key to solving the problem (Kobayashi, Hayashi and Sone, 1995). However, the only way to identify such malt at present is to carry out a small-scale fermentation test. The lower popularity of high gravity brewing means that good filterability aided by a low glucan content or high glucanase activity of the malting barley is required. The popularization of unpasteurized bottled or canned beer also requires a good filterability, because sharp filtration is necessary for aseptic filtration. Consumer sensitivity towards safety and natural foods means that we are unable to use additives such as gibberellic acid or enzymes. Therefore, brewers desire a malting barley with a naturally high diastatic power.

Table 1. Characteristics of the brewing method and beer quality in Japan.

- Use of solid (not liquid) adjuncts
 - Less additives, even before fermentation
 - Normal (not high) gravity brewing
 - Low temperature fermentation
 - More bottled or canned unpasteurized beer than pasteurized beer
-

However, the recent sharp increase in the yen rate has produced an increase in cheap imported beers from overseas in the beer market. For this reason, brewers have to reduce the production cost by the introduction of the rationalized brewing methods, which have already been adopted by overseas brewers. Therefore, it can be predicted that the brewers in Japan will make requests similar to the North American brewers. One recent example of producing a low priced beer is the use of a low malt concentration, less than 25%. Malt itself is not very expensive in Japan because most malts are imported; however, the tax rate of the low malt beer is nearly half that of common beers, which are brewed using malt as more than two-thirds of total grist and are taxed at a rate almost equivalent to half of the final price. It is possible to use high nitrogen malt for the production of the tax-saving beer, since most flavor compounds are produced during fermentation in proportion to the amount of amino acids consumed (Inoue and Kashiara, 1995). If such a malt with a low glucan content became available, it would also be welcomed by brewers in south Asian countries, the only part of the world where extensive growth of the beer market is shown. They have no domestic barley, but they face a shortage of hard currency to import malt. Therefore, they are trying to brew beer with less malt and more adjuncts.

Future targets for breeding malting barley

More than ten years have already passed since success in breeding an antocyanogen-free barley mutant, achieved by the Carlsberg Laboratory in Denmark, shocked the world. It seems that the mutant is not completely satisfactory and thus has not become widely used, but it has a great potential for

the production of a beer with eternal clarity.

Another possible target for increased beer quality will be a lipoxigenase-free malting barley. Using such a malt, a beer with long-lasting freshness could be produced. Lipoxigenase-free soy bean mutants are already available, so the barley mutants are expected to become available in the near future.

A high lysine peptide concentration is known to be effective in producing good head retention by forming a potential detergent with the hop bitter substances present in beer. If a high valine barley malt could be bred in addition to a high lysine barley, it would contribute to the reduction of the maturation period, because the length of maturation is predominantly determined by the concentration of the diacetyl precursor produced by yeast, and which is suppressed by the valine in wort. The mashing time could be shortened if the heat stability of barley beta-amylase could be improved. Beta-amylase is one of the most heat-sensitive saccharifying enzymes that produce fermentable sugars. The introduction of genetic engineering to breeding malting barley could result in several innovational improvements. For instance, if the key factor that induces premature flocculation of yeast during fermentation described above could be identified and controlled, a reproducible fermentation could be achieved and a constant quality of beer could be produced without any artificial control, which would also reduce the labor required.

Nowadays the brewers in Japan are using cheaper and higher quality imported malt and are using less than 15% of domestic malt. In addition, the beer market is nearly saturated, as in North America. Under these circumstances, the brewers are actively developing beers with new characteristics to the market, such as dry beer, bottled or canned draft beer, etc. I look forward to the launch of malting barley with new characteristics to support our efforts.

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Global production and markets for barley in the 21st century

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Background

In addressing the topic of global production and markets for barley in the 21st century, I am going to draw from a report entitled *CWB Grain Trade Forecast to 2004-05*. This forecast was completed in 1995 from detailed projections of wheat, coarse grain and barley production and demand by region/country. A base period of 1989 to 1993 was used and projections were made to the years 1999/2000 and 2004/05.

World grain production

In looking at world barley production into the 21st century, it is also worthwhile looking at production projections for wheat and other coarse grains. This is relevant because other crops compete for available cropland. The Canadian Wheat Board (CWB) estimates of future world grain production are listed in Table 1. Wheat is forecast to increase from an average of 559 million tonnes in the base period to 593 million tonnes and 625 million tonnes respectively, five and ten years hence. Coarse grain production is expected to rise to 842 million tonnes by 2000, below the record 862 million tonnes set in 1992, but well above the base-period average of 813 million tonnes. By 2005, production is expected to exceed 910 million tonnes. Barley production is

Table 1. World production of major grains (million tonnes)

	1989-93 Average	Projections	
	Base Period	1999/2000	2004/05
All wheat	559	593	625
Durum	29	27	29
Coarse grains	813	842	910
Barley	169	177	186
Total	1,371	1,435	1,535

Totals may not add due to rounding.

expected to rise from 169 million tonnes in the base period to 186 million tonnes in 2004/05.

The forecast assumes that there are few significant new production technologies on the horizon that might sharply raise grain yields beyond existing trends.

CWB trade forecast for wheat and coarse grains

Wheat trade is expected to rise from 104 million tonnes in the base period to about 125 million tonnes by 2005, with significant import growth for Latin America, Asia Pacific, the Middle East and Africa.

World coarse grain trade is expected to decline from the base period average of 93 million tonnes to around 85 million tonnes by the year 2000. This is well below the 100+ million tonnes levels seen between 1980 and 1990. Looking beyond the year 2000, coarse grain trade is expected to trend higher, reaching 98 million tonnes by 2005.

Trade forecasts for wheat and coarse grain (including barley) imply that major exporters will have some difficulty in supplying all of the market demand. The world's major grain exporters have, in most cases, highly developed economies with extensive livestock and food-processing sectors that supply both domestic and export markets. Because of these activities, the major exporters are likely to find that their domestic use of feed grains will continue to expand. This expansion will provide active competition with the export market.

The coarse grain trade forecast of 98 million tonnes in 2005 assumes that exporters will significantly expand production and exports to meet import demand. Projected exports for all major competitors leave a considerable quantity to be supplied by the so-called minor exporters. Specifically, countries other than the five major exporters are expected to generate coarse grain exports in the five-year and ten-year forecast periods of 15 million tonnes and 20 million tonnes, of which China is projected to generate 4 million tonnes to 6 million tonnes.

CWB trade forecast for barley

Total barley

World barley trade is projected to fall significantly, from 18.5 million tonnes in the base period to 16.5 million tonnes in 2000, before recovering to 17.6 million tonnes in 2005. With trade at this level, barley should comprise just under 18 per cent of the projected world coarse grain trade of 98 million tonnes in 2005. Potential barley import demand in 2005 would be closer to 20 million tonnes, based on past trade patterns, but limited supply in the major exporting countries and relative prices for feed barley and corn are expected to limit trade to 17–18 million tonnes, as listed in Table 2.

Various developments in each of the four major exporting countries will tend to restrict exports during the forecast period:

- **In Australia**, a rapidly expanding beef industry is expected to create substantial

annual increases in feed grain consumption, about half of which will be barley. Since 1990, feedlots have been expanding at a rate of roughly 20 per cent per annum, resulting in total feed grain consumption of over 6 million tonnes in 1995/96. Feed grain consumption is expected to approach 10 million tonnes well before the end of the 10-year forecast period. Given a relatively stable barley area and modest increases in yields, barley exports over the next decade are not expected to exceed current levels of 2 to 3 million tonnes.

- In **Canada**, increases in rail transportation costs and expanding domestic feed barley use will likely limit future barley exports. Strong per-acre returns from malting barley and a feed barley export program limited to unsubsidized markets suggest exports of up to 3 million tonnes.

Canadian barley area is expected to stabilize at roughly 10 million acres and production about 12 million tonnes. In view of competition from other crops for area in western Canada, a barley area of more than 10 million acres would seem unlikely. Therefore exports higher than 3 million tonnes would only be possible through higher-than-projected yields.

Bulk malting barley exports are projected at 1.4 million tonnes. Feed barley exports to Japan, the U.S., and several other non-subsidized markets are expected to make up the remaining 1.6 million tonnes. Projections for Canadian malting barley exports may be a little conservative. Current malting barley exports are above the projected levels, mainly because of record malting barley exports to the U.S. in the past two years (assisted by some disease problems with the U.S. crop), and strong demand from Latin America and China.

- In the **EU**, CAP reform has lowered grain prices, thereby contributing to a lower barley area and slightly higher feed barley use. Exports have already dropped significantly from the peak of 9.9 million tonnes in 1991/92 and are expected to be about 7 million tonnes by 2005.
- In the **U.S.**, barley area is in long-term decline. In 1995, it reached the lowest level in several decades, at 6.4 million acres – less than half what it was in

Table 2. World barley imports by region (thousand tonnes)

	1989–93 Average	—Projections—	
	Base Period	1999/2000	2004/05
Europe	1,448	650	670
Former USSR ¹	4,940	1,130	1,010
Middle East	6,469	7,440	8,440
Africa	1,275	1,570	1,420
Asia Pacific	2,757	4,050	4,290
Latin America	580	790	930
Others/Unspecified	<u>1,023</u>	<u>860</u>	<u>860</u>
World Total	18,492	16,490	17,620

¹ Includes trade between members of the former USSR in all periods. Totals may not add due to rounding.

1960 (13.8 million acres). In recent years, the provisions of the U.S. farm program have been the main factor contributing to barley's decline as a cropping option. Proposed changes to the 1995 farm bill are unlikely to increase the U.S. barley area in the future. Nevertheless, the fact that potential demand from the world market for barley is higher than is likely to be supplied does suggest strong world barley prices versus corn. This should encourage increased U.S. barley exports, which are projected to rise to about 2.5 million tonnes by 2005.

Regional import demand highlights

European barley imports are forecast to drop from 1.4 million tonnes in the base period to 670,000 tonnes by 2005. The drop in imports between the base years and the five/ten-year projection reflects a major decline in imports by Poland, Romania and other East European countries. In Western Europe, particularly the EU, it is expected that there will be only small import demand for high quality malting barley.

Imports by the **former USSR** have fallen to extremely low levels in recent years. Imports are likely to remain low, projected at roughly 1.0 million tonnes in 2005, down from 4.9 million tonnes in the base period. Most of the remaining imports will be supplied from within the region.

Saudi Arabia continues to dominate **Middle East** and world imports, with import volume increasing as domestic grain production declines. Barley imports by some other markets (e.g., Iran, Bahrain, Jordan) will be constrained by the available export supply. In total, the Middle East is expected to account for nearly half of world barley imports by 2005.

Imports of barley by sub-Saharan **Africa** will be limited to small quantities of malting barley, while North Africa will increase total imports to 1.4–1.6 million tonnes from 1.3 million tonnes in the base period. Imports by Algeria and Morocco will not reach their potential due to supply constraints.

Asia Pacific barley imports should grow substantially (from 2.8 million tonnes to 4.3 million tonnes) due to liberalized imports of feed barley and growing demand for malting barley. Chinese barley imports are projected to increase to 1.5 million tonnes by 2005 from about 700,000 tonnes during the base period. If the recent growth in Chinese malting barley imports continues at the same pace, this import projection may even be conservative. Japanese demand is expected to trend higher, based on recent experience. Taiwan and South Korea are also expected to be important markets in the region.

Three markets – Brazil, Colombia and Mexico – have dominated the **Latin American** market in the past, and this is expected to continue. Demand for barley in Latin America is almost exclusively for malting barley. Total imports in the region are projected to expand from 580,000 tonnes to 930,000 tonnes by 2005, with the three main markets accounting for more than three-quarters of the total. Again, if recent growth in Latin American beer consumption continues at the same pace, these import projections may also be conservative. Latin American feed barley imports are expected to remain negligible.

Malting barley

While only a minor increase in total world barley trade is projected, trade in malting barley is expected to jump 64 per cent from 2.3 million tonnes in the base period to 3.8 million tonnes in 2005. The regional demand projections are listed in Table 3. Asia Pacific will account for 2.0 million tonnes or 52 per cent of this 3.8-million-tonne total, followed by Latin America with 24 per cent. China and Taiwan are expected to continue to be the key malting barley markets in Asia Pacific, followed by South Korea and Japan. In Latin America, Brazil, Mexico and Colombia comprise about three-quarters of total imports into the region. The major malting barley importer in "other" regions is the U.S. Projected imports of about 400,000 tonnes (all from Canada) are probably conservative, and could be significantly higher, especially if the U.S. continues to experience some disease problems in the Red River Valley.

Table 3. World malting barley imports by region (thousand tonnes)

	1989-93 Average	—Projections—	
	Base Period	1999/2000	2004/05
Europe	233	60	80
Former USSR ¹	182	130	170
Middle East	51	40	70
Africa	43	90	170
Asia Pacific	1,033	1,690	1,960
Latin America	507	760	900
Others/Unspecified	237	400	400
World Total	2,285	3,170	3,750

¹ Includes trade between members of the former USSR in all periods. Totals may not add due to rounding.

Feed barley

After averaging 16.2 million tonnes in the base period, world feed barley trade is expected to decline to 13-14 million tonnes in 2000 and 2005. A region-by-region listing of import demand is included as Table 4.

The reduction in European imports is likely to continue. Imports are projected to fall from 1.2 million tonnes in the base period to under 600,000 tonnes by 2000 and continue at about this level until 2005. The decline in imports in the five-year projection period is mainly due to greater integration in Europe and declining feed markets in Eastern Europe.

The former USSR regularly imported over 5 million tonnes of feed barley during the base period. Barley imports by former Soviet republics are currently well below that level, but are expected to rebound to roughly 1 million tonnes by the turn of the century. Most of this will consist of trade among the countries of this region.

Middle East feed barley imports will comprise well over half of the world

total during the forecast period, up from about 40 per cent in the base period. Saudi Arabia currently imports over 4 million tonnes of barley per year, and its demand for barley is constant, regardless of price. With the government of Saudi Arabia abandoning its policies on domestic wheat and barley production to protect valuable underground water reserves, feed barley imports by Saudi Arabia could grow even further in the coming ten-year period.

African demand for barley is generally viewed as more price-sensitive than that of key markets in the Middle East. As a result, Africa is likely to reduce imports in the second half of the forecast period as supplies tighten. By 2005, Africa is expected to import about 1.3 million tonnes of feed barley, or 9 per cent of the world total.

The increase in Asia Pacific feed barley imports from 1.7 million tonnes in the base period to 2.3 million tonnes in 2005 reflects import liberalization in some markets (e.g., South Korea) and growing demand (e.g., Japan).

The projected overall decline in world feed barley trade is due to:

- limited production response in exporting countries (at traditional price premiums to corn)
- the unwillingness of most buyers to pay large premiums for barley compared to other feed grains
- strong demand from maltsters, who are able to bid supplies away from potential feed barley markets.

Value-added and special barley markets

Barley trade projections were only done for feed and bulk malting barley. World demand and trade for malt will also continue to expand in response to growth in beer consumption in developing economies. There will also be opportunities for increased trade in special barley types such as hullless barley for animal feed and human food, and barleys with special starch characteristics of interest for nutraceutical, pharmaceutical and industrial applications. As

Table 4. World feed barley imports by region (thousand tonnes)

	1989-93 Average	— — — Projections — — —	
	Base Period	1999/2000	2004/05
Europe	1,215	590	590
Former USSR ¹	4,758	1,000	840
Middle East	6,418	7,400	8,370
Africa	1,232	1,480	1,250
Asia Pacific	1,725	2,360	2,330
Latin America	72	30	30
Others/Unspecified	<u>786</u>	<u>460</u>	<u>460</u>
World Total	16,207	13,320	13,870

¹ Includes trade between members of the former USSR in all periods. Totals may not add due to rounding.

breeders and scientists develop varieties with specific commercial application and functionality, marketing agencies like the CWB will assist in supplying such new niche value-added markets as they become apparent. This is already happening in the Japanese food market.

Conclusions

In conclusion, this forecast contains a positive message for plant breeders and other scientists working worldwide to enhance the yield and quality of barley for both animal and human consumption. The implication is that there will be a role for higher yielding feed varieties but also for malting varieties with the specific quality parameters required for an ever-expanding world beer demand.

The future of oat and barley as functional foods

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Introduction

The use of food as medicine is on the rise in the US, as is the use of supplements and herbs. Consumers are trying to increase their intake of fiber-containing foods (44%) and fortified foods (14%) not only to get the nutrients they need but also to improve their health. Consumers want to feel better and to add years to their lives.

Oatmeal has been recognized as the first “functional food.” Oat consumption continues to grow in the US, with per capita use rising 130% between 1980 and 1992. These higher consumption levels are linked to an increased consumer awareness of the health benefits of oat and the positive response of consumers to national dietary recommendations to increased fiber consumption.

Oat is best known for lowering serum cholesterol. Forty-one studies over 30 years have documented this effect. Barley properties are similar to those of oat, but the research documenting a cholesterol-lowering effect is not nearly as extensive.

Recent research suggests that oat plays a role in other physiological processes, such as moderating the effects of hypertension, regulating blood glucose and insulin levels, managing weight and promoting gastrointestinal health. This impact is attributed to the soluble fiber fraction, beta-glucan. This paper discusses the status of health claims internationally with special emphasis on the US, the Quaker Oats petition for a health claim for oat and coronary heart disease, leveraging the health-promoting effects of oat and barley products, and the research necessary to support these claims.

International health claims regulations

Regulations governing health claims vary widely around the world. In the Philippines, a food is considered misbranded if it carries a health claim without research substantiation. Korean regulations do not allow explicit health claims;

e.g., discussion of the prevention and treatment of a specific disease. However, the regulations allow messages about health maintenance. Canadian regulations allow both general claims – e.g., protein is a “factor in the maintenance of good health” – as well as specific claims for the biological role of nutrients – e.g., “protein helps build antibodies.” The Australian National Food Authority is considering the use of health claims and recently asked for comments on the Review of the Food Standards Code, Concept Paper on Health and Related Claims. In the UK and the EU, health claims are general and address overall health. Japan is further along in dealing with “functional food” claims. The Japanese Ministry of Health Welfare has approved “Specific Health Maintenance Food Components,” listed with their efficacy; e.g., soybean oligosaccharides and bifidus growth.

Health claims were first legally allowed in the US when the Nutrition Labeling and Education Act (NLEA) of 1990 was enacted. Health claims for foods are tightly regulated. Supplements also are subject to stringent regulations. For supplements, however, more leeway is allowed regarding statements of nutritional support; e.g., statements that describe the role of a nutrient in preventing a deficiency state.

Current requirements for health claims in the US

The use of health claims on food product labels should help consumers make wise food choices about products that may prevent disease and maintain health. The strictly regulated health claims in the US describe the relationship between diet and specific health issues (FDA, 1995). Eight health claims are approved: two for coronary heart disease, three for cancer, one for hypertension, one for osteoporosis and one for folic acid. For example:

Diets low in saturated fat and cholesterol and high in grains, fruits and vegetables that contain fiber, particularly soluble fiber, may reduce the risk of heart disease, a condition associated with many factors.

The US Food and Drug Administration believed the number of products carrying health claims would increase once NLEA was enacted. Unfortunately, however, health claims are not being used to their full potential. Most food manufacturers do not use food labels to give people diet/disease information. Health messages are lengthy and are not consumer-friendly, and they appear on the back of the product package where they are not easily seen.

US health claim petitions, using the oat health claim as an example

The regulations implementing NLEA in the US encourage petitions for new health claims (FDA, 1995). The petition process requires a complete explanation of how the substance conforms to the general health claim requirements. The substance must be associated with a disease or health-related condition for which the US general population or a subgroup is at risk. The substance must be a food or a food ingredient.

A summary of the scientific data, along with an interpretation of the findings,

must be included. The quality of the scientific investigations conducted is important; e.g., they must be well-designed and should include well-controlled clinical trials. The petition must demonstrate that the substance confers a health benefit, along with an optimal level that has a positive effect.

The analytical data should show that the substance can be analyzed and that the substance appears in foods qualifying for the claim. Association of Official Analytical Chemists methods should be followed.

A model claim, which is a brief statement of the relevant conclusions of the scientific data, must accompany the petition. A statement detailing how the substance helps the consumer achieve a total dietary pattern or goal must be included. Appendices accompanying the petition include copies of the literature search, published papers, all data to support the claim and a notation of any adverse side effects.

In 1995, The Quaker Oats Company submitted the first petition to the Food and Drug Administration for a food-based health claim. The petition describes the role of oat in the reduction of coronary heart disease, supported by a summary encompassing 30 years of research and 41 studies. A number of studies validated the mechanism – e.g., the soluble fiber beta-glucan as well as the effective amount of beta-glucan needed to see a mean serum cholesterol reduction of 5%.

As stated in the proposed FDA regulations (FDA, 1996), in order for a food to carry the oat claim, it must

- contain 1 gram of beta-glucan and 20 grams of oatmeal or 13 grams of oat bran/serving (Reference Amount Customarily Consumed)
- be low in fat (≤ 3 grams), saturated fat (≤ 1 gram) and cholesterol (≤ 20 milligrams)
- not exceed established disqualifying levels for health claims – e.g., 13 grams of fat, 4 grams of saturated fat, 60 milligrams of cholesterol and 480 milligrams of sodium per serving.

Shorter, more effective consumer language is included in the proposal in the form of a split claim, e.g., a short, concise claim on the front of the package with more detailed information on the back panel.

- *Front panel language:* A diet high in oatmeal may reduce the risk of heart disease (See back/side panel for more information about oat and heart disease).
- *Back or side panel language:* Diets high in oat bran/oatmeal and low in saturated fat and cholesterol may reduce the risk of heart disease.

Consumer communications

Consumers in the US are very health conscious. Nutrition plays a major role in their efforts to maintain health and prevent disease. However, consumers in the US are frustrated with conflicting news reports and are confused about building a healthy diet. One way to decrease consumer confusion is to increase the use of effective health messages on food labels. Communications research, including consumer understanding of the oat and coronary heart disease claim, will be covered. The most effective way to communicate health messages will be recommended in the oral presentation.

The future of oat and barley as functional foods

Functional foods and the phytochemicals in these foods are a major focus in preventing and treating chronic disease and maintaining health in the US. A *functional food* is any modified food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains. *Phytochemicals* are substances found in edible fruits, vegetables and grains that may be ingested daily by humans in gram quantities. They exhibit a potential for modulating human metabolism in a manner favorable for cancer prevention.

Oat products as well as barley can confer a number of physiological benefits. Not only do they have good nutritional profiles, but they also are some of the most concentrated sources of soluble dietary fiber. Although oat and barley contain a number of other components that may be beneficial to health, the soluble fiber fraction – beta-glucan – is responsible for the majority of their effects. Evidence from both epidemiological observations and clinical intervention trials supports the role of oat in reducing total blood cholesterol and LDL-cholesterol, known risk factors for heart disease. Furthermore, oat appears to play a role in other physiological processes, such as moderating the effects of hypertension, regulating blood glucose and insulin levels, decreasing obesity, and promoting gastrointestinal health.

Phytochemicals in oat and barley may help reduce the risk of cancer. These phytochemicals – e.g., lignins, saponins, polyphenolics and isoflavonoids – may provide additional health benefits.

Leveraging the market – What research is needed?

Two additional well-designed clinical trials would be sufficient to support the role of oat in blood glucose control. Clinical trials are needed across various groups to determine the dose response and mechanism of action for blood pressure and weight control. Further exploratory work is needed to ascertain which cancer biomarkers are affected by oat. Barley research lags behind oat research in these areas.

Not only do most regulatory agencies require well-controlled research to support health messages, but communications research is critical to ensure that consumers appropriately interpret the message. To reinforce the increased consumption of whole-grain breads and cereals, messages in the US must be integrated with public policy guidance, e.g., the Food Guide Pyramid.

Acknowledgement

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Food applications for barley

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Barley – A food crop from antiquity

Archaeologists have discovered that barley was a staple food as far back as 18,000 years ago (Wendorf et al. 1979), so it was probably the first cereal crop to have been cultivated (Darby et al. 1977). In the Bible, Deuteronomy (ca. 1400 BC) describes the promised land as a “land of wheat and barley, and vines, and fig trees, and pomegranates.” Barley (*hordeum*) was the main food cereal of the Roman Empire and St. John’s Gospel (ca. 90 AD) records how five thousand were miraculously fed from “five barley loaves and two small fishes.” Barley continued to be the main food cereal of northern Europe until the sixteenth century. However, even in ancient times, barley was looked on with disfavour as “peasants’ food.” As European agricultural practices improved, particularly during the seventeenth and eighteenth centuries, wheat bread gradually supplanted barley, and today very little barley is used for human consumption (except in the form of beer and whiskey). However, barley has always enjoyed a “healthy image.” It has been the basis of numerous herbal remedies. Hippocrates made many references to it and believed that “the decoction of barley is the most judicious choice of all food extracts in the treatment of acute diseases” (Precope, 1952).

Barley is now returning to favour as an excellent source of soluble dietary fibre (McIntosh and Oakenfull 1990).

Dietary fibre – “A concept rather than a substance”

Following Denis Burkett and Hugh Trowell, who pioneered the scientific study of this important area of human nutrition, nutritionists define dietary fibre operationally as “any substance of plant origin which is undigested by the human alimentary enzymes (Trowell 1972).” No one would seriously dispute this definition – but it presents problems to chemists. As K.W. Heaton has recently pointed out (1990), dietary fibre is “a concept rather than a substance.”

Attempts have been made to define dietary fibre in purely chemical terms as *non-starch polysaccharides* (NSP) (Furda 1989), but this definition omits lignin and resistant starch (Figure 1) (Blakeney 1993). Lignin has bulking characteristics; resistant starch has some of the nutritional properties of soluble fibre (Annison and Topping 1994). Solubility presents an additional problem. Again, the term is not clearly defined. Any method for determining soluble fibre involves some sort of arbitrary definition, depending on the analytical method (Southgate 1995).

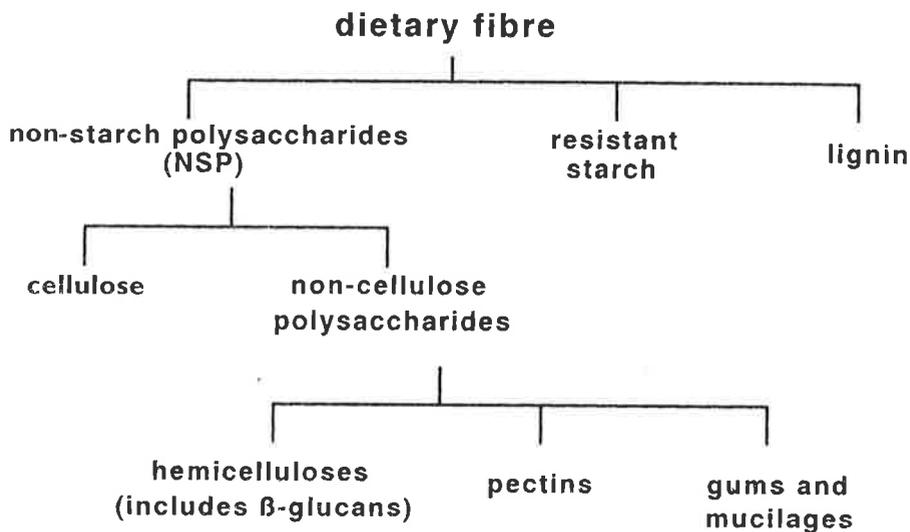


Figure 1. Chemical composition of dietary fibre. The non-cellulose polysaccharides (hemicelluloses, pectins, gums and mucilages) tend to be water soluble.

In general terms, the soluble dietary fibres are the non-cellulose fraction of NSP – hemicelluloses (which include the (1–3),(1–4) mixed linkage beta-glucans of barley and oat), pectins, gums and mucilages (which include materials such as guar gum, added to some manufactured foods to modify texture).

Sources of soluble dietary fibre – Barley and oat bran compared with fruit and vegetables

Figure 2 shows the levels of dietary fibre (soluble and insoluble) in a few commonly eaten fruit and vegetables compared with whole barley flour oat bran and wheat bran. The cereals are the richest source of dietary fibre – after legumes (peas and beans). Of the cereals, oat and barley are the richest sources of soluble fibre because of their mixed linkage beta-glucans.

Health benefits from soluble fibre – A review of the evidence

Soluble and insoluble fibre differ in two respects that seem to have important nutritional consequences: soluble fibre increases the viscosity of the digesta, and it ferments more rapidly than insoluble fibre in the large bowel. Different effects appear at different stages in the food's passage through the gut (Oakenfull 1993). Firstly, viscous polysaccharides appear to slow the rate of gastric emptying and thereby influence food intake (Oakenfull 1993). Then, in the small intestine, it seems that viscosity reduces the rate of nutrient absorption (Oakenfull 1993). For example, Jenkins and his colleagues (1978) have shown the viscous polysaccharides guar gum and pectin to be beneficial in controlling hyperglycaemia. In addition, soluble fibre may influence cholesterol metabolism

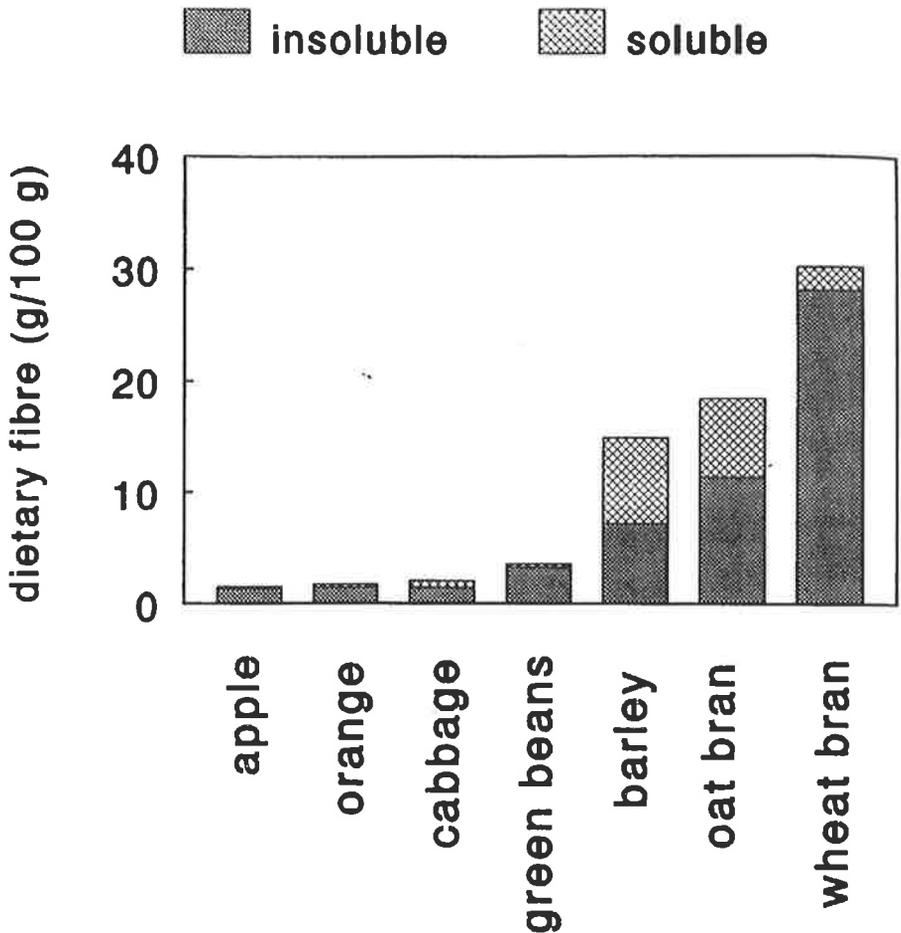


Figure 2. Levels of soluble and insoluble dietary fibre in some commonly eaten fruit and vegetables compared with whole barley flour, oat bran and wheat bran. The data are given on a wet weight basis for the foods as eaten.

by inhibiting reabsorption of bile acids.* This is still a controversial area, and Eastwood and Morris recently concluded that there is in fact little evidence to suggest that viscous polysaccharides inhibit absorption from the small intestine (Eastwood and Morris 1992). In the large bowel, all fibre fractions are broken

*Bile acids are surfactants that aid the dispersion and digestion of dietary fats. They are synthesised in the liver from cholesterol, and normally after doing their job are reabsorbed from the small intestine and returned to the liver in the portal blood stream. Viscous polysaccharides might reduce the efficiency of this recycling process. Bile acids would then be lost by faecal excretion and replaced by metabolizing cholesterol, thereby lowering the plasma cholesterol concentration.

down to a greater or lesser extent by the enzymes of the gut microflora (lignin is least affected) (Topping and Illman 1986). Fermentation depends on the accessibility of the polysaccharide molecules to the micro-organisms, which depends, in turn, on chemical structure and physical properties, particularly solubility. Soluble fibre is very accessible; it ferments rapidly and is gone before it has time to pass far through the bowel. Insoluble fibre ferments more slowly in a process that continues throughout transit. Thus, solubility has a profound effect on the kinetics of release of metabolites, particularly short-chain fatty acids. It is often suggested that the high (but transient) concentration of short-chain fatty acids may be a factor in controlling cholesterol metabolism, although recent evidence suggests otherwise (Evans et al. 1992). The lower (but more persistent) concentrations of short-chain fatty acids (particularly butyrate) produced by insoluble fibre appear to help protect against colorectal cancer (Young 1991).

There is clearly much we still do not know about dietary fibre. Nonetheless, it is now firmly established that soluble fibre has a cholesterol-lowering effect (Truswell 1995) – even though we do not yet fully understand the mechanism (or mechanisms) by which this effect occurs.

Health benefits specifically from barley

As we have seen, barley resembles oat bran in being rich in beta-glucans. The cholesterol-lowering properties of oat bran have been extensively studied. Very little doubt remains that oat bran can lower plasma cholesterol – provided enough of it is eaten. Moreover, this reduction is greater in those with more elevated plasma cholesterol levels (Ripsin et al. 1992). Fewer studies have been done with barley, but the collective evidence for cholesterol-lowering activity is again very convincing. In addition, barley products have been shown to lower serum glucose and insulin responses to a meal (Liljeberg et al. 1992).

Lowering of cholesterol has been repeatedly demonstrated in animal studies. In chicks, for example, barley and oat produced substantial reductions in plasma cholesterol compared with a diet based on corn (Prentice et al. 1982). We found a similar effect in an experiment with rats (Oakenfull et al. 1990). The animals were fed diets containing 70% of either barley or wholewheat flour. The diets were otherwise equivalent in terms of fat, protein and total dietary fibre. All diets contained cholesterol (1%) to make the animals hypercholesterolaemic. In the animals fed barley, the total plasma cholesterol was 52% lower than in those fed wheat and 28% lower than in those fed a basal diet free from dietary fibre (Figure 3). Animal studies have also confirmed that the cholesterol-lowering activity can be explained by the mixed linkage beta-glucans present in barley. Figure 3 includes plasma cholesterol data for rats fed two different levels of isolated barley beta-glucans. Feeding the isolated beta-glucan mimics the effect of feeding an equivalent amount of whole barley. In addition, there appears to be a limit to the active level of dietary beta-glucan. Above this limit, additional beta-glucan has no additional cholesterol-lowering effect (Oakenfull et al. 1990). Although the mechanism of the cholesterol-lowering effect remains uncertain,

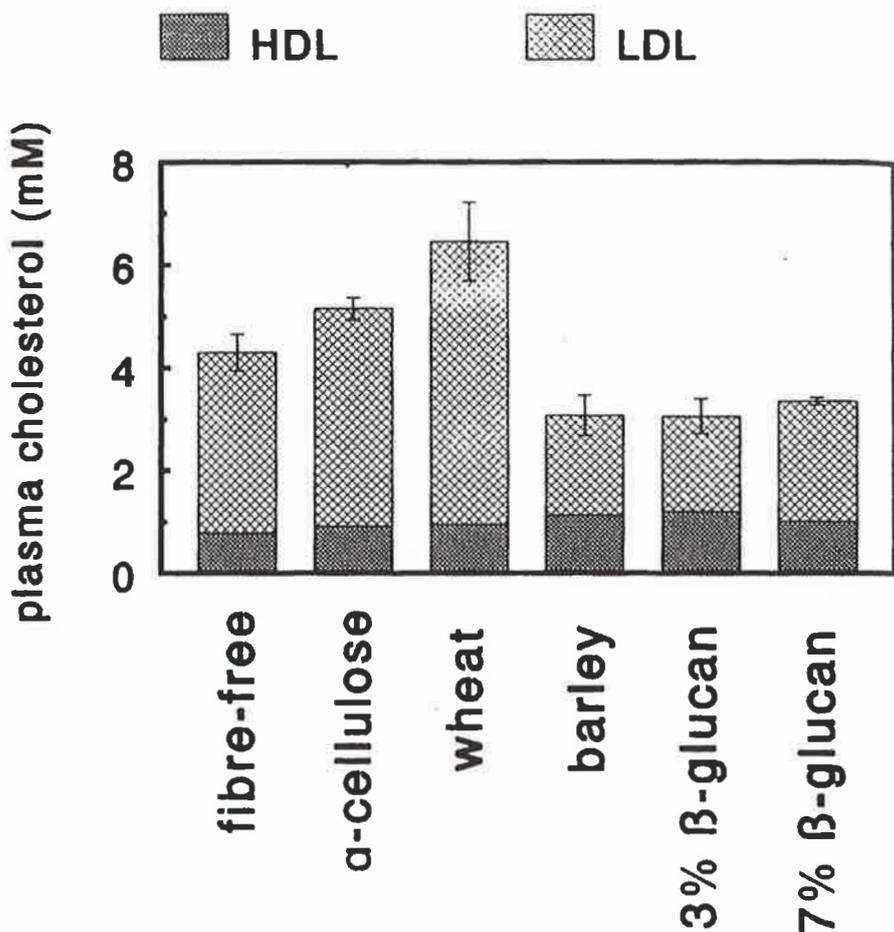


Figure 3. Plasma cholesterol concentrations in rats fed barley, wheat or isolated barley beta-glucans. Concentrations found with a basal, fibre-free diet and with purified alpha-cellulose are given for comparison. Other than the fibre-free diet, all diets were equivalent in terms lipid, protein and total dietary fibre. All animals received 1% dietary cholesterol.

it clearly depends on the physical integrity of the beta-glucan molecule. When oat bran is treated with endo-beta-D-glucanases to hydrolyse the beta-glucans, the cholesterol-lowering activity (in rats) disappears (Tietjen et al. 1995).

Fewer human trials have been reported, but they are nonetheless convincing – particularly when considered in conjunction with the numerous human studies carried out with oat bran. Newman and her co-workers (1989) at Montana State University have carried out a trial in which two groups of male volunteers were given a variety of foods based either on barley or wheat. Both diets provided the same daily intake of dietary fibre (42 g). With the wheat diet, the mean plasma

cholesterol increased by 12% from a pretreatment level of 4.54 mM. With barley, there was a small, but not statistically significant, decrease of 3% from a pretreatment level of 4.61 mM. Thus, there is a strong indication of a significant difference between the effects of wheat and barley on plasma cholesterol and some indication that barley may lower plasma cholesterol. In a more rigorously controlled experiment carried out in Australia, McIntosh and his colleagues (1991) compared barley with a corresponding wheat diet in a group of 21 mildly hypercholesterolaemic men (plasma cholesterol 5.5–7.0 mM). Again, both diets provided the same intake of dietary fibre (38 g/day) and each diet was maintained for two months in a cross-over designed experiment. The barley diet gave a mean plasma cholesterol 6% lower than the wheat diet. The effect of the low density lipoprotein (LDL) fraction was greater (7%), and the difference was satisfactorily significant. (LDL cholesterol is the fraction associated with increased risk of heart disease.)

Application of food science and technology to the development of barley-based food products attractive to consumers

Barley is suitable for use in many food products, such as baked goods, pasta, rice alternatives and breakfast cereals. However, the mixed linkage beta-glucans have a high water-holding capacity that affects the density, texture and volume of products. In yeast-leavened breads, barley flours can be substituted for part of the wheat flour, but its use is limited because it lacks gluten. Barley breads have a low loaf volume and a compact structure. Pasta can be made with barley flour (Nakamura et al. 1986). Muffins made with 100% barley flour were acceptable to consumers (Newman et al. 1990). It is also possible to incorporate 30% barley flour into pastry without compromising consumer acceptability (McNeill et al. 1988).

Cultivar and environment can strongly influence levels of beta-glucans (Xue et al. 1991; Narasimhalu et al. 1995). Milling technology can also be used to increase the level of beta-glucans. For example, air classification and sieving can produce barley fractions enriched in beta-glucans and tocopherols (Sundberg and Aman 1994; Knuckles and Chiu 1995; Yoon et al. 1975). Extrusion cooking increases the apparent dietary fibre content of cereal-based foods because it generates resistant starch. Extrusion cooking has been specifically shown to increase the proportion of resistant starch in wholemeal barley flour (Østergård 1989). (However, an oat-bran-based extruded cereal was recently reported to be less effective in lowering plasma cholesterol than the equivalent unprocessed bran (Hicks et al. 1995).)

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Non-food uses of oat and barley

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Oat and barley have traditionally been grown around the world as a major source of animal feed. Less than 10% of oat production is utilized for human food and, although specific statistics are not kept for such purposes, a much smaller amount likely finds its way into non-food uses. Since one of the major industrial uses of barley is for brewing, and this is covered elsewhere in these proceedings, no further treatment will be given to this topic in this presentation. In approaching a review of the non-food uses for oat and barley, exclusive use has been made of the patent literature since 1980; the scientific literature has not been used, since, although there are many articles that allude to potential commercial uses for oat and barley fractions and components, most have not been advanced to the stage of protectable intellectual property and/or actual commercial practice. Examples will also be presented based upon the recent commercialization of one of the authors' research findings.

Oat

Historically, perhaps the best known non-food application of oat is its use in skin care products, principally as a cleansing agent in face masks and in bath formulations to relieve itching (Stanton, 1951). Some early cosmetic claims suggest that oatmeal could be used as a hypoallergenic agent in the treatment of geriatric dermatoses, eczema, measles, chicken pox, pityriasis rosea and sunburn (Meister, 1963). The benefits of the use of oat flour as an ingredient in some early cosmetic products has been adequately summarized (Miller, 1977). There are still several well known commercial brand products in use today employing powdered oat flour as an ingredient. Both finely divided oat flour and an extract of oat has also seen some use during the past 50 years as a source of anti-oxidants, but BHA and BHT have all but reduced this use to the curiosity level. However, with a rising interest in additives derived from plant sources, and the introduction of some new processes for fractionating oat, there is a re-emergence of the use of oat fractions and components in cosmetic products. A novel oat fractionation process developed by Burrows et al. (1984), and later improved upon by Collins and Paton (1992), formed the basis for the development of this technology by Paton in collaboration with the POS Pilot Plant Corporation and its business arm, Nuvotech Ventures International, during the period 1992-95. The developed technology was ultimately licensed to Canamino

Inc. of Saskatoon for the manufacture of several fractions for use in the cosmetics sector. There is a strong market opportunity, in the face of stiff opposition by animal rights activists, to replace animal-derived cosmetic ingredients with those of plant origin. This technology produces a bran-free oat flour and a flour-depleted oat bran as primary products. In turn, the flour is the starting material for the production of a highly refined oat starch and a protein concentrate that undergoes hydrolysis to yield a soluble protein. The bran itself has utility, but may also be extracted to produce a line of beta-glucan powders with purity in the range 65–85%. The oat starch has uses as a partial or, in some cases, total replacement for talc in a wide range of dusting powders. There is a perceived link between talc and asbestos in terms of risk to human health from inhalation. The bran has application in facial scrubs and, in addition to its exfoliating properties, conveys a super-moisturizing characteristic from its high beta-glucan content. A novel feature of the process used to make the bran is the fact that the beta-glucan component has been rendered in a readily hydratable form, and thus the bran, on contact with water, possesses a smooth feel to the touch. The oat protein hydrolysate finds utility as an ingredient of shampoos. Proprietary formulations of several of these oat fractions also find use in pet and large-animal care products. Another interesting fraction is one that concentrates the active principles conferring the anti-irritant behaviour in a solution of either propylene or butylene glycol. One very large market use for this fraction is in combination with oat starch as the internal dusting agent for surgical gloves: the dual function of powder smoothness and anti-irritant is displayed.

The beta-glucan in solution has a molecular weight in excess of 1 million daltons and possesses a medium-high viscosity. Interesting applications are being developed for this product as an active ingredient of wet surgical dressings, where the beta-glucan confers its moisturizing effect but is also believed to promote healing in combination with other components of the formulation. The principal use of beta-glucan is in its incorporation into creams and lotions as a super-moisturizer. The substance that beta-glucan is replacing entirely or in part is hyaluronic acid, a high polymer derived from the combs of roosters. Beta-glucan has a structural backbone similar but not identical to hyaluronic acid.

A very recent patent application has been filed by Cioca et al. (1994) for sun-screening compositions containing cereal extracts and an oat extract specifically. They have found that in a formulation with a well-accepted sun-blocking agent, titanium dioxide, there is produced a synergistic effect of the oat extract when the latter is used at a level of 0.1–2.0% of the formulation. Cosmetic preparations such as skin lotions, cold creams, lipsticks and the like are formulated to include the oat extract.

Another new family of products, introduced by Nurture Biotech Inc. of Montana, US, is based on oat proteins, processed by a proprietary technology. These products appear to be based on a series of patents that were issued to the Dupont Co. in the early 1980s (Oughton, 1980). Here, oat groats are ground in an organic solvent and differentially centrifuged to give protein bodies of a discrete particle size, nominally less than 50 microns. Nurture has discovered that these particles display preferred time release properties that makes them suited to applications in cosmetics,

pharmaceuticals, environmental clean-up and pesticide/agricultural chemistry applications (Gupta, 1991). In a further Nurture patent application in 1995, Potter et al. (1995) disclose an oat oil composition with useful dermatological properties; evidence is presented showing that human skin, when exposed to ultraviolet radiation in the 290–320 nm range, showed a 30% reduction in peroxidation of skin lipids when oat oil was applied. Applying the same quantity of linoleic acid to skin resulted in a 20% increase. Information is also given on the incorporation of oat oil into a dermatologically effective soap. The latter term is used to describe the moisturizing, mildness, rinsability, non-film-forming properties, while resulting in no appreciable destruction of the stratum corneum. The rate of water loss through the skin was determined using 6 different human subjects. The average rates of reduction using Vaseline, 100% oat oil, 100% canola oil and 10% and 20% blends of oat oil in canola oil were 59%, 38%, 43%, 36% and 28% respectively. No discussion of these relative performances was given in the document. The patent application of Marshall and Bixby (1995) discloses the use of these same oat protein bodies as a carrier and controlled release agent for agricultural chemical sprays. Surprisingly, no data is given in this document, but it is stated that spray head nozzles contain less residues that can lead to clogging when oat protein bodies are used in the formulation.

In a patent application filed by Lenz and Paisley (1993), a means is provided for the fractionation of oat to produce a stable, water-soluble protein. Oat, in the form of fine flour, flake or oat meal, is slurried in alkaline water (pH 10–12) and maintained at a temperature within the range 45–60°C for 1–3 hours in the presence of a protease. This action solubilizes the protein, which, following removal of the insoluble solids and the fat by centrifugal means, is membrane filtered, concentrated and dried to give the final product. The product has a nominal protein content in the range 50–60%, and contains less than 1% fat. When dispersed in water, the product has a minimum solubility of at least 85% with a residual insoluble solids level of less than 0.1%. Although no cosmetic formulatory claims are made, reference is made in the text to targeted use of selected plant proteinaceous matter in clear cosmetic hair and skin care products. A recent patent application by Malkki et al. (1995) describes the preparation of a fine granulated oat starch. The process involves treating an isolated oat starch with a surface active agent or a lypolytic enzyme preparation to cause disintegration of starch aggregates. Projected uses for the product include incorporation into biodegradable plastics and as a surface treatment agent. Arraudeau et al. (1989), in a patent assigned to L'Oreal, disclose a cosmetic make-up composition that includes 0.5–40% of a mineral or organic matter selected from the group consisting of talc, rice, wheat, corn or oat starches, wheat germ powder and kaolin. In a patent application by Evans et al. (1990), the preparation of a surfactant from oat is described that has uses as an emulsifier in the food, agricultural, chemical, cosmetic, pharmaceutical, building, textile and tanning sectors. The process involves extracting oat with an aliphatic alcohol of at least 2 carbon atoms, removing the solvent and further treating the intermediate with methanol. The methanol is removed from the soluble fraction and is in turn treated with acetone. The acetone-insoluble material is the product. Yet a further patent (Rudov, 1988) describes the preparation of a pharmacologically effective extract for external application, derived from the

juice of the leafy green parts of graminaceous plants, including oat. The juice is freshly prepared from plants at the unjointed stage of plant development by squeezing, crushing or grinding under sterile conditions, and the extract is immediately treated to prevent the growth of microorganisms. This treatment takes the form of concentrating and drying the juice or by adding a preservative to the liquid concentrate.

Qureshi (1991) has described a process for the stabilization of tocotrienols, tocopherols and tocotrienol-like compounds in plant tissue and a means of recovering these. Oat is among the plant materials of choice. This family of compounds is found in the deodorizer distillate resulting from the refining of the oil component of the plant material. Although this patent also includes claims for these compounds in a pharmaceutical formulation for the treatment of hypocholesterolemia in humans and as dietary supplements, no evidence was given of specific data in support of these claims. A patent issued to Kiebke (1993) describes the use of coarse grains in a mixture used to provide a biodegradable cat litter product. The hydrating agent is given to be gluten that binds the animal waste into a hardened clump. Another patent of the same type is that of Ducharme et al. (1989), which incorporates cereal grain hulls, including oat hulls, into absorbent compositions useful as animal litters or as oleophilic binding agents. The oat hulls are a minor component of the formulation, the active ingredient being a cyclodextrin. Cowan et al. (1991) describe compositions of matter suited to use as drilling well fluid control agents. An additive is claimed wherein ground oat hulls, of particle size 74 to 590 microns, are formulated in an amount of 50–98% by weight with 2–50% of a particulate selected from the group consisting of corn cobs, hydrophobic water-wettable cotton, ground citrus pulp, ground cotton burrs and mixtures thereof. Such compositions are found to reduce the seepage loss of the fluid to fluid-permeable earthen formations. The conversion of cereal flours, including oat, into an acetyl derivative and its subsequent incorporation into formulations that constitute biodegradable plastics is disclosed by Elion (1993). The starch acetate is produced without the use of an organic solvent as the mobile phase and under conditions of reactant addition and mixing, which minimize the generation of side reaction products. When used in combination with other biodegradable fillers such as crustacean shells, mouldable solid utility products can be produced that biodegrade at a rate of 5–10% per month in standard tests.

Barley

A review of barley, including some of the potential industrial applications for co-products generated, has been published by MacGregor and Bhatti (1993). Although the use of barley in the brewing and malting trade is well known, a non-malting application is the production of alcohol and spirits. An integrated process for the production of barley starch and ethanol has been outlined by Lehmuusaari and Ham (1987) and is being practised in Finland by Oy Alko Ab (Alko Ltd.) at a plant in Koskenkorva. The integrated process begins with dry milling the barley, removing husks and soaking the flour, followed by sieving to remove bran. Following further aqueous separation to remove protein, two starch streams are prepared. The “A” starch stream is dried and sold for applications in

the pulp and paper industry, while the second cut or "B" starch is mashed, with the resultant production of ethanol. The ethanol is further distilled at Alko plants in Nurmijarvi or Helsinki to produce the renowned Finlandia vodka or Koskenkorva spirits. Some of the ethanol production is utilized by wine and liqueur makers. The Koskenkorva integrated plant uses 150,000 tonnes of barley annually and produces 15,000 tonnes of ethanol, 30,000 tonnes of starch and a quantity of liquefied CO₂.

In the United States, a process for the production of products from waxy barley was initially patented by Goering and Eslick (1982). Subsequent pilot plant trials resulted in process modifications and updates to the patent (Goering and Eslick, 1989 and 1991). Although many of the co-products can be applied in the food industry, the main focus was the production of high maltose syrup that could in turn provide a suitable base product for conversion into alcohol. The process design is flexible, and can produce a starch stream as well, depending on market demands. Commercialization of the technology has been attempted in Dillon, Montana, with varying degrees of success. Processes for the co-production of ethanol and improved human food products from cereals grains (including pearled barley) have also been patented by Thacker and Dodgin (1994). The process involves enzymatic conversion and removal of starch from the grain, followed by separation of the liquor and subsequent fermentation to produce industrial fuel alcohol. Although the literature cites applications of barley starch in coatings for the paper industry, few patents were identified in our search. Use of barley starch to prepare a cationic starch after reaction with 3-chloro(hydroxypropyl)-trialkyl ammonium chloride has been patented by Bergh et al. (1994), and yields a composition that can be used as a binder in coating colours as well as in the paper coating process. A process has been described by Rayas and Ng (1995) whereby barley flour can be subjected to a process aimed at protein removal, with the resulting solution then used to form edible and biodegradable films through a process of casting on a surface.

Other miscellaneous non-food applications of barley include those already referred to in the oat section: cat litter formulations and fresh juice from plants for the purpose of creating cosmetic or medicinal ointments. More specific to barley, a patent has been granted to Ogilets (1986) for the use of an oil extract made from barley shoots in combination with extracts of St. John's Wort to produce a cream having anti-inflammatory properties for cosmetic use. A process that incorporates barley protein, or an alcohol-modified protein hydrolysate of the same, combined with a number of ingredients common to cosmetic manufacture has been described by Nisshin Oil Mills (1987). The method is intended to efficiently produce a cold cream, vanishing cream or lotion that has high stability and a pleasant feel when applied to the skin.

As the search for new and unique market niches for products continues, further opportunities will undoubtedly be created for the utilization of value-added components derived from plants, and oat and barley in particular.

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Novel food – Is there a market for barley and oat?

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Some years ago I visited an exhibition at the Smithsonian National Museums called “The change of the seeds” that told the story of the changes that happened due to the travels of Christopher Columbus. Changes that may interest this audience include three foods, to that time unknown, that he brought home to Europe: potatoes, tomatoes and maize. They were “novel foods” of that time. People had to learn by trial and error how to use, prepare and consume these novel foods without harm to their health, since some were poisonous, such as potatoes, which contained solanine. The health hazard from these foods originated mainly from their natural properties and not from human manipulation. Improvement was at that time difficult to achieve – the only option was to exclude the food items from the diet.

Today, modern and improved technological and biotechnological processes and their applications have made it possible for us to design foodstuffs with highly specific nutritional and health properties, at the same time avoiding toxic problems, by, for example, changing the genetic programming of plants and animals. This new group of foods are characterized by adjectives such as “new,” “novel” and “novelty,” and would appear to be food with a higher “content of knowledge.” They are, however, normal foods that can be consumed by everyone, but have been produced in such a way that they have a documented positive effect on health. On the other hand, they do not fit in with the regular way of distinguishing between foods and drugs.

The foods for the future are here!

Consumers have been offered a completely new type of food, food with scientifically documented properties. This is food where traditional nutrients have been replaced with new components – food where the technological, nutritional and toxicological properties have been changed. But this new food has resulted in a moral and ethical debate. Not only may farmers and food manufacturers be able to increase their production capacity and change the nutritional value and inherent toxicological properties, in this food the natural properties have been changed so profoundly that it demands new legislation.

For the moment the European Community (EU) has not taken any definite decision about legislation governing novel foods. The United States already has novel food items on the market, and is trying to put pressure on Europe to accept

these products. However, The EU and the World Trade Organization (earlier known as GATT) are far from reaching agreement in many cases. Although much scientific effort is being put into formulating assessment protocols in Europe, the US, Japan and elsewhere, it is already fairly clear that “novel foods” will have to be assessed initially on a case-by-case basis, based on scientific and objective criteria. Within the EU there is also a profound disagreement, and many EU countries might have to make drastic changes in their food policy. While England already has allowed several novel-food products, countries like Denmark and Sweden are fighting against liberalization of the laws.

It is, however, important to bear in mind that many traditional foods contain anti-nutritional or toxic components. The techniques being used to create novel foods could also be used to understand the structure of traditional foods and to improve their nutritional value.

What is a novel food?

New physical, chemical and biotechnological methods allow us today to change food items, not only for technological purposes, but also to improve their nutritional value and decrease inherent toxicological properties. Therefore, the terms “novel foods” and “non-traditional foods” have been invented to emphasize not just the novelty, but also the ethical questions. The English term “novel food” seems to have infected all languages and is used untranslated in most countries. It is important not to confuse the terms “novel food” and “functional food.” Functional food is based on perfectly normal substances from nature or from our own body, but has been isolated, prepared, concentrated or been used to create or give certain medically documented health effects.

The concept of “novel food” needs to be clarified in the sense of being a “food creation” appearing for the first time. According to the *Oxford Reference Dictionary*, novel means “a new kind, strange, hitherto unknown.” A food may be novel as a result of the use of novel raw materials, novel processing or preparation techniques, or novelty in terms of its role in the diet. Novel food organisms or products derived from such organisms may result from recently developed techniques such as genetic modification or from more conventional plant and animal breeding techniques.^{1, 2}

- **Novel foods** are foods or food ingredients that have not previously been used for human consumption to a significant degree and/or that have been produced by extensively modified or entirely new food production processes.
- **A novel process** is a process that has not previously been used in the processing of foods.

Novel foods have also been divided into the following groups:

- never consumed by humans before
- to be consumed in much higher quantities than before
- to be consumed as before, but transformed through new physical, chemical and biomedical methods, leading to:
 1. substantial changes in the content of naturally inherent toxicants beyond limits of natural variation

2. substantial changes in the content of nutrients important for the overall dietary intake below limits of natural variation
3. new functional gene(s) inserted that has recognized cellular functions or gene products

In this respect we need to have information about the origin of the food; the composition, type and levels of natural toxicants and nutrients; and the quantities of the food likely to be consumed. The inserted genetic material has to be defined and the fate of the genes inserted followed. It will not be an easy task to characterize the novel plant materials. To get a background for defining a novel plant, repositories for natural and traditional existing plants must be established. Today, knowledge of the levels of natural components, both nutrients and toxins, is limited.

The definition of a novel food process is intended to cover processes that could bring about significant toxicological or nutritional changes in the food. Prior to their introduction, the processes of freeze-drying, pasteurization, extrusion and irradiation are examples of what would have been regarded as novel processes. However, small temperature changes or changes to the design of a process vessel would not be regarded as novel.

Novel cereal grains: Biotechnology is the key

There is today no lack of food in the Western world, and an increased production of agricultural products is not necessary to satisfy the primary requirements. The spectrum of types of food products is to a great extent determined by the availability of raw materials. Today's product development is moving in the direction of producing raw materials with a design and composition suitable for the production of tailored food products.

Cereals have been bred and improved for food, feed and beverages for many thousands years through selection of spontaneous mutations and random hybrids. Genetic transformation is an additional tool for the breeder to introduce novel genes and cereals with new properties. Genetic transformation has been demonstrated in maize, rice and wheat, and recently also in barley and oat.³ The genetics of barley, and to some extent also oat, have been extensively studied, resulting in detailed mapping of the chromosomes. Extensive mutation research has been carried out, showing that it is quite possible to make mutants in barley because it is diploid. Barley's great potential is due to its "dormancy." Sequences for proteins coding for lipid transfer protein have been found in Norway, and a genetic library is to some extent available. In Denmark, genes affecting structure and composition of endosperm proteins have been found. It is this enormous potential that the cereals possess for biotechnology that opens the new future for barley, among other crops.

Improvement of proteins and lipids for use in food

Crossability between different species of cereals has been proposed as a key for developing new crop species. Characteristics of several wheat x barley and barley x rye hybrids have been developed. This has, for example, given barley higher protein content and hence better baking properties. An improvement of the protein quality has also been shown by altering the protein synthesis, by

crossing of genes in barley similar to the gene opaque-2 in maize (Bates et al.). This gives the possibility of producing barley with an improved nutritional protein value in countries where barley is a main food crop. Changes of only one gene have managed to produce protein that is 90% equivalent to skim milk protein. This suggests a tremendous potential of "undiscovered" genes that can be activated in special order to make cereal proteins more nutritionally adequate.⁴

Oat lipids have been shown to be a very potent factor for decreasing cholesterol in humans, which is important in prevention of coronary heart disease. Recent research has also shown oat lipids to be important in dermatology. To make use of the health benefits, it has been an important task to include the oat lipids in food products. Therefore the physical properties of oat lipids have been investigated in order to understand their functional significance during processing. Oat lipids contain high levels of polar components and the liquid-crystalline phases formed in interaction with water exhibit important functional properties during, for example, baking. Addition of 2% polar oat lipids has been shown to increase bread volume by about 40%. This increase has shown to be almost linear in the range of 1–2% of added amount of lipid.⁵ Today, several companies are about to offer oat varieties with up to 15% polar oat lipids to make use of the improved baking properties.

Hulless barley bran

Hulless or naked barley as well as waxy or low-amylose barleys have been rediscovered. Both low- and high-amylose hulless barley contain more dietary fibre than does normal amylose hulless barley. Hulless barley has a great potential in food both for preparing bran and flour for use in bakery products. There has been a renewed interest in hulless barley as a source of soluble fibre, which has been shown to effectively lower cholesterol. Novel food products based on hulless barley seem to have important potential not only for their health properties, but also due to their functionality and taste. One such product is hulless barley bran, which has applications in ready-to-eat cereals, high-fibre bakery products and for extraction of beta-glucans to be used in high-fibre products. The hulless bran is by definition a novel food from an ancient grain, and will give food products with desired nutritive properties.⁶

Components with antioxidant properties

It is recognized that populations who eat a lot of cereals tend to have a lower incidence of cardiovascular disease. Studies have shown that barley in particular is effective in lowering lipid levels in animals. The hypocholesterolemic agents have been identified as tocotrienols and seem to have significant clinical implications. Tocopherol isolated from green barley leaves seems to have antioxidant capacity. The attention plants have received as sources of biological active substances makes barley an interesting cereal in this aspect. Once these antioxidative compounds have proven to be non-hazardous, they will undoubtedly be concentrated in barley, most probably by biotechnological means, for use in foods and beverages.⁷

Oat has also been shown to have antioxidant effects, attributed, as in other cereals, to the presence of phosphatides and tocopherols, but also to that of caffeic and ferulic acid.⁸ As the food industry is interested in reducing lipid oxidation to extend shelf life, and to satisfy consumer demand for health benefits due to antioxidants, increased content of these components would be desirable.

Novel food products with barley or oat on the market today

In February 1995 the UK Advisory Committee for Food Legislation examined the problem of distinguishing between novel foods and food additives.⁹ The committee was asked to consider the legislative position of the product alkaline-hydrogen-peroxide-treated oat fibre, which was proposed as a source of additional dietary fibre in baked goods such as white bread and cakes. The committee concluded that this was to be characterized as a novel food, due to its properties and the fact that it was produced by a novel process. In the future the authorities will have to face many discussions like this.

Products with increased dietary fibre

Consumers seeking foods that contribute to both gastrointestinal function and control of blood cholesterol have induced several cereal companies to introduce a wide variety of breakfast cereals and bread with enhanced dietary fibre content. In the last few years waxy hulless barley has been used for this purpose, i.e., barley especially-produced as a result of breeding. Several large companies are incorporating barley, flaked or rolled. Barley has an equally good if not better cholesterol-lowering response than oat. Continued work by plant geneticists can most likely result in superior barley varieties with high nutritional value, good functional properties and desirable agronomic characteristics.

The Montana Agricultural Experiment Station, using back-crossing and mutation procedures, developed a high-protein, waxy starch, short-awned, nude barley called Prowashonudpana (PWSNP), which has become a successful product of the US company ConAgra.¹⁰

PWSNP gives the highest levels of beta-glucans and soluble fibre of any product available on the market today. The content of total dietary fibre is 38%, of which 15% is beta-glucan. This product has not only improved nutritional value, it also has excellent functional properties suitable for foods and beverages.

Oat milk: A new milk substitute

A novel food product to be found on the market in Sweden, and also recently in England, is oat milk. This has been called a high-tech product, a product generated by scientists at the University of Lund in Sweden. "Oat milk" is made from steamed-flaked oat that is wet-ground in water and mixed with rapeseed oil. Enzymatically active barley extract is added as a processing aid. The extract modifies the major components of the oat, the starch, rendering it digestible and ready-to-eat. The modification also gives oat milk good taste and suitable functional properties. "Oat milk" is meant to be an optional product, competing with soy milk, for persons allergic or intolerant to milk. The development of oat

milk and related products represents a technological advance, so this product is classified as a novel food.

Oat milk has a high content of polyunsaturated lipids. The high amount of natural vitamin A protects it from oxidation during storage. The composition of nutrients is well balanced, with a high content of dietary fibre. The product allows for the convenient addition of vitamins, minerals and flavour.

Oat milk has also demonstrated several health benefits for normal human subjects: concerning plasma insulin and weight reduction, decreasing total blood cholesterol and LDL cholesterol, and increasing HDL cholesterol.¹¹

ProViva: Novel food or functional food?

The physiology and microbiology of the intestinal mucous membrane and its effect on health is a subject of great interest for many scientists and physicians, and for the food industry. The balance between protective and harmful bacteria is crucial for our health and well-being. It has been shown that it is possible to supply protective bacteria via food. A research group at the University of Lund, Sweden, showed that an isolated strain of lactobacilli was growing in products rich in dietary fibre; this was in contrast to earlier isolated lactobacilli that favoured dairy products. A product was developed using oat as a base. This product, ProViva, successfully introduced to the Swedish market, has shown health benefits for persons with intestinal inflammatory problems and provides possibilities for patients with organ collapse to receive nutrients. But the most important market for the product is as a probiotic product based on oat, scientifically proven to be effective. This product, like "oat milk," is also suitable for people who should avoid milk in their diet.

Novel food in the future

New and creative development in combination with biotechnology and improved food processing technology will undoubtedly introduce vast amounts of new food products in the future. It will be a difficult task for the authorities to control these products if the legislation is accepted only in some countries.

Even if many of these novel foods represent an improvement for the consumer, risk evaluation of food additives incorporated in large amounts must be different from risk evaluation of conventional food additives. As the daily intake of these macronutrients could be large, the interpretation of the results of the tests could be difficult. Human studies are also needed to a much greater extent.

Concerning the novel food products on the market so far, including barley and oat, no harm has yet been seen. The approaches up to now have been well documented, and the cereal industries seem to be heading in the right direction.

Notes

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Image analysis for cereal improvement

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Because so many important food quality and grading factors are based on assessment of physical characteristics such as size, shape, color, and texture, image analysis (computer and machine vision) systems have been used routinely for many years for rapid and precise quantitation of diverse materials produced by the food industry. In its simplest form, image analysis is merely the computerized analysis of information obtained in digital form from various camera systems, spectral scanners, lasers (Chen et al. 1989) or nuclear magnetic resonance systems (McEntyre et al. 1995). In some instances the potential to secure automated measurements using digital cameras has led directly to automated on-line or at-line quality assurance systems for candies, bread and flour products, vegetables, seafood and a wide range of other food products or raw materials. For nearly 25 years, steady improvements in both computer and camera quality have resulted in cost reductions, speed improvements, and wider application. Structure, color, and other physical attributes of food can be measured both inexpensively and rapidly, to provide the processor with tighter controls during material handling.

Like many other segments of the food industry, digital image analysis of grains has been the subject of many studies over at least two decades. Sapirstein (1995) has reviewed the fundamentals of digital image analysis, and has described in some detail the hardware and software requirements in developing systems for grain analysis. Although Sapirstein's discussion focused primarily on wheat identification and characterization, very similar systems have been used to explore the relationships between quality and structure in other grains, including oat and barley.

Curiously, despite a long and extensive use of digital image analysis in grain research, there has been very little exploitation of the technologies during either grain handling or processing. In part this has been due to the need to integrate a large number of parameters in, for example, grain grading programs, and to a poor understanding of grain *structure* as it relates to quality. While crude differences in kernel shape or color may be measured and related to single quality parameters quite easily (e.g., Gebhart et al. 1993, Symons and Fulcher 1988a, 1988b; De Koeyer et al. 1993), subtle differences among varieties, and even among classes within a major grain class, are often quite difficult to define (Zayas

et al. 1986; Pietrzak and Fulcher, 1995). Some attempts to differentiate otherwise indistinguishable varieties of winter wheats using digital image analysis have been successful, but of little advantage for routine use (Symons and Fulcher 1988c, 1988d). The latter studies required the measurements of a large number of kernel traits.

Because grain quality is the product of a large number of characteristics, perhaps the major limitation to more widespread use of imaging systems in the industry relates to the fact that we have relied traditionally on linear statistical definitions that rarely accommodate synthesis of multiple inputs. Simple linear regression, for example, is the analytical method of choice in assessing the contribution of a *single* physical attribute on grain quality, although increased reliance on principal components analysis using software packages such as *Unscrambler* (Camo AS, Trondheim, Norway) has improved substantially our analytical opportunities for predicting grain quality.

Similarly, the relatively recent introduction of artificial neural networks (ANN) and fuzzy logic to image analysis systems (Eerikainen et al. 1993, 1994; Egelberg et al. 1994; Ruan et al. 1995; Egelberg et al. 1995) has further enhanced the ability to predict grain quality using an integrative approach to data handling. The *GrainCheck*TM 310 (AgroVision AB, Lund, Sweden), for example, is an image analysis system designed specifically for grain analysis. It is equipped with a digital color camera, a robust sample delivery system, and ANN-based software. It also contains a system for weighing samples, and it automatically defines shape, size, color and density characteristics of each sample. It is likely that it will find widespread application in monitoring and quality in grain handling systems, mills, product characterization, and perhaps breeding programs. ANN analysis of *multiple* quality inputs derived from digital image analysis should greatly enhance our ability to predict quality and performance of grain samples, both accurately and rapidly. The GrainCheck system is described by Egelberg et al. (1995).

While much of the emphasis on digital image analysis in the grain industry has concentrated on whole kernel characteristics, the use of this approach for routine measurement of *component* quality (e.g., of cellular storage compounds such as starch, bran, and lipids) has been relatively rare. One of the reasons for this apparent neglect is the fact that structural analysis of cereal components requires the addition of microscope systems to the image analysis unit. Also, cellular materials are usually only detectable using microscopy after they have been stained or otherwise made visible by time-consuming chemical procedures. While this approach is common in many cell research facilities, it has not been widely adapted to the food industry, although a few such systems have been developed for single component evaluation. One of these systems, the I440 Microscope Imaging System (Dipix Technologies, Ottawa, Ontario), has been available for some time to allow reasonably rapid measurement of a number of grain components, including starch granule size and shape, starch damage levels, lipid droplet concentration and distribution, and bran/hull content and particle size in ground products. For the most part, recent improvements in sample

preparation have removed initial difficulties, and it is now possible, for example, to measure starch granule size and shape in a small sample of ground grain in a matter of minutes. The high speed video capture board and rapid scanning stage of the I440 allows measurement of 300–400 fields of view in a few minutes (3–6 minutes, depending on the sample and other requirements). Figures 1A and 1B, for example, show starch granule size distributions in typical barley (1A) and oat (1B). Each figure represents data from approximately 20,000 granules, classified according to size. The bimodal distribution of the granules in the barley is readily apparent, while the much smaller, unimodal characteristic of the oat sample is quite different. Because the automated measurement procedure is now quite rapid, it is likely that routine use in breeding and quality monitoring programs that relate to starch quality can be improved considerably. Oliveira et al. (1994) have used a similar approach to evaluate the genetic aspects of starch granule traits in barley, and Harrigan (1995) has discussed both the instrument and its applications quite thoroughly. The microscopic approach to automated measurement of bran particles in flour, hulls and bran in oat and barley is expanding, as are applications in oat and barley processing.

The examples cited in this brief discussion represent but a fraction of the available information relating to digital image analysis of grain components at both the kernel and cellular level. The recent addition of both automated systems and principle component analysis/neural networks to the grain industry will substantially improve the speed and accuracy of grain quality evaluation. There are few if any substantial barriers to routine use of either macroscopic or microscopic image analysis systems in breeding programs, disease evaluation, component quality, or classification. As computer and video costs continue to decline, their use at all points in the grain development system will escalate.

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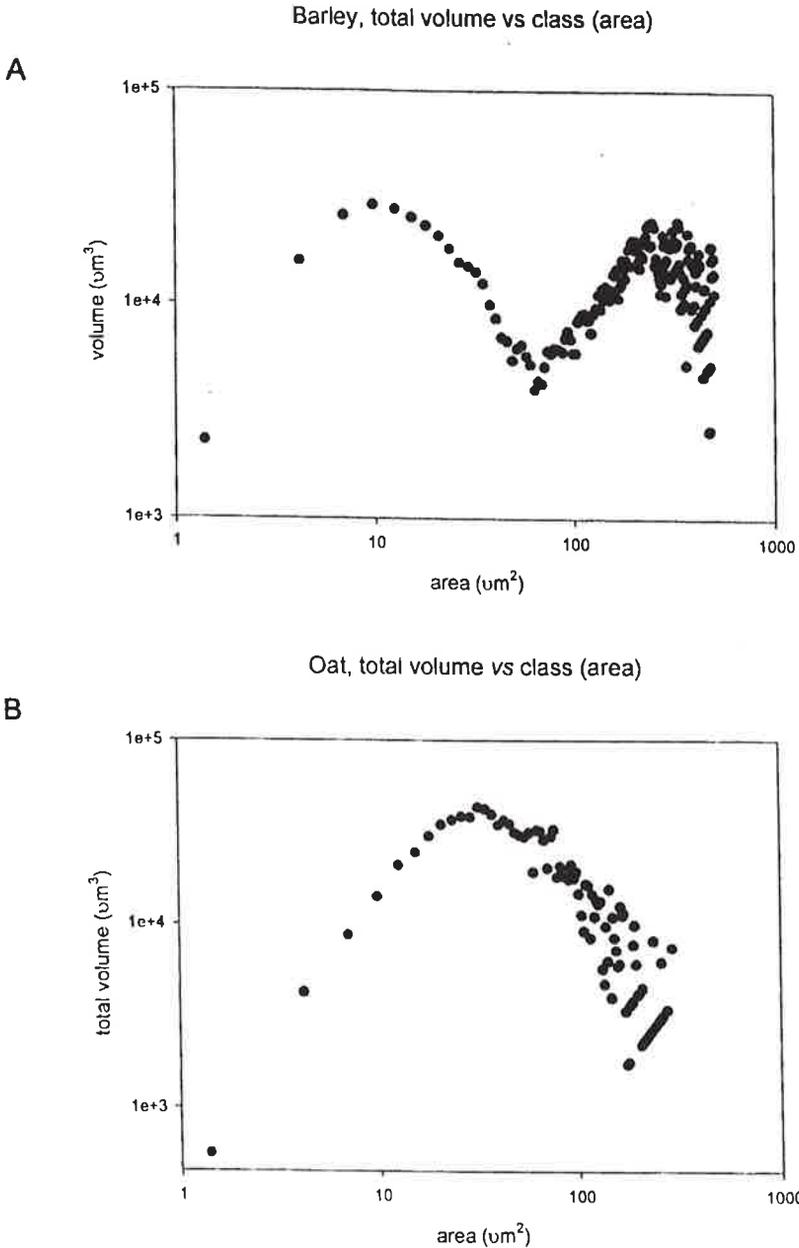


Figure 1. Comparative starch granule size distributions in barley (1A, top figure) and oat (1B, bottom) kernels obtained by rapid digital analysis using the I440 microscope imaging system. The distinctive bimodal distribution of granule sizes in barley is evident in 1A, while the unimodal distribution of oat starch is also obvious in 1B. Analysis time was approximately 3–5 minutes after initial grinding.

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Oat and barley as livestock feed – The future

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The historical approach to breeding cereal grains for livestock feeds has been to maximize yield per unit of land base. Plant breeders now are acquiring (or have) new capabilities for introducing novel traits into feed ingredients, and we also have new demands from a changing livestock industry. New demands for feed relate to nutritional quality, but perhaps not in the traditional sense. We have tended to regard quality in strictly nutritional terms – energy levels, amino acid composition and digestibility, to name a few. For the purpose of this presentation “nutritional quality” will be defined more broadly – simply put, what will command a premium in ingredient selection for feed in the years to come, and how might barley and oat breeders adapt their crops to meet emerging feed requirements.

Consumer preferences

As feed costs escalate, we encounter increasing consumer resistance to pay the premium associated with meat products that are perhaps preferred esthetically, but less efficient in feed conversion. Beef consumption has declined on a per capita basis due to competition from other meats, and with time it is likely that the grain fraction of the diet of beef animals will also decline. These changes continue to evolve and may increase in rate if the “tolerance level” of succeeding generations to poultry meat is cumulative. While barley in particular has gained a new-found acceptance into poultry diets with widespread application of beta-glucanase as a feed additive (Campbell and Bedford 1992), this shift has been restricted to local barley use – barley has not been accepted as poultry feed where imported barley competes with imported corn. Although the same technology also applies to oat, it has not found acceptance to the same extent. A pessimistic viewpoint would be that continued growth of monogastrics at the expense of ruminants will impinge on the barley/oat feed market; from an optimistic perspective the value of feed ingredients in monogastric feeds is higher than for ruminants.

Nutrient-dense diets

Nutrient-dense feeds (as typified by a corn/soy diet) have always been preferred in intensive livestock production, and this preference is likely to grow with improvements in animal genetics and nutrition as well as changing animal

demographics. Conventional barley and oat are accepted into such diets, but only at a substantial discount. While it appears logical to expect fibre to have low economic value in accordance with its low nutritional value, its value in nutrient-dense diets is actually highly negative. Conventional barley or oat is discounted by the cost of concentrated protein and energy required to overcome the dilution effect of the fibre, which is invariably expensive. While the need to continue to perfect hullless varieties is obvious, any success in improving the energy level through higher oil content, as well as higher or more digestible protein, will also make the crops more competitive in nutrient-dense feeds.

Nutrition and animal population

Feed efficiency is one determinant of the location of animal production units. Low feed input relative to the mass of animal product produced favours near-market production locations since transportation costs of the raw ingredients are comparatively less than the finished product. Feed ingredients have low moisture content, low spoilage potential, and are more conducive to economies of bulk handling than animal product. It is not a recent observation that intensive animal production coexists uncomfortably with urban populations; what is new is the growing awareness of these problems and their inclusion as a factor in diet formulation and ingredient selection (Williams and Kelly, 1994). This is already occurring on a wide scale – the volume of manure produced by livestock is enormous in terms of human equivalents.

Diet and waste

Nutrient-dense feeds obviously increase value at market destination with lower transportation costs; a less obvious effect is on manure output. Indigestible fibre will concentrate in the manure – thus a 10 per cent increase in dietary fibre could increase manure 30 per cent. While manure and odour are an immediate concern with a very vocal public, awareness has increased regulatory pressures to reduce specific water and soil pollutants arising from animal waste.

Ammonia release and ideal protein

Ideal protein is the concept of providing amino acids in animal feed in a ratio as close as possible to animal requirements, which enables nutritionists to reduce total protein provided in the diet. This reduces the metabolic cost of urea excretion, and lowers N-loss as a pollutant. Although the concept of providing very high quality protein is hardly new, recent availability (at competitive pricing) of second and third limiting amino acids (threonine and tryptophan) have made this a reality for swine diets. There is similar interest in poultry nutrition. Protein containing high levels of non-essential amino acids, such as proline and glutamic acids in hordein fractions of barley, will have even lower value, which could slip to negative value with ecotaxation in sensitive regions.

Phytate and phosphorus pollution

Excreted phosphorus arising from undigested phytate has also come under

increased regulatory scrutiny. High phytate crops have been discriminated against in some regions, and phytase feed additives have been developed (Simons et al. 1990), with partial success. It would clearly be preferable to develop low phytate crops, which could carry the benefit of reducing crop fertilization needs, as is currently being attempted with canola (Georges 1996).

Trace residues in animal feeds

Concern is frequently expressed over the repeated, heavy manure application on lands adjacent to animal facilities. This has become an issue in terms of heavy metals in grains destined for human food items; animal feeds are potentially a greater concern because animal products provide the opportunity to accumulate throughout the food chain.

Processing and grain characteristics

Interest in feed processing as it affects nutritional value and feeding characteristics has never been higher than in the feed industry today. Much attention has been directed towards engineering/technological aspects, and differences in processing behaviour between major feed ingredients. The development of certain processing attributes could be a major incentive in ingredient selection for animal feeds; for example, a premium is often paid for wheat in pelleted feeds due to its gluten content, which acts as a binder. This has assumed a new significance with the explosive growth in aquaculture, where pellet stability in water adds a new dimension. More extensive cooking, achieved through extrusion (or expansion), is increasingly popular in feed preparation, beyond its original applications in pet and fish feeds. One reason for extrusion is that high levels of fat are achievable while still maintaining a formed end product with minimal fat leakage. Feed ingredients with a high carrying capacity for fat would enjoy a premium in this market.

Pelleting or extrusion are normally associated with centralized facilities – the capital costs would be prohibitive for farm-mix feeds, for example. Less intensive operations would only grind the grain prior to feeding. Even with this simple operation there is realization that particle size distribution is an important determinant of animal productivity (Wondra et al. 1996). Grinding is essential, however, because generation of excessive fine particulates is detrimental for all species. For example, fines have been being linked to ulcers in swine and bloat in ruminants. Tempering, steam flaking or even pelleting are processing methods that endeavour to expose the endospermal nutrients without generation of fines.

While these traits are frequently discussed with reference to grain quality, there have been no systematic efforts to improve feed grains such as barley and oat. Desirable feed processing attributes are comparable in principle to desired characteristics in the human food and perhaps malting industries.

Novel traits

Recombinant DNA technology theoretically provides an opportunity to introduce many biological products in most plant systems. Many biologicals produced with

fermentation technology are used in feeds – some major products are vitamins, antibiotics and essential amino acids. When considered individually, vitamins are fairly low value at the inclusion levels employed in feed, and hence would not be a logical candidate; nor would antibiotics (or growth promotants), which have the added disadvantage of withdrawal requirements to avoid end product contamination, and are not necessarily compatible across species. This would be a regulatory nightmare, and if such products are to be produced in barley or oat it would be as a highly specialized crop designed to replace classical fermentation rather than for use as general feed. Although barley and oat may be the host plant for production of many such products, a primary consideration would be biological compatibility.

There are novel traits that are feed-specific and could increase value for barley and oat. For the most part, this would entail adapting barley and oat for specific target markets. Higher levels of limiting essential amino acids could displace synthetic amino acids added to the diet, and make the crops more compatible in diets formulated to low protein levels, as discussed earlier. Which amino acids would be most desirable again depends on animal species, as well as the value of individual synthetic amino acids. Presently, second and third limiting amino acids for swine (threonine and tryptophan) are considerably more costly than lysine or methionine (for poultry). Of course, past efforts to increase limiting amino acids in barley have not been commercially successful in the case of high lysine barley, illustrating that when introducing new traits there is always the cost associated with loss of other desirable features.

Fat is also a valuable nutrient in feeds, not only for the energy density that it provides but also for physical characteristics it imparts in finished feed relating to dust suppression, palatability and feed lubrication. Increasing fat levels would add value to barley and oat, as would modifying the fatty acid profile. There is growing interest in manipulating the fatty acid content of animal products with respect to omega-3 fatty acids, which is perceived to reduce risk of cardiovascular disease associated with consumption of saturated fats. Whole linseed is presently finding markets in North America for laying hens for the purpose of producing a healthier egg (Ajuyah et al. 1990; Farrell 1995). There are also more established markets for these fatty acids in fish feeds and pet foods. While fish have a high requirement for omega-3 fatty acids, their role in pets is partly cosmetic. Consumption of these fatty acids imparts a glossy coat, which is desired by discerning pet owners. A high omega-3 oat might find similar preference over conventional oat among horse owners.

Other biologicals that could be considered as candidates for introduction to barley and oat are various pigments. Feed-derived pigments are responsible for colouration of end products – for example, barley or oat will be discounted in markets accustomed to poultry pigmented with xanthophyll from corn by the cost of the added pigment. Xanthophyll addition would be a negative attribute for swine, where the hardness associated with saturated fat, and whiteness associated with lack of pigment, is a competitive advantage for barley over corn.

More exotic pigments are more valuable. Astaxanthin and canthaxantin are required by salmonids for the typical red/orange colouration of salmonid flesh. These

are presently provided as synthetics or from yeast-derived (Pfaffia yeast) product, and constitute a major component and cost of fish feed. With addition of these pigments to an ingredient (such as oat) containing moderate oil that facilitates their absorption, extraction and concentration may not be necessary.

Conclusions

In surveying the potential in barley and oat breeding in feed-related areas, it is apparent that, beyond conventional opportunities such as continued efforts in hulllessness, further developments in added value will probably depend on tailoring the crops to specific market segments. Barley faces an additional challenge – a large component of the barley marketed for feed is barley that fails to meet malting criteria. Any developments of specific feeding attributes will necessitate specific marketing as opposed to bulk marketing. Even moderate departures from conventional barley or oat require separate storage and transportation, and a willingness on the part of the end user to handle another ingredient. These factors must be addressed in assessing the economic viability of new directions in barley and oat breeding, as well as technical feasibility. Whether or not such initiatives would be worthwhile will also depend on overall feed demand – high demand and high prices as we are presently experiencing favour the historical approach of simply maximizing nutrient yield.

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Barley feed quality for beef cattle

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To define characteristics that determine barley feed quality for beef cattle is difficult. Most grain characteristics that are easily and routinely measured have not proved to be highly related to animal performance. Producers often use test weight as an indicator of barley feed quality, while research has not found a strong relationship between the two. We compared light weight (.54 kg/L) and heavy weight (.67 kg/L) Busch 1202 barley in finishing (85% barley) diets for beef steers, and found no difference in intake, gain or efficiency (Table 1). Hunt (1995) also reported that barley test weight was not a good indicator of feed quality, especially in the higher test weight ranges.

Rate of digestion

Rate of digestion in the rumen has been used as an indicator of feed quality. It is presumed that extremely fast rates of digestion lead to increased incidence of bloat, acidosis and founder, and to reduced animal performance. Kennelly et al. (1995) reported a correlation ($r = .72$; $P = .02$) between *in situ* DM digestion (ISDMD) after 12 hours of incubation and the percentage bloating when animals were fed 10 barley varieties. Hunt (1995) fed Russell and Steptoe barley varieties with widely differing rates of digestion, but no differences were found in animal performance. However, we found greater ADG and DM intake by cattle fed barley varieties (Chinook and Merlin) with faster rates of digestion compared with slower digesting varieties (Baronesse and Medallion; Table 1). If feeding management can be manipulated to prevent bloat and/or acidosis, then it appears that barley with high rates of digestion may result in improved animal performance.

Barley vs. other feed grains

Corn has often been reported to be the most profitable feed grain for finishing beef cattle, and frequently the statement is made that barley would be a more desirable feed grain if it could be made more similar to corn. However, Owens et al. (1995) reviewed the effects of grain source on feedlot performance in 565 comparisons involving over 23,000 cattle, and concluded that cattle fed barley gained faster than cattle fed corn, milo or wheat. These authors commented, "We were surprised at the remarkably high rates of gain for cattle . . . fed barley." In addition, they found that, although the metabolizable energy (ME) values for

corn and milo in their review were similar to the values predicted by the NRC (1984), the ME value for barley was greater than expected (3.34 vs. 3.04 Mcal/kg). Our mean predictions of NE_m and NE_g for barley based on 15 estimations (Table 1) is 2.16 Mcal/kg NE_m and 1.48 Mcal/kg NE_g . These values are similar to values of 2.14 Mcal/kg NE_m , and 1.47 Mcal/kg NE_g estimated for dry rolled barley by Zinn (1993). However, the energy value of barley can vary between varieties. Boss and Bowman (1996a) reported higher NE_m and NE_g values for Medallion barley than for Gunhilde and Harrington barleys.

Differences between barley varieties

Our research has demonstrated differences in animal performance between barley varieties (Table 1). Steers fed Harrington barley gained weight 8% faster, and had an 8.6% greater intake of digestible starch than steers fed Gunhilde or Medallion (Boss and Bowman, 1996a). In addition, steers fed Harrington had greater microbial protein synthesis and flow to the small intestine, and greater efficiency of microbial growth than steers consuming Medallion barley (Boss and Bowman, 1996b). In another study (Table 1), steers consuming Steptoe gained similarly to those fed corn, while performance by steers fed Harrington

Table 1. Performance by beef steers fed diets based on different barley varieties

Item	ADG, kg	DM intake, kg	Feed efficiency	NE_m , Mcal/kg	NE_g , Mcal/kg
Busch 1202, .54 kg/L	1.68	10.52	6.3	2.08	1.42
Busch 1202, .65 kg/L	1.72	11.07	6.4	2.00	1.34
Corn	1.43 ^c	10.00 ^c	7.0 ^c	2.26	1.55
Gunhilde, .64 kg/L	1.18 ^a	7.70 ^{ab}	6.5 ^b	2.32	1.61
Harrington, .63 kg/L	1.30 ^b	8.20 ^b	6.3 ^{ab}	2.35	1.64
Medallion, .62 kg/L	1.22 ^a	7.30 ^a	6.0 ^a	2.51	1.77
Baronesse	1.17 ^a	11.28 ^b	9.6	1.86	1.22
Medallion	1.27 ^{ab}	9.88 ^a	7.8	2.22	1.54
Chinook	1.48 ^c	11.73 ^b	7.9	2.06	1.40
Merlin	1.40 ^{bc}	11.13 ^b	8.0	2.10	1.43
Corn	1.61 ^b	12.2 ^c	7.9	2.09	1.42
Harrington	1.42 ^a	9.9 ^a	7.0	2.19	1.51
Morex	1.41 ^a	10.3 ^{ab}	7.3	2.13	1.46
Steptoe	1.66 ^b	11.4 ^{bc}	7.0	2.16	1.49
Corn	1.63 ^b	14.8 ^b	9.1 ^b	2.05	1.39
Gunhilde, .62 kg/L	1.50 ^a	12.2 ^a	8.3 ^a	2.15	1.47
Harrington, .65 kg/L	1.50 ^a	12.4 ^a	8.2 ^a	2.19	1.51
50%Gun/50%Har	1.54 ^a	12.8 ^a	8.3 ^a	2.13	1.46

^{a,b,c} Means in a column without a common superscript are different ($P < .10$).

and Morex was lower. Since varieties differ genetically, understanding the control of genetic differences in feed quality characteristics may help us to understand what grain characteristics to select for in developing new barley varieties with superior feed quality.

Improving barley feed quality

We are using the North American Barley Genome Mapping Project's (NABGMP) populations to identify, map and validate quantitative trait loci (QTL) for barley digestibility and processing characteristics. This will ultimately allow deployment of genes for improved feed quality via marker-assisted selection. The genetics of DM and starch digestibility of barley has been studied by analyzing 150 doubled haploid (DH) lines from a Steptoe/Morex cross developed by the NABGMP (Kleinhofs et al., 1993). The 150 DH lines were grown in replicated yield trials under dryland and irrigated environments, and each of the lines and the parents were subjected to ruminal fermentation, with the residues analyzed for DM and starch content. Agronomic and malting quality characteristics were also measured. Population means, ranges and coefficients of variation for each measured trait are presented in Table 2. Substantial variation exists among barley genotypes for starch, CP, malting characteristics, digestibility and particle size. The effect of environment (dryland vs. irrigated) on various traits and the heritability estimates for those traits are presented in Table 3. Environment and genotype had an effect on all measured traits. The heritability estimate for DM digestibility was .50, a promising value for a trait that likely depends on the action of several genes.

The data were subjected to QTL analysis using a medium saturation restricted fragment length polymorphism (RFLP) map developed by the NABGMP from the same DH lines and Mapmaker-QTL (Lander et al., 1987). Major QTL markers were identified for particle size after dry rolling (cracked barley), DM and starch digestibility of ground and cracked barley (to simulate processing used prior to feeding), ADF content, starch content and starch index (starch content x digestibility) of ground and cracked barley.

Interestingly, QTLs for particle size after dry rolling were located on chromosome 3, as were QTLs for DM and starch digestibility of cracked barley (Figure 1). When DM digestibility was regressed against particle size after dry rolling, linear relationships ($P = .0001$) were found for DM digestibility of dry rolled barley after 3 and 6 hours of ruminal incubation ($R^2 = .32$, $Y = 76.11 - .0251 X$, for 3 hours; and $R^2 = .33$, $Y = 90.61 - .0202 X$, for 6 hours). We know that grain digestibility is inversely related to particle size, so finding QTLs for both particle size and digestibility of cracked barley in the same region indicates that particle size has a large effect on the digestibility of barley that is cracked. Physical characteristics of barley after processing can also affect intake. Barley that had larger particles after dry rolling resulted in increased intake by steers.

Our data indicated that the primary region affecting digestibility of ground barley (no particle size effect) is located on chromosome 4 (Figure 2). This

Table 2. Population mean and range for traits in 150 DH Steptoc/Morex barley lines grown under dryland and irrigated conditions

Item	Dryland			Irrigated				
	Min	Max	Mean	CV	Min	Max	Mean	CV
Starch, %	38.3	60.8	49.8	7.5	41.0	76.0	55.2	10.2
CP, %	12.0	17.5	14.2	7.6	10.3	16.6	12.1	8.1
ADF, %	3.4	6.8	4.9	11.4	-	-	-	-
Particle size, um	1074.7	2295.5	1359.4	13.5	-	-	-	-
<i>In situ</i> digestibility, %								
DM, 3 h cracked	23.2	57.1	42.5	16.0	-	-	-	-
DM, 6 h cracked	44.7	74.5	63.5	8.6	-	-	-	-
DM, 6 h ground	68.6	82.8	77.3	3.1	67.1	85.0	76.1	5.4
DM, 24 h ground	-	-	-	-	75.4	91.8	84.9	4.4
Starch, 3 h cracked	24.0	69.1	50.5	18.9	-	-	-	-
Starch, 6 h ground	88.3	98.2	95.3	1.9	90.2	99.3	95.8	2.8
Wort protein, %	3.3	6.8	4.8	13.7	3.4	6.1	4.4	12.6
Soluble/total CP, %	25.6	42.9	33.3	11.3	27.6	46.8	36.1	10.5
beta-glucan, %	.3	2.4	1.1	34.4	.4	2.4	1.2	37.4
alpha-amylase, 20°	18.9	46.9	30.0	14.5	20.1	47.6	32.2	14.9
Malt extract, %	70.1	80.2	75.5	2.2	72.1	81.9	77.7	2.1
Plumpness, %	59.9	97.4	88.3	7.8	44.8	97.1	85.3	10.5
Kernel weight, mg	30.7	46.6	38.2	7.2	29.6	46.1	37.7	8.2
Kernel color	52.0	81.0	69.1	7.4	54.0	83.0	69.9	8.6
Wort color	1.1	2.4	1.5	13.5	1.0	1.9	1.4	10.5
Wort clarity	1.0	3.0	2.7	20.0	1.0	3.0	2.8	13.3
Diastatic power,°	44.0	202.0	95.0	25.7	63.0	170.0	96.3	20.2

Table 3. Heritability estimates and environmental effects in DH Steptoe/Morex barley lines

Item	Dryland	Irrigated	SE	Pr > F Environment	Pr > F Genotype	Herita- bility
Starch, %	49.8	55.2	.34	.0001	.003	.58
CP, %	14.2	12.1	.05	.0001	.0001	.90
<i>In situ</i> digestibility, %						
DM, 6 h ground	77.3	76.1	.21	.07	.001	.50
Wort protein, %	4.8	4.4	.03	.0001	.0001	.71
Soluble/total CP, %	33.3	36.1	.26	.0001	.0002	.70
beta-glucan, %	1.1	1.2	.02	.02	.0001	.69
alpha-amylase, 20°	30.0	32.2	.26	.0001	.0001	.73
Malt extract, %	75.5	77.7	.10	.0001	.0001	.77
Plumpness, %	88.3	85.3	.55	.0001	.0001	.48
Kernel weight, mg	38.2	37.7	.17	.02	.0001	.67
Kernel color	69.1	69.9	.31	.04	.0001	.53
Wort color	1.4	1.4	.01	.01	.0006	.42

would imply that independent selection could be made for both digestibility and processing characteristics. The ADF content of barley mapped to a similar region on chromosome 4 as digestibility of ground barley. Barley CP, soluble/total CP, and wort CP all exhibit QTLs on chromosome 4 in the same region as the QTLs for digestibility are found. Another set of QTLs were identified for digestibility of ground barley on chromosome 5 in a region that contains QTLs for the hordein proteins.

Grain characteristics that map to the same loci as digestibility appear to have a relationship with endosperm degradation, both during germination and in the rumen. This suggests that specific proteins may be critical in determining the ability of ruminal microorganisms to digest barley. These components might include amylase and/or proteinase inhibitors.

Starch vs. protein

Early barley feeding trials conducted at Huntley, MT, demonstrated no advantage in gain or efficiency to feeding high amylose Glacier barley (40% amylose) compared to normal Glacier (20% amylose) in either wintering (44% barley) or finishing (80% barley) diets to beef steers. Hunt (1995) and others have suggested high starch content, rather than the type of starch, is important in determining feed quality for barley. Boss and Bowman (1996a) found a linear relationship ($P = .0001$; $R^2 = .37$; $Y = .52 + .22 X$) between ADG and digestible starch intake for steers fed corn or one of three barley varieties. As digestible starch intake increased, animal performance improved.

In correlation analysis of the 150 Steptoe/Morex genotypes, grain CP was negatively correlated with DM digestibility ($r = -.28$; $P = .0007$), starch

Intake

Intake is an important criterion for barley feed quality. Factors affecting intake are numerous, and often difficult to identify; however, nutritionists agree that higher intakes result in greater animal performance. When diets based on corn and on Harrington and Steptoe barleys were compared, gain and intake by cattle fed corn and Steptoe were greater than when Harrington was fed (Milner et al., 1995). We compared feeding behavior by these steers, and found that steers fed Steptoe spent a longer time eating and had more numerous feeding events during the day than steers fed Harrington. Intake of barley diets often is lower than intake of similar diets based on corn, and this can result in reduced animal performance (Boss and Bowman, 1996a). Varietal differences in feeding behavior may be related to intake and animal performance.

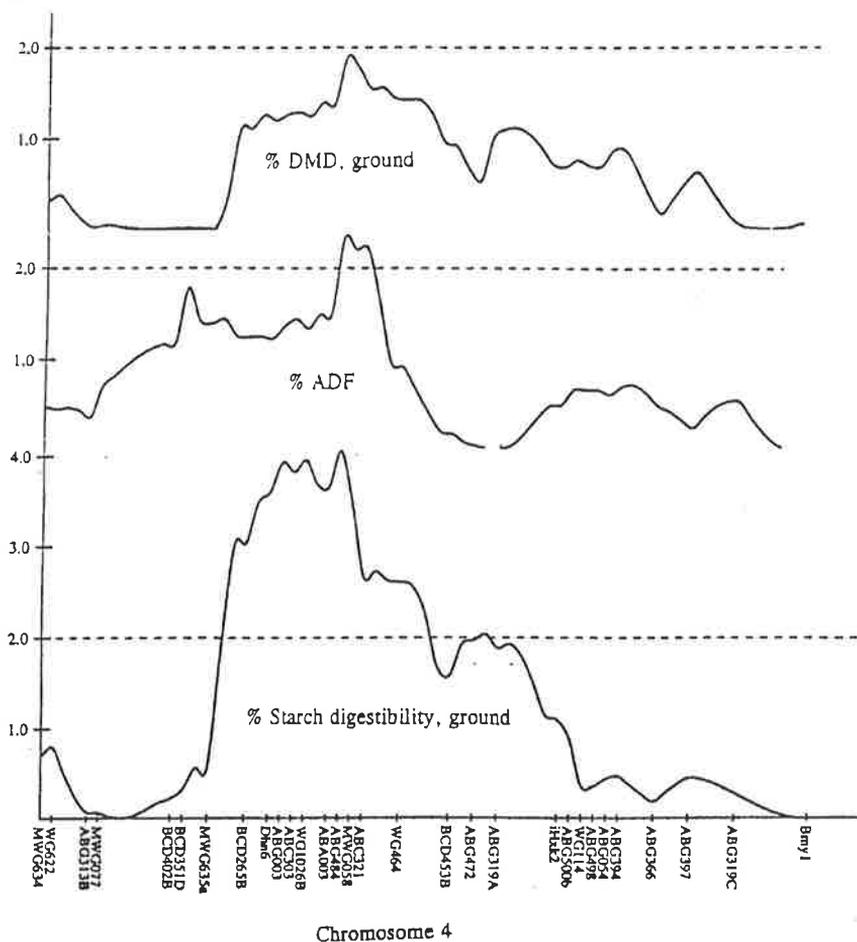


Figure 2. LOD score at marker locations on barley chromosome 4 for ADF, DM and starch digestibility of ground barley

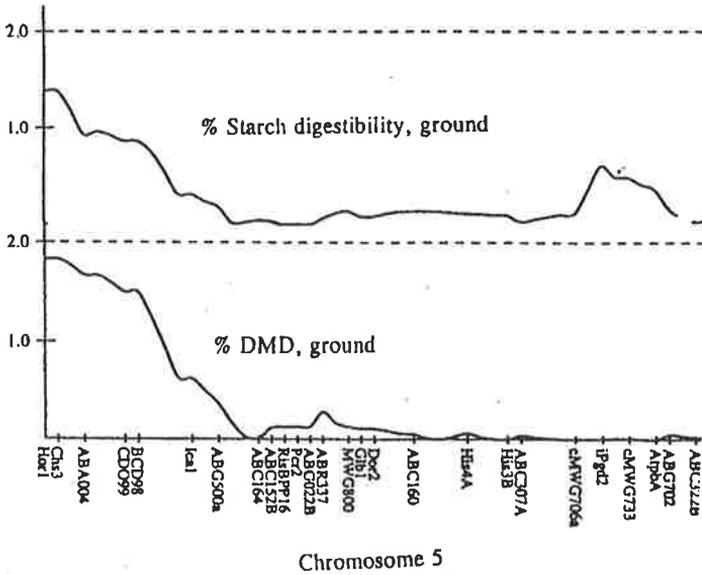


Figure 3. LOD score at marker locations on barley chromosome 5 for DM and starch digestibility of ground barley

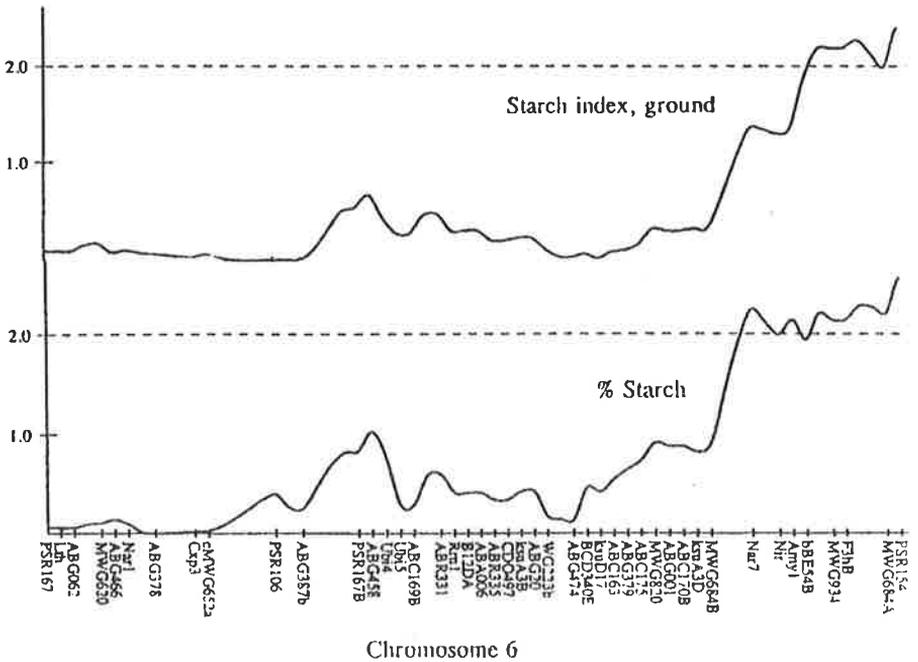


Figure 4. LOD score at marker locations on barley chromosome 6 for starch and starch index of ground barley

Summary

Various barley characteristics under genetic control appear to be related to animal performance. The identification of genes or regions on barley chromosomes that control digestibility or processing characteristics would allow the rapid selection of barley varieties specifically designed for feed quality characteristics.

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Barley and oat improvement for non-ruminants

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Over the years the cereal grains barley and oats, particularly barley, have been used extensively as feedstuffs for monogastric animals. The relatively high fiber content of barley and oats has been a limiting factor with regard to their utilization in the diets of rapidly growing animals. In addition, antinutritional factors, particularly the content of beta-glucan, have decreased the use of these feedstuffs. Advances in genetic selection and the advent of the use of exogenous enzymes have expanded the usage of barley and oats as a quality feedstuff for all classes of monogastric animals. This paper will review the composition of barley and oats in terms of their nutritional quality. Improvements in nutritive quality will be highlighted and future potential advances will be identified.

The chemical composition of barley and oats, as summarized from several sources by Slominski (1991), is given in Table 1. From a nutritive standpoint, starch provides the largest portion of bioavailable energy (BE) to the animal, followed by protein and to a lesser extent fat and soluble sugars. The dietary fiber components, while representing a significant component (18.7–28.1% DM for barley and oats, respectively), contribute little to the energy content of the grain, acting primarily as a diluent (i.e., cellulose and lignin) or as a potential antinutritive component (i.e., beta-glucan and arabinoxylan). The fiber components of barley and oats have been reported to be a sensitive predictor variable for BE value, but other chemical parameters have been indicated as having weak or no relationships (Bhatty et al., 1974; Coates et al., 1977; and Zhang et al., 1994). The ability to predict the BE of cereals is of importance because of the relatively wide range in values as a consequence of genetic and environmental factors. In recent studies, Campbell (1996) demonstrated that two-rowed barley had higher BE than six-rowed barley grown in the Canadian prairies. Similarly, Jeroch and Dänicke (1995), in summarizing data from several European sources, indicated a higher metabolizable energy value for poultry in two-rowed barley than in six-rowed types. Campbell et al. (1994) demonstrated considerable environmental influence on the composition of six different varieties of barley grown at 12 locations throughout Manitoba during several years. In that study, genetic-environmental interactions were also evaluated and fiber components as estimated by neutral detergent fiber showed a greater effect than did protein. The implication of this in terms of the importance of fiber as a

Table 1. Chemical composition of barley and oats (% DM)

Component	Barley	Oats
Protein	12.6	14.1
Sugars		
Glucose and fructose	0.3	0.2
Sucrose	1.4	1.1
Galactooligosaccharides	0.3	0.3
Fructosans	0.6	0.3
Starch	60.4	46.8
Dietary fiber		
Cellulose	5.0	8.7
Beta-glucan	4.7	3.0
Arabinoxylan	6.7	8.9
Lignin and others	2.3	7.5
Fat	3.0	4.7

Source: Slominski, 1991 (data from Henry and Saini, 1989; Henry, 1985; Bach Knudsen et al., 1986; Bach Knudsen, 1994; Aman et al., 1985, Aman and Newman, 1986; Aman and Graham, 1987; Theander et al., 1989; Sibbald and Price, 1976).

predictor of nutritive quality requires further study.

The composition of selected amino acids in barley and oats is given in Table 2. The values for lysine, threonine and methionine are low relative to the requirements of growing animals; oats demonstrate a better amino acid profile than barley, especially for lysine. This latter difference may be explained by a lower contribution of prolamine protein in oats than barley (Lockhart and Hurt, 1986). Improvements to the nutritive content of cereals by increases in protein content have been somewhat counter-productive due to the negative correlation between the content of essential amino acids and protein percentage, especially for lysine (Boila et al., 1996). In this regard the potential adverse effect on protein quality through the development of high-protein cultivars appears to be of less concern in oats (Peterson and Brinegar, 1986). Another approach to improved quality has been the selective breeding for high-lysine content, although this has generally resulted in lines with inferior agronomic characteristics (Eggum et al., 1995). Recently Eggum and coworkers reported that a new high-lysine barley mutant showed promise in terms of both nutritional and agronomic characteristics. In addition to an enhanced content of lysine, the high-lysine barley mutant also demonstrated a reduced content of the amino acids glutamic acid and proline (Table 2). Since these latter amino acids do not contribute directly to the nutritive quality of the protein, their reduction represents an advantage due to the potential for a decrease in nitrogen pollution via animal waste. Further work in this area is warranted.

Starch is a major constituent in barley and oats, and contributes significantly to the BE value of these cereals for animals. In human nutrition the bioavailability

Table 2. Content of selected amino acids in barley and oats (g/100 g protein)

Amino acid	Barley ¹	Oats ²	High-lysine barley ³
Lysine	3.15	4.20	4.73
Threonine	3.00	3.30	3.86
Methionine	1.88	2.50	1.71
Glutamic acid	23.44	23.90	15.20
Proline	11.07	4.70	6.63

¹Boila et al., 1996; ²Peterson and Brinegar, 1986; ³Eggum et al., 1995.

of starch is of concern and in this regard there appear to be important implications of the amylose and amylopectin contents (Bijörch et al., 1990). Because of the high bioavailability of starch in swine and poultry and due to the lack of a potential for grain processing to affect starch quality in livestock feeds, these concerns are of less significance in animal diets. However, due to the availability of barley and oat varieties with varying amylose and amylopectin concentrations, further research in the animal area will undoubtedly occur. Realistically, relative to other characteristics of nutritive quality, starch composition should be given a low research priority.

As indicated above, the major influence of the dietary fiber component of barley and oats on nutritive quality is as a diluent in terms of energy density and as a potential antinutritive factor. Of the known antinutritive factors (trypsin inhibitors, tannins, alkylresorcinols, viscous polysaccharides) only the polysaccharides, beta-glucan and arabinoxylan are considered of significance from a physiological point of view (Jeroch and Dänicke, 1995). Barley and oats in comparison to other cereals contain a relatively high content of beta-glucan (Table 3). While the total content of arabinoxylan in barley and oats is comparable to that of other cereals, the soluble component of these polysaccharides is considerably less (Table 3). It has been well established that the antinutritive effects of beta-glucan and arabinoxylan relate directly to the soluble fraction of these polysaccharides.

While the content of beta-glucan may vary substantially due to genetic and

Table 3. Total and water-soluble beta-glucan and arabinoxylan contents of cereal grains (g/kg DM)

Cereal	—Beta-glucan—		—Arabinoxylan—	
	Total	Soluble	Total	Soluble
Barley	49.5	32.8	64.7	5.5
Oats	38.3	24.2	86.9	5.7
Wheat	7.4	6.5	75.3	13.4
Rye	21.5	7.7	96.5	29.5

Source: Adapted from Jeroch and Dänicke, 1995.

environmental effects, it would appear that the most effective means of overcoming the antinutritive influence is through the use of exogenous dietary enzymes (Campbell and Bedford, 1992). In the last decade, considerable advancement has occurred in the commercial utilization of dietary enzymes to enhance the nutritive value of cereals for monogastric animals, particularly poultry (Annison and Choct, 1991; Chesson, 1993). The dietary enzymes improve the nutritive value of the cereals by depolymerizing the soluble polysaccharides that inhibit nutrient digestion. In this regard, relatively few cleavages of the polysaccharide chain are required to achieve the effect. As a consequence, a direct contribution to the energy content of the feed does not result, since complete digestion of the polysaccharide to allow absorption of the liberated monosaccharide sugars does not occur in the small intestine. However, an indirect effect on feed energy content through enhanced hindgut fermentation may occur. Advances in enzyme development may in the future result in the generation of enzyme mixtures capable of achieving complete depolymerization of the cereal polysaccharides. The importance of this from a nutritional standpoint is dependent on the constituent sugar content of the polysaccharides, and future strategies for genetic improvement of barley and oats need to be coordinated with developments in the use of exogenous enzymes to enhance nutrient content of cereals.

Despite the availability of genetic lines of both hulless barley and hulless oats, and research data indicating the potential nutritional value of hulless material, there is not widespread production and use of hulless varieties as feedstuffs for swine and poultry. Table 4 summarizes the production performance of animals fed diets containing hulless barley or oats in comparison to control conventional diets. In western Canada there has been a renewed interest in hulless barley, and further research is needed to document the advantages of this crop in terms of handling and storage characteristics, enhanced nutrient density and availability, and reduced potential for environmental pollution (Hickling, 1995). With increased acceptability of this crop at the commercial level, the opportunity to exploit the genetic potential of

Table 4. Productive performance of animal fed hulless barley or oats

Test animal	Test cereal	Control diet	Performance trait	Hulless as % of control
Growing pigs	Hulless barley	Barley	Feed:gain	92.9
Growing pigs	Hulless barley	Barley	Feed:gain	94.8
Laying hens	Hulless barley	Wheat	Feed conversion	101.8
Roaster chickens	Hulless oats	Corn	Feed:gain	102.0
Broiler chickens	Hulless oats	Corn	Feed:gain	101.4
Broiler chickens	Hulless oats	Oats	Feed:gain	88.0
Growing pigs	Hulless oats	Corn	Feed:gain	97.2

Source: Data summarized from published literature.

hulless barley will increase. Research information is needed on nutrient availability and response to exogenous enzyme use for both swine and poultry. This information needs to be coordinated with genetic improvement strategies to allow for maximum improvement in the nutritive value of barley. Due to a lesser acceptability of oats at the commercial level, the use of hulless oats as a niche crop might be a more appropriate strategy.

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Hulless oat – Building a commercial future

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Introduction

Optimism for naked oats was expressed at the 4th International Oat Conference held in Adelaide in 1992. A combination of new varieties and market development in the United Kingdom (Valentine and Clothier, 1992; Cuddeford et al., 1992; Mason, 1992), Canada (Burrows et al., 1992) and in Australia (Farrell et al., 1992) was the basis. Marshall and Weaver (1992) were cautious about the future for the crop in the United States. The aim of this paper is to review progress since the 1992 conference and to present a strategy to build a stronger future for hulless oats.

Progress since 1992

Varieties released

At least six new varieties have been released in North America and the United Kingdom since 1992 and five lines are planned for release in 1996 or 1997 (Canada, the US, Australia). Breeders claim that these varieties improve key traits including yield, percentage of naked seed, disease resistance, lodging resistance, grain size and density.

The two most prolific programs have been led by Vern Burrows (Agriculture Canada, Ottawa) and John Valentine (Welsh Plant Breeding Station [WPBS], Aberystwyth). The Ottawa program has released five varieties since 1990, with registration of a further two pending. Since the release of Rhiannon, Kynon and Pendragon, the WPBS program has released Neon and Ripon (spring types) and Krypton and Harpoon (winter types), with two further releases pending. The major advantages of these lines are increased percentage of naked grain, shortness and stiffness of straw (Neon, Bullion), grain yield (Ripon, Bullion), better winter hardiness (Harpoon) and resistance to stem eelworm (Harpoon). In yield trials conducted in 1994, the “untreated” yield of the three winter

cultivars was 71–72% of the control (husked) group, while the spring types yielded 79–81% of the husked controls (NIAB, 1995).

Two US programs have released or are about to release their first naked varieties. 'Paul' was released by the North Dakota breeding program in 1994, while two breeders lines (88Ab3073, 86Ab1616) are planned for release from Idaho. Paul has good crown rust, stem rust and BYDV resistance, and groat yields are competitive with current husked varieties. No breeding of naked oats is conducted in Ohio, Minnesota, South Dakota or Michigan. Breeding efforts may result in a release from Iowa before the year 2000 (Holland, pers. comm.)

Breeding efforts in South Australia have concentrated on improving yield (compared to Bandicoot, which achieves 65–70% of the husked control Echidna), percentage of naked grain and disease resistance. The next planned release meets all of these objectives and has a larger grain size than Bandicoot. However, it does not have reduced trichomes. In Western Australia, a small program is underway, with approximately five crosses made per year. Lines enter advanced trials in 1996.

In Brazil, a crossing program was initiated in 1993 (Federizzi, pers. comm.) to produce adapted cultivars, but releases are not expected in the near future. In South Africa, attempts have been made to commercialize "Bandicoot" naked oats. Bandicoot is well adapted to Cape Province in photoperiod and plant type, but, in contrast to Australia, it has proved very susceptible to crown rust. A large poultry producer (Rainbow Chickens) and a research agronomist (J. Platt, Zylem CC) have developed production packages for Bandicoot for chicken feed. Naked oat germplasm with resistance to South African crown rust races is under evaluation. In Finland, a program began in the early 1970s and advanced breeders lines are now under test (Peltonen-Sainio, 1994). Breeding programs are also underway in Germany, Russia, China, France, Hungary and the Czech Republic.

Area sown

On the world scene, naked oats are still a very small crop. Estimates of areas sown in other countries were obtained from a survey in March 1996. Approximate areas are: Canada 10–20,000 ha; US <2000 ha; Australia 4,000 ha; UK 4500 ha; France 350 ha. Naked oats are grown in central Europe and in Russia, but the authors were unable to obtain estimates of the area.

Characterization of feed value

The collaborations and contributions of breeders, economists and animal nutritionists on three continents has led to a comprehensive data set for naked oats in rations for pigs, poultry, dairy cattle, beef cattle and horses (Table 1). This research allows commercial feed formulators to insert accurate values for the feed value of naked oats into their ration formulation software. In Australia, Bandicoot naked oats is included in "AUSPIG," a national database of nutrition information accessible to all feed formulators.

There are large differences in the composition of naked oats grown on different continents (Table 2). Canadian and US varieties tend to be very high in protein

Table 1. A sample of the key publications on the use of naked oats in animal diets

Animal	Reference
Pigs	Morris and Burrows (1986); Friend et al. (1989); Van Barneveld et al. (submitted); Friend et al. (1988)
Poultry	Cave and Burrows (1993); Brenes et al. (1993); MacLean et al. (1994); Farrell et al. (1992)
Cattle	Givens and Brunnen (1987)
Horses	Cuddeford et al. (1992)
Sheep	Martin (1990)
Turkeys	Farrell et al. (1992); MacLean et al. (1994)

Table 2. Comparison of the nutritional profile of naked oats from Australia, UK and Canada

Trait	Unit	Bandi-coot ¹ (Aust)	Rhi-annon ² (UK)	Tibor ³ (Can.)	Terra ⁴ (Can.)	Pen-nuda ⁵ (US)
Crude Protein	% DM	13.7	11.9	18.4	13.4	20.1
Ether Extract	% DM	10.0	9.7	5.8	6.6	5.1
Lysine	g/kg DM	6.0	5.0	6.5	5.9	-
Neutral detergent fibre	% DM	14.8	14.1	-	-	-
Acid detergent fibre	%	2.6	5.1	-	-	-

¹Barr and Teague, 1995; ²Doyle and Valentine, 1988; ³Friend et al., 1988; ⁴Christison and Bell, 1980; ⁵Marshall, pers. comm. (from 1995 C.N.O.T.)

(>18%) and low in fat (<6%), whereas UK and Australian grain is lower in protein (11–13%) but considerably higher in fat (9.7–10%).

Building a commercial future

A survey of breeders of naked oats in March 1996 combined with a literature search identified eight factors that limit the acceptance and growth of the naked oat crop.

Developing markets

Most breeders involved in improving naked oats rated the lack of markets as one of the key limitations to expansion. Great efforts have been made in the UK (WPBS, Superioat Co. Ltd.), North America (Burrows, Marshall, Weaver et al.) and Australia (Barr and Teague, 1995) to overcome the lack of high price and speciality markets. A continuing problem is the lack of product to fully exploit these markets. This problem can be solved by simultaneously making the crop more competitive agronomically and convincing farmers that markets do exist.

Improving yield

The yield potential of the naked oat crop is one its most serious limiting factors, with most current varieties yielding 65–80% of the husked control varieties (Anon., 1995; Barr and Teague, 1995; Marshall, pers. comm.). Jones et al. (1986) calculated in the naked oat Rhiannon that the yield of groat plus lemma/palea was below the yield of groat plus lemma/palea in covered oats.

Increasing the genetic yield potential of naked oats is a crucial yet daunting task as genetic variation for yield in the husked oat germplasm pool is also limiting in some environments. In comparison, much greater resources are invested in breeding husked oats in almost all programs. Further, the germplasm pool in naked oats is relatively narrow, with many current lines only 3 to 5 generations removed from ancestral Chinese naked cultivars. In moisture stress conditions, we have circumstantial evidence that naked oats are inferior to husked varieties. Our hypothesis is that the thin, papery lemma and palea of the naked oat are a less effective barrier to desiccation than the more heavily lignified tissue of the husked oat.

What is required to improve the yield potential of naked oats? The following strategies must be considered: broadening the genetic base of naked oats; instigating more aggressive breeding systems for improvement of yield such as recurrent selection; physiological studies that examine the components of yield in naked oats (e.g., Saastamoinen et al., 1992); and studies specifically seeking to understand the grain filling process and mapping genes conditioning the naked trait to examine if the adjacent regions of the chromosomes are conserved (O'Donoghue et al., 1995). It is possible that flanking regions are conserved and carry genes (pleiotropic or closely linked) that limit naked oat yield.

Most hullless varieties have been released into production systems developed for husked oats, and a lack of specialized “management packages” has often been cited as a limitation. Two exceptions are the “Naked Oat Husbandry Guide” for the UK (Hayes, 1992) and “Bandicoot Naked Oats – a farmer’s guide” (Barr and Teague, 1994) for southern Australia.

Increasing the percentage of naked oats

Early naked oat lines often showed incomplete and variable expression of the naked trait. McKenzie et al. (1981) noted that cv Terra showed approximately 5% covered seed, as does cv Tibor (Burrows, 1986). The high-yielding variety AC Belmont averaged 88% naked grain in the 1995 Coop Naked Oat Tests (Marshall, pers. comm.). This problem reduces the value of the naked oat grain by a) precluding the direct use of the grain for rolled oat products, b) making dehulling and/or recleaning a required step, c) (marginally) lowering feed value, due to high fibre content of the husk, or d) spoiling the appearance of the sample. This variable and low expression of the naked grain trait can be contributed to at least five causes:

Genetic: The naked grain trait is conditioned by one major and at least two minor genes (Valentine and Clothier, 1992; Atiyya and Williams, 1976).

Modifying genes are almost certainly important as well. Most breeders of naked oats have found genetically homogeneous lines, which may average between 90% and 100% naked grain over environments with large genotype x environment interactions. Recent releases (e.g., Harpoon (UK) and Baton (Canada)) have very high levels of expression of the naked trait, in excess of 98%. Bandicoot grown in Australia usually has less than 5% husked oats, yet when grown in UK trials it produced over 50% husked grain (Mason, pers. comm.).

Seed admixture: If covered oat varieties are mixed with naked oats, the frequency of husked oats increases over generations due to their higher average yield and germination.

Outcrossing: We have observed outcrossing between adjacent naked and husked varieties. The florets of naked oats may open under some environmental conditions, allowing foreign pollen to enter.

Application of 2,4-D amine, dicamba and MCPA herbicides: The major gene conditioning the naked trait is regulated by auxin and when auxin-like herbicides are applied at critical growth stages, the expression of the naked trait can be severely affected. In one experiment conducted in South Australia in 1993, the percentage of naked grains was reduced from 94% in the untreated control to 83% where 1 L ha⁻¹ of 2,4-D amine was applied. Herbicides that have different target sites do not affect the expression of the naked trait. These include the aceto lactate synthase inhibitors (chlorsulfuron, flumetsulam), bromoxynil, terbutryn, diuron and diflufenican. Marshall (pers. comm.) also reports this problem with 2,4-D amine, 2,4-D ester, MCPA and MCPA + dicamba. No problems were observed with bromoxynil or low rates of MCPA + dicamba.

Environmental causes: Marshall (pers. comm.) believes that the interaction of environmentally induced stress (due to low soil temperatures, water logging or early drought with developmental stage) may cause varying expression of the naked grain trait. Hence, it is common for large genotype x environment interactions to affect the percentage of naked oats. For instance, Belmont ranged from 72% to 95% naked in the 1995 Coop Naked Oat Tests.

We are confident that breeding and agronomic strategies are available to overcome all the causes of incomplete expression from the list of limiting factors.

Improving the feed utilization of naked oats

Most breeders of naked oats have targeted high-priced speciality feed markets, where the high energy content of naked oats obtains a premium. These have included weaner pigs, broiler chickens, race horses, young cattle, wild birds and aviary birds. Recent research has better defined the role and optimized the use of naked oats in animal diets (Table 1).

The beta-glucans in naked oats cause lowered food intake, depressed growth rates and “sticky faeces” in chickens (Burrows et al., 1992; Farrell et al., 1992). Marked responses in growth rate, feed conversion ratio and fat digestibility were achieved by the addition of beta-glucanase (Brenes et al., 1993; Farrell et al., 1992) with additional responses to phytase and protease. Burrows et al., (1992) reported that addition of antibiotic and vitamins A, D3 and E also improved the

performance of broilers where “sticky faeces” syndrome was evident. Subsequent research by Farrell and Takhar has shown that pelleted feeds were superior to mashed feed and that older birds were better able to digest the fat in naked oats. Layer hens perform well on Bandicoot naked oats, producing larger eggs with superior yolk colour and an elevated level of polyunsaturated fatty acids (Barr and Teague, 1995). Burrows et al., (1992) also report increased egg size due to inclusion of naked oats in diets. Artificial enzymes are now commonly used in poultry diets and represent only 2–4% of the compounded food cost. Hence, the need for an enzyme in order to achieve maximum productivity should not be a constraint. Pig and poultry research has led to market demand in eastern Canada and to a small extent in Australia. However, in Australia, it has not been possible to obtain the predicted metabolizable energy from naked oats. Cuddeford et al. (1992) presented encouraging data for race horses that were fed diets containing naked oats. This research plus market development from several groups has facilitated the development of markets for horse feeding in the UK, Japan and North America. The Japanese market is supplied by the UK and with Bandicoot from Australia. This is a high priced but small volume market.

Reducing the trichomes on naked oats

Oat groats have silicon-impregnated hairs called trichomes adhering mostly to the distal end. In hulled oats they are covered by the husk, and relatively few are removed during harvesting. In naked oats, the trichomes are exposed, and during threshing and handling they are easily dislodged. These “free” trichomes cause serious problems, including: severe physical irritation, severe hay fever, allergic and asthmatic responses, lowered “palatability” in some livestock feeds, and low morale in plot harvester and grain handling crews! By adhering to all metal, even vertical, surfaces on harvesters, fires have been started under Australian conditions. In a survey of Australian growers of Bandicoot naked oats, the itch was rated in the top three disincentives for the crop (along with yield and marketing). Trichomes may also cause storage problems by creating conditions favourable for storage insects and disease.

The most attractive method of dealing with the problem is to breed trichome-free lines. Burrows has pursued this goal vigorously and has recently registered a variety low in trichomes. This is an important milestone in the development of the naked oat crop. Wesenberg and Marshall (pers. comm.) have also reported low trichome lines in material genetically distant from the Ottawa lines. Barr and Pelham (unpublished data) have surveyed Australian oat germplasm and found very limited variation in trichome numbers. Only the diploid oat Saia had low levels of trichomes. In the absence of genetic solutions, there are four suggestions to ameliorate the problems caused by trichomes.

- disposable, plastic-impregnated, all-in-one paper overalls to reduce the itch to those operating harvesters or handling grain. Air-assisted, filtered dust hoods reduce hay fever and asthma. Talcum powder in skin folds prior to exposure reduces trichome penetration and irritation.

- careful attention to harvester settings to remove as many hairs as possible during harvest.
- seed cleaning systems using deawner drums and strong aspiration to remove hairs and dust.
- experimental treatment of grain with 7 litres of refined canola oil per tonne of grain, applied to the grain in an auger stream or in a pickler, to bind the dust and hairs. The oil technique certainly greatly reduces the dust problem and improves the physical appearance of the grain. Small-scale trials show no effect on the palatability of grain to chickens, but germination is very markedly reduced, so this technique *should not be used* for seed or grain for malting. The cost is estimated at \$A9.10 per tonne.

Storage

Various authors have highlighted potential problems with the storage of naked oats. Problems can be attributed to either the high oil content causing rancidity, exposed embryo causing reduced germination or grain insects. In experiments conducted in South Australia, a loss in viability of 1–2% per month was observed in Bandicoot, with no change in the husked oat variety Echidna (Barr and Teague, 1995). Consequently, growers were advised that germination testing prior to seeding was warranted, with seeding rates adjusted accordingly. The second trial measured rancidity over a 42-month storage period. The free fatty acid levels were comparable and stable in Bandicoot and Echidna at 6 and 18 months, but increased in Bandicoot at 30 and 42 months. One sample that had been attacked by grain insects had very high free fatty acid levels (27% on fat) (Barr and Teague, 1995). Experience in the UK and Canada suggests that the crucial factor in storage is grain moisture. If grain moisture is below 14%, and the grain is carefully harvested, then few special problems occur. If grain moisture is higher than 14%, then naked oats will not store as well as husked oats. Welch (1977) found the level of hydrolytic rancidity only exceeded husked oats if the grain was severely bruised.

Improvement in grain size and uniformity

A pleiotropic effect of the genes conditioning naked grain is a multiflorous spikelet. In contrast to husked oat, where there are usually two or three florets per spikelet, in naked oats there may be up to eight (Atiyya and Williams, 1976). Grain size decreases along the spikelet in hulled oats and even more markedly in naked oats. The small grains in the distal florets are a problem in naked oats because they are often lost in the grain separation stream of the harvester, must be screened out of naked oats used for human food, and represent an inefficient allocation of photosynthate, given their strong likelihood of abortion or loss. Uniformity of grain size is a key quality parameter in oats for human food. Fortunately, there is genetic variation for floret number in naked oats and many breeders have selected varieties that typically have three to five florets per spikelet. Improvement of grain size *per se* becomes more straightforward when

the floret number has been reduced. Recent releases in several countries have made progress in this trait. For instance, Harpoon has larger grain than its predecessor Kynon in the UK trials (NIAB, 1995). In South Australia the breeder's line OX88.045-11N has an average grain weight of 22.4 mg, compared to Bandicoot's 21.7 mg (33 comparisons). A problem related to grain weight and trichome level is that of grain density. High density is usually linked to better freight rates and better grain quality. Many new releases claim higher grain density (e.g., Harpoon, Ripon and Krypton (UK)).

Improving germination and emergence

Low germination and subsequent poor crop establishment contribute to lower yield. In southern Australia this is a problem, particularly for waterlogged, heavy soil types. Further investigations are required to determine if this is a coleoptile or "bursting" problem. Most breeding programs would at least practice indirect selection for improved emergence by discarding lines that do not emerge well.

Damage by seed cleaning and harvesting is more likely in naked versus husked oats due to the exposed embryo. Extra care is therefore required during these processes. Selection for better kernel conformation is one strategy to decrease the likelihood of embryo damage. Vitavax (R) seed dressing has improved emergence of naked oats.

Summary

At least 14 new naked oat varieties have been released since the Fourth IOC, with improvements in grain size, uniformity, percentage of naked oats, freedom from itch and modest yield increases. Animal nutritionists have now gained an understanding of the strengths and weaknesses of naked oats for most animals where naked oats show potential. Marketers have further developed outlets for the crop. However, the crop is still very small on the worldwide scene, primarily because the grain yields are too low relative to competitive crops. Before the marketers can really promote the crop, the yield problem must be solved.

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Hulless barley: Development and utilization

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Introduction

Hulless or naked barley (HB) was rediscovered in western Canada in the early 1970s when the author and his colleagues at the University of Saskatchewan were investigating the nutritional quality of barley germplasm, then available in our department. The following paragraph is taken verbatim from a published paper (Bhattu 1975): "the hull content of barley was the major factor influencing its digestible energy content. Thus it would seem desirable to develop hulless cultivars of barley for swine and poultry feeding." Vigorous efforts by barley breeders followed, first at the University of Saskatchewan and later at the Field Crop Development Centre, Lacombe, Alberta, to develop HB cultivars for use in swine and poultry feeds and later for use in human foods as a source of dietary fiber. The first 2-rowed and 6-rowed cultivars of HB were registered in Canada in 1982 and others later (Table 1), including three waxy or low amylose starch cultivars, CDC Candle, Merlin and HB803.

The more recent HB cultivars registered in the United States are Azhul, Waxbar and Merlin waxy barleys. Both in Canada and the United States, 2-rowed cultivars predominate because of their plump kernel, white aleurone, soft endosperm and a generally high beta-glucan content, which are all desirable in

Table 1. Registered cultivars of hulless barley in Canada

Type and Name	Year Registered	Type and Name	Year Registered
2-Rowed		2-Rowed	
Scout ^a	1982	HB803 ^b	1995
Condor	1988	CDC Fleet	1996
CDC Richard	1990	6-Rowed	
Phoenix	1993	Tupper	1982
CDC Candle ^b	1994	CDC Buck	1990
CDC Dawn	1995	Falcon	1993
Merlin ^b	1995	CDC Silky	1994

^a Deregistered in 1992

^b Waxy or low amylose starch

food applications of HB. Potential new applications of HB include: preparation of food malt, which is distinct from brewer's and distiller's malts (Bhatty 1996); production of ethanol, for which HB is equally if not more suitable than wheat (Ingledew et al. 1995); extraction and enrichment of beta-glucan (Bhatty 1995; Sundberg et al. 1995); preparation of native and modified starches (Vasanthan and Bhatty 1995); and preparation of roller-milled bran and flour for use in bakery and non-bakery products (Bhatty 1993; Sundberg and Åman 1994).

Hulless barley production on the prairies started slowly in the 1980s, and reached close to 300,000 tons in 1995, doubling every year since 1993. This brief history of HB development and production in western Canada does not imply that HB was not known in North America or elsewhere. The superiority of HB over hulled barley, wheat and corn in supporting swine growth was reported almost 75 years ago in the United States (Joseph 1924). Other attributes of HB include:

- It is an ancient grain that probably preceded hulled barley.
- It has a single recessive gene (nn) that is responsible for the hulless character.
- It has waxy (ww) or normal starch.
- It can be low or high in beta-glucan.
- There is an absence of trichomes (hairs) on the seed surface.
- It can be used to produce food malt, and possibly brewer's and distiller's malts.
- It can be directly processed for use in human foods.
- It is free of any known anti-nutritional factors.

Nutritional potential

The nutritional potential of HB for humans lies in its soluble fiber (SF), tocotrienols and possibly other compounds as yet undiscovered. Tocotrienols inhibit 3-hydroxy-3-methylglutaryl (HMG)-COA reductase activity. Mevalonic acid, the product of this reaction, is generally considered to be the rate limiting substrate for the synthesis of cholesterol. High-protein flour prepared from brewer's spent grain containing little or no SF inhibited HMG-COA reductase activity more than wheat germ, oats or brewer's grain (Qureshi et al. 1991). Barley hulls, the aleurone layer and the germ are rich in tocopherols and tocotrienols (tocols) compared to the whole kernel; malting of barley has essentially no effect on tocols, which are enriched in brewer's spent grain (Peterson 1994).

Soluble fiber undergoes extensive fermentation in the colon, resulting in the production of a large bacterial mass and a low fiber residue. The hypocholesterolemic effect of oat SF has been generally accepted. Barley SF has been reported to exhibit similar properties in animal and human studies. The hypocholesterolemic effects of different barley products was, as expected, highly variable due to different products and the experimental species used. The barley products tested included ground waxy and non-waxy barleys, with or without the addition of beta-glucanase (Fadel et al. 1987), HB of 30% extraction (Ranhotra et al. 1991), ground waxy HB (Newman et al. 1992), barley bran flour (Lupton

et al. 1994), bakery products containing waxy HB flour (Newman et al. 1989) and dehulled whole barley (Kahlon et al. 1993). HB bran of 30% extraction reduced total cholesterol in rats by 41% and increased HDL by 68% (Ranhotra et al. 1991). Nevertheless, more medically credible evidence using human experiments is needed to corroborate the hypocholesterolemic effects of HB bran. Several mechanisms for the hypocholesterolemic effect of SF have been proposed: (1) binding of bile acids, which results in reduced serum cholesterol; (2) fermentation of SF by colonic bacteria and the production of short-chain fatty acids, which may inhibit cholesterol synthesis; (3) increased breakdown of low-density lipoprotein cholesterol; and (4) indirect effects as fiber replaces some dietary saturated fat and cholesterol (Glore et al. 1994).

Hulless barley milling: Bran and flour

Although whole grain products such as flakes, grits and pearls may be commercially produced and used in food products, roller-milled barley products such as bran and flour have the most potential in food applications. The bran may be used in ready-to-eat cereals and high-fiber bakery products such as cookies and muffins. Barley flour can be blended with wheat flour for making pastry, cookies, muffins, cakes and flatbreads, or used as a food thickener. Traditionally, hulled or HB has not been roller-milled, like wheat and oats, to produce bran and flour. Rarely have true barley bran and flour been produced and investigated. Barley reacts differently than wheat to conventional roller-milling. The bran is brittle and shatters, regardless of tempering conditions, unlike wheat bran, which separates as large, stable flakes. Because of the brittleness of barley bran, roller-milled HB flour has a higher ash content and is sometimes darker than wheat flour milled under identical conditions (Bhatty 1986, 1987, 1993). HB flour was nearly as white as wheat flour, and had higher ash and beta-glucan. Starch damage was low, though higher than in wheat flour. The barley flour had higher water, mixograph and farinograph absorptions due to its higher beta-glucan content.

HB bran is more like wheat bran in structure, except for the tri-cellular aleurone layer and chemical composition, especially SF. HB bran thus consists of the pericarp and its outer and inner layers, the seed coat (testa), epidermis, aleurone and the subaleurone layers. The germ consists of the embryonic axis and the scutellum and is a separate part of the seed, but is often included in the bran. HB bran of about 30% extraction is a true bran containing the seed coat, germ, aleurone and subaleurone layers. At 30% extraction, beta-glucan enrichment is, on average, about 37%, yielding bran that may contain 7–14% SF, far higher than the level in oat bran. Because of its high SF, HB bran is like oat bran, hypocholesterolemic and hypoglycemic. Unlike oat bran, HB bran is lower in ether extract lipids (about 4%), may be prepared without steaming or stabilization of HB, has a longer shelf life and produces low calorie bakery products (Bhatty 1995).

Beta-glucan extraction

Hulless barley is an excellent source for beta-glucan extraction. Beta-glucan was extracted with sodium hydroxide from one sample of hulless barley (Azhul) bran in the laboratory and from one sample of hulless barley (Scout) bran in a pilot plant and subsequently purified (Bhatti 1995). Sodium hydroxide solvent extracted 96–98% of the beta-glucan from the brans and beta-glucan recovery after purification varied from 73 to 77%. The Azhul barley bran preparation contained 76% beta-glucan, 11% pentosans and about 1% protein, starch, ether extract and ash. The pilot plant preparation from Scout barley bran contained only about 50% beta-glucan and a high ash content (16%) due to poor washing of the extracted material. Its protein, starch and ether extract contents were also higher. The apparent viscosity of the beta-glucans was highly concentration-dependent and the pilot plant preparation was the most viscous. The apparent molecular weight of the beta-glucans was estimated at 2×10^6 . Calculation of power law constants of 1% beta-glucan solutions suggested low shear sensitivity of the preparations. In other studies, beta-glucan has been enriched by dry-milling and sieving (Knuckles et al. 1992) or by dry milling and air classification (Sundberg et al. 1995).

Starch separation by pin-milling and air-classification

Small and large granule starches have been separated from different barleys by pin-milling and air-classification (Vasanthan and Bhatti 1995). Scanning electron microscopy of the pin-milled barleys and their air-classified fractions showed that the first two air-classifications separated small granule starch into fine 1 and fine 2 fractions; the large granule starch was separated into coarse 1 and coarse 2 fractions. The third pass yielded a fine fraction (F3) rich in large granule starch. Further purification of F3 fractions by a wet process yielded almost pure large granule starch with an extraction efficiency about 30% higher than that obtained by conventional laboratory starch extraction from barley. Furthermore, the process yielded protein and beta-glucan as by-products at high levels of purity. The small and large granule starches have different functional properties. Large granule starch may be modified for use in foods and industry and substituted for corn starch, since their physicochemical properties are generally similar. Cationized large granule barley starch compared favorably with corn starch for use in the pulp and paper industry.

Conclusions

Hulless barley has been rediscovered, particularly in North America. Several cultivars have been registered, including waxy or low amylose barleys. There is a renewed interest in HB as a source of soluble fiber implicated in hypocholesterolemia and hypoglycemia in non-insulin-dependent subjects. Hulless barley may be processed for use in foods and for industrial applications of starch. Promotion of HB in foods and industry requires sustained research effort at several fronts.

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Core collections in germplasm conservation: Evaluation and use

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Introduction

The sizes of germplasm collections in many cases limit their accessibility, and thus their utilization in plant breeding and the quality of their management. To improve this situation it has been proposed that a limited set of accessions with as much genetic diversity as possible be selected from the collection. Such a selection would offer a good starting point to search for specific traits, and could be used for in-depth evaluation, increasing knowledge about the entire collection. Frankel (1984) introduced this concept, calling the resulting collection a “core collection.” It was further developed by Frankel and Brown (1984) and Brown (1989a, 1989b).

Since those early publications many scientists and curators have been working with the concept, resulting in a body of practical experience. Many core collections have been compiled, and are currently used in research and breeding programmes. Core collections have been screened for disease and insect resistances and stress tolerances, such as late leafspot resistance in a peanut core collection (Holbrook and Anderson 1995), or acid soil tolerance in an alfalfa core collection (Bouton 1996). Core collections have also been used to study physiological effects on a wide range of genotypes, for example genotype x environment interactions in a core collection of french perennial ryegrass (Charmet et al. 1993).

The concept evolved from a theoretical model into a workable method. In this paper the current interpretation of the concept will be discussed, and a general method for creating a core collection will be described. The Barley Core Collection will be used as an illustration.

What is a core collection?

Frankel (1984) defined a core collection as “a limited set of accessions of a crop species and its wild relatives which would represent, with a minimum of repetitiveness, the genetic diversity of a crop species and its wild relatives.”

This definition was modified by Brown (1994) to “a limited set of accessions derived from an existing germplasm collection, chosen to represent the genetic spectrum in the whole collection, and including as much as possible of its genetic diversity.” In applying the concept some questions have arisen:

- What is “a limited set”? Population genetic calculations based on non-realistic models and arbitrary values for parameters can not yield the number of entries that should be included in the core collection. This number is fundamentally arbitrary and should therefore be based on practical considerations.
- Are core collections always derived from “an existing collection”? There are several examples of core collections not derived from existing collections. The Barley Core Collection (Knüpffer and Hintum 1994) is a new collection with material from various sources, and the *Brassica oleracea* core collection (Boukema and Hintum 1994) is composed of material from several specific European gene-bank collections.
- Should a core collection contain “as much as possible of the genetic diversity in the collection”? If so, a core of the average gene-bank collection would be dominated by the wild species, which are often more diverse than the cultivated material. Many existing core collections, however, try not only to represent the diversity but also the distribution of diversity in the collection.

The concept of core collections has evolved, and the current core collections will often not meet the original definitions. A core collection can now be defined as “a germplasm collection optimally representing specific genetic diversity.” This implies that the size, type and origin of the core collection will depend on the requirements. A core collection can form part of one or several existing collections, but it can also be newly created. It can represent the diversity in a complete genus, but also the diversity in a small part of a gene-pool. It can contain as diverse material as possible, but it can also give higher priority to a certain type of material.

Method for creating a core collection

It is a comforting idea that a random selection from a collection might, to some extent, be considered a core collection: the simplest and most efficient. It will be a better representation of the genetic diversity than a sequential set, like, for example, the accessions with numbers 8201 to 8485 (Vaughan 1991). A simple improvement that can be proposed (Spagnoletti, Zeuli and Qualset 1993) is equidistant sampling in terms of accession number; for example, include all accessions with accession numbers ending with a zero if ten percent are to be selected for inclusion. However, the selection of a more efficient core collection is slightly more complicated.

A general procedure for the selection of a core collection can be divided into four steps: (1) definition of the domain (2) division into genetically distinct types, (3) allocation of entries over the types, and (4) choice of entries. These steps will be considered more closely.

- Definition of the domain. The material to be represented by the core collection

will vary widely, and might be the complete U.S. germplasm collection of peanut (Holbrook et al. 1993), a restricted set of annual *Medicago* (Diwan et al. 1994), the cultivated *Brassica oleracea* in European collections (Boukema and Hintum 1994), a set of lentil accessions from Chile, Greece and Turkey (Erskine and Muehlbauer 1991) or the entire gene pool of *Hordeum* (Hintum et al. 1990). However, it might also include, or even be restricted to, material with specific traits, such as local maize populations with good combining ability (Radovic and Jelovac 1994) or *Pisum sativum* germplasm with disease resistance (Matthews and Ambrose 1994).

- Division into genetically distinct types. This can be accomplished stepwise, first making the important major divisions like that in distinct taxa, and subsequently splitting these groups in smaller ones, etc. This exercise might end with groups such as “white cabbages from France” (Boukema and Hintum 1994), “lentil landraces from Greece and Turkey” (Erskine and Muehlbauer 1991), “*Medicago arabica*” (Diwan et al. 1994) or “pea landraces from Mongolia” (Matthews and Ambrose 1994). In several examples of core collections, one or more groups are added, consisting of genetic stocks, reference material, or accessions with specific traits such as disease resistances (Hintum et al. 1990, Matthews and Ambrose 1994). These groups, though not comparable with the groups representing types of material with a common genetic background, can play an important role in representing specific genetic diversity. And what is more, they increase the practical value of a core collection considerably, and will thus increase its use.
- Allocation of entries over the types. For this purpose, again a stepwise procedure can be followed, deciding after each division of a group how the entries in the group should be allocated to each new group. This is a subjective procedure and should be based on the relative importance of the groups as determined by the judgment of the germplasm user and the diversity in the group. If relative importance of the groups cannot be determined, it is possible to use an allocation algorithm such as the constant, proportional or logarithmic strategies, which are based on the number of accessions in the groups (Brown 1989b) or other strategies based on information of marker systems (Schoen and Brown 1994).
- Choice of entries. The final choice from the groups of accessions corresponding to the types defined earlier can be done randomly, or if additional data are available, on the basis of these data – for example, after a multivariate analysis (Basigalup et al. 1995) or a pedigree analysis (Hintum and Haalman 1994) of the accessions in the group. Also, practical considerations can play a role, such as the availability of the seeds and the quality of the documentation of the sample.

The barley core collection

The Barley Core Collection (BCC) is a so-called “synthetic core collection” (Brown 1994), since it is not part of any existing germplasm collection, but

created as a new collection. It started as a collaborative initiative of the barley working group of the European Cooperative Programme for the Conservation and Exchange of Crop Genetic Resources (ECP/GR) in 1989 (IBPGR 1989). This group concluded that creating one synthetic core collection instead of several independent barley core collections in the larger genebanks would have some important advantages. Its concept was elaborated by a working group, which was extended into an international BCC committee in 1992. Creating and managing the BCC is, and will be, organized as an international effort; it will try to get as many curators and scientists involved as possible. (Knüpffer and Hintum 1994, Hintum 1992, Hintum et al. 1990).

The BCC was defined as a limited set of entries selected from genebank collections optimally representing the genetic diversity of barley. It is supposed to increase the efficiency of germplasm evaluation and utilization, to provide for a manageable and representative set of barley entries for use in research and breeding, and to provide standardized material for scientific investigations. The BCC should not consist of more than 2000 entries.

The domain of the BCC is the entire *Hordeum* gene pool. The first division in distinct types, and allocation of entries over the types, resulted in the following groups (Figure 1):

- Cultivars, represented by ca. 500 entries, divided over six macro-geographical regions, according to the importance of barley breeding in that region.
- Landraces, represented by ca. 800 entries, divided into five macro-geographical regions, according to the genetic diversity expected to occur in the corresponding regions.
- *Hordeum spontaneum*, wild barley in the primary gene pool, represented by 150 to 200 entries, of which two-thirds originate in the central and one-third in the marginal areas of distribution.

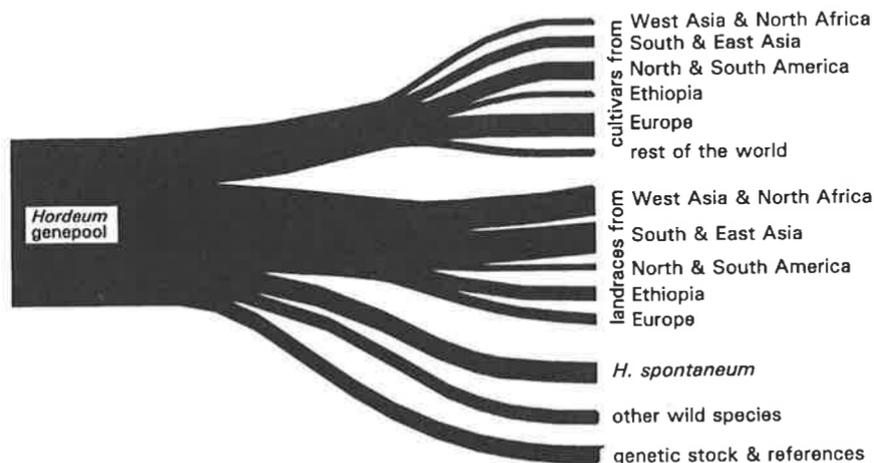


Figure 1. First part of the diversity tree of the Barley Core Collection

- Other wild *Hordeum* species, represented by 60–100 entries, roughly two per species, and some more for *H. bulbosum*, which forms the secondary genepool.
- Genetic stocks and reference material, including standard or reference material and well-defined marker materials.

The subsequent division of these groups and the final choice of entries has been made by scientists and curators with specific knowledge of and experience with these groups. This is coordinated on a macro-geographical region basis.

The BCC is about to become operational. Once it is generally known and used, the information about its entries will be compiled in one freely available and easily accessible database. This database will contain an ever-growing wealth of information that is expected to increase our knowledge of the genepool and increase the utility and accessibility of barley germplasm collections.

Conclusion

The concept of core collections has, due to its success, evolved from its original definition to a much wider interpretation. The methodology applied in the compilation of core collections varies widely, but can be generally described with four steps: definition of the domain, division of the domain into genetically distinct types, allocation of entries over the types and choice of core collection entries. The Barley Core Collection is a good example of a core collection; it is the result of an international initiative that will allow a more effective combination of the results of research on barley worldwide.

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Conservation and use of wild relatives of barley

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Plant genetic resources

Conservation and sustainable use of plant genetic resources (PGR) are presently of utmost importance for global agriculture and mankind. The Convention on Biodiversity declared plant genetic resources to be subject to national sovereignty; most countries ratified that statement. Negotiations concerning access to PGR are now being dealt with by the Food and Agriculture Organization's Commission on Genetic Resources. This will, it is hoped, lead to a binding, multilateral agreement with free access to PGR and then to international programs for conservation and sustainable utilization. This treaty will concern all genebank collections. Future collection, access and utilization of wild and cultivated barley germplasm will have an influence on breeding as well and research activities worldwide.

Barley is the second-largest crop represented in the world genebanks. According to the FAO's newly finished State of the World report, there are around 485 000 accessions of wild and cultivated barleys stored. The actual number of unique accessions is impossible to state since the figure includes duplications and accessions without adequate passport data. One important task for genebanks will be to identify duplicates in the world holdings (Hintum & Knüpffer 1995). Another major obstacle for an optimal utilization of barley germplasm is the scarcity of evaluation data on genebank material.

Another cause of inefficient utilization of plant genetic resources is the increasing gap between the genebanks and practical breeding. When breeding is transferred from public to private hands, long-term breeding or prebreeding is decreased. During the last couple of years, several countries have deliberately decreased public input into prebreeding, formerly done by universities or public institutions. There must be effective interfaces between genebanks and practical plant-breeding programs. For barley, this is effectively done by ICARDA, which has prebreeding programs, including barley landraces and wild forms (*Hordeum vulgare* ssp. *spontaneum*). For long-term efficient use of genetic resources in barley, it is important that national programs for plant genetic resources also include prebreeding activities.

Several genebanks in the world have direct financial problems due to state

cutbacks. A far-sighted obligation is a necessity for genebank practices.

This paper discusses utilization of exotic germplasm and wild taxa in breeding and surveys the current conservation status of this material in *in situ* and *ex situ* collections.

The primary gene pool of barley

Like other crops, the main gene source for barley breeding consists of advanced breeding lines, varieties and mutant stocks. These are well represented in genebanks throughout the world. The primary gene pool also comprises landraces and more primitive forms of cultivated barley. Landraces are still cultivated and fairly abundant in some parts of the world (southwest and central Asia, North Africa), but the number is diminishing at an accelerating rate. Recent collecting in these areas has resulted in substantial genebank holdings, such as in the genebank at ICARDA, the Ethiopian genebank and the Chinese genebank. It is, however, not known to what extent these collections represent the entire genetic diversity of a given area. "White spots" in the *ex situ* collections are thus not yet possible to locate. Large efforts are being made to collect, evaluate and conserve barley material, but there should be more systematic planning and surveying to determine the major gaps in the world's holdings. To preserve the large variation found within individual landraces, "on-farm conservation" systems have been developed in certain areas.

In most developed countries the old landraces have more or less vanished. Modern plant breeding started more than 100 years ago, before systematic and efficient collecting was undertaken. Subsequently, older varieties were wiped out before the importance of the preservation of genetic resources was realized. Landraces are hence rare in European collections. At present, it is not possible to estimate the genetic erosion in Europe due to the disappearance of older material. All European breeding programs started with selections and recombination breeding based on landrace materials. A great deal of the genetic content of the landraces is still present in modern cultivars. A broad investigation should be initiated to study the decrease of genetic diversity in modern breeding lines compared to older landraces, and the eventual losses of gene material over time.

Hordeum vulgare ssp. *spontaneum*

The progenitor of cultivated barley, ssp. *spontaneum*, is interfertile with cultivated barley. It thus belongs to the primary gene pool of the crop and has as such a special interest for utilization in breeding programs.

Spontaneum is still abundant in its native distribution area, the eastern Mediterranean eastwards to Afghanistan, in natural as well as in more disturbed habitats (cf. Bothmer et al. 1995). Brittle rachis types occur outside this area, especially in North Africa and China, but these areas constitute secondary distributions or the material represents segregation products. Introgression between wild ssp. *spontaneum* and the cultivated ssp. *vulgare* occurs frequently.

The Fertile Crescent, especially Israel, is the area where the highest genetic diversity is found (Jaradat 1992, Nevo 1992).

Due to the close relationships to cultivated barley, *ssp. spontaneum* has been subject to intense evaluation studies. The most studied field is resistance to pest and diseases, such as aphids (Weibull 1994), powdery mildew (cf. Kintzios & Fischbeck 1996), scald (Abbott et al. 1992) and rust (Jana & Nevo 1991). Other areas include stress tolerance, such as drought (Grando & Ceccarelli 1995) and quality traits (Jaradat 1991).

Since *spontaneum* belongs to the primary genepool, it has also been repeatedly utilized in long-term barley breeding programs with good success; e.g., in the WANA region by ICARDA (Ceccarelli 1989), Scandinavia (Lehmann & Bothmer 1988) and England (Ellis et al. 1991) in backcross programs.

An interesting approach to utilizing exotic germplasm in barley breeding is the creation of a "dynamic genepool" (Veteläinen 1994). It is a composite cross, including 40 different accessions, of which 20 are modern North European varieties and breeding lines. The remaining 20 are exotic landraces and *spontaneums* chosen for resistance characteristics. The genepool is designed to comprise six generations of recombination, where all lines are represented equally in controlled, convergent crosses without selection. The genepool in the sixth generation is used for breeding as well as for research purposes.

Despite complete compatibility in crosses, landraces and *spontaneum* are laborious and time-consuming to use in practical barley breeding programs. A phase of prebreeding with at least three back-cross generations is necessary between the evaluation of desirable traits and the conventional breeding program, due to the content of unadapted and "wild" characteristics in the more exotic germplasm (Lehmann & Bothmer 1988).

As for landraces, special collecting of *spontaneum* has been undertaken in recent years and substantial holdings are built up in genebanks, such as at ICARDA and in the United Kingdom, Israel and Turkey. However, no general survey of the genetic diversity of *spontaneum* has been undertaken, which is a prerequisite for the planning of further collecting of the taxon for *ex situ* preservation.

There does not seem to be an immediate threat to the survival of *ssp. spontaneum* in nature. Plans for long-term *in situ* conservation reserves have been made in some countries (Israel and Turkey).

The secondary genepool of barley

Only the single species *H. bulbosum* belongs to the secondary genepool of barley. It is native to the Mediterranean area and extends eastwards to Iran. It is a perennial, tall-grown species with an outbreeding reproductive pattern including a self-incompatibility system. *H. bulbosum* occurs in a diploid cytotype in the western part of the Mediterranean and in a tetraploid form in the eastern part. The border between the two cytotypes is very sharp and goes through central

Greece (Jørgensen 1982). *H. bulbosum* forms mostly sterile hybrids with barley, but these show high chromosomal meiotic pairing. Several sets of data indicate that *H. bulbosum* shares the same genome (I) with cultivated barley and, apart from ssp. *spontaneum*, it is the closest wild relative (see Bothmer et al. 1995 for references).

H. bulbosum has been of interest to breeders since the 1970s. This was due to the chromosome elimination system and production of doubled haploids (homozygous lines in barley). This mechanism has been widely studied, and the system has been developed for routine utilization in breeding (cf. Chen & Hayes 1989). The success rate for production of haploids depends on genotypic as well as on environmental conditions (cf. Thörn 1992). *H. bulbosum* has also been used in attempts to produce doubled haploids in wheat, but the haploid frequency has been too low to be applicable on a commercial scale (cf. Bozorgipour & Snape 1990). Allo-plasmic lines of barley produced through chromosome elimination have been shown to have a large kernel (Aida et al. 1981).

In later years, *H. bulbosum* has gained increased attention as a gene source in barley breeding. Several traits found in *H. bulbosum* may be of interest for transfer to barley, such as resistance to powdery mildew (cf. Pickering et al. 1995); leaf and stem rust as well as spot blotch resistance (cf. Dyck et al. 1992; Pickering, pers. comm.), and resistance to barley mild mosaic virus (Michel 1995).

Xu and Kasha (1992) succeeded in transferring powdery mildew resistance from *H. bulbosum* to *H. vulgare* by using partially fertile, triploid interspecific hybrids that were backcrossed to barley. Research groups in Germany and New Zealand are presently working with an approach similar to that of Xu and Kasha (op. cit.) and have produced fertile disomic substitutions, with the aim that it be included in regular barley breeding programs (Michel 1995; Pickering et al. 1995; Pickering, pers. comm.).

Since *H. bulbosum* is of interest for breeding purposes, a strategy must be carried out concerning *in situ* as well as *ex situ* conservation and availability in active collections. For the Barley Core Collection, ca. 10 accessions will be included from the entire range of distribution, which has not yet been achieved. *H. bulbosum* is rather poorly represented in genebanks. Particular collecting expeditions have been undertaken in Spain and Israel. From these countries a fair number of accessions are available. For other areas, collecting missions should be initiated. Due to the out-breeding habit, the multiplication of *H. bulbosum* is more complicated than for in-breeding species, which hampers the efficiency of genebank handling.

The tertiary gene pool of barley

The remaining species of the genus *Hordeum* belong to the tertiary gene pool of barley. The genus comprises ca. 30 species and 45 taxa distributed in most temperate areas of the world (cf. Bothmer et al. 1995). *Hordeum* is a diverse genus with, for example, annuals and perennials, diploids and polyploids, in- and out-breeders. Several taxonomic and phylogenetic studies of the genus have

been undertaken, and the general knowledge is fairly good. Almost all species are crossable with cultivated barley, but there are little chromosome homology between the I genome of *H. vulgare* and *H. bulbosum* and the H genome of most other *Hordeum* species (some species, like *H. murinum* and *H. marinum*, still have unidentified genomes; cf. Bothmer et al. 1995).

Several of the wild species included in the tertiary gene pool of barley have proven to contain traits of interest for breeding. These include, for example, resistance to powdery mildew (Gustafsson & Claesson 1988), leaf rust (Rubiales et al. 1996), typhula blight (Bothmer & Hagberg 1983), loose smut (Nielsen 1987), aphids (Weibull 1987) and scald (Brown 1990). However, further screenings and evaluations should be carried out on the wild species.

Since these species belong to the tertiary gene pool of barley, it has proven difficult to use them as gene donors. Primary hybrids are in most cases easily obtained, whereas backcross programs have failed due to high hybrid sterility (Bothmer & Linde-Laursen 1989). Further studies of gene transfer to barley are necessary.

A particular case is the South American diploid *H. chilense*, which is native to Chile and a small area in Argentina. It crosses readily with both tetraploid and hexaploid wheats and this intergeneric amphiploid, named Tritordeum, has been a subject for a large domestication program by Spanish scientists and breeders (cf. Alvarez et al. 1992 and A. Martín, pers. comm.). The goal is to create a new crop of importance for the food industry. The program includes evaluation of several traits, such as disease resistance to greenbug (Castro et al. 1995), rust (Rubiales & Nix 1992) and powdery mildew (Rubiales et al. 1993). It also contains screening for quality traits, especially for bread making (Alvarez et al. 1992). For success in developing new crops, it is necessary that the program be planned and financed on a long-term basis, which is the case with the Spanish project.

Most of the wild *Hordeum* species are fairly common and cover large distribution areas, but the status for these species is not completely known (Bothmer et al. 1995). Some species are rare, and as species or cytotypes they are threatened or vulnerable. These include the hexaploid cytotype of *H. brachyantherum* ssp. *brachyantherum*, which is known only in a single locality in California. *H. intercedens* and *H. arizonicum* are decreasing in their distribution areas in the southwestern United States. *H. erectifolium* is known in a single locality in Argentina and *H. guatemalense* is endemic to a very small area in northern Guatemala. Both species are very vulnerable. *H. secalinum* is decreasing in most parts of its distribution area in central and northern Europe.

For long-term conservation, investigations of genetic diversity are needed as a basis for selecting areas or populations for *in situ* conservation. For the Barley Core Collection, two accessions of each taxon/cytotype are included. For long-term preservation, at least five populations of each taxon, representing a maximum of diversity, should be included. Further collecting should be initiated in those areas where no collections have been made previously. Major holdings of wild *Hordeum* species are held by Agriculture Canada (Ottawa) and the Department

of Plant Breeding Research (Svalöv, Sweden). Special collections of individual species are held by ICARDA (wild species of southwest Asia) and the Departamento de Genética, Cordoba, Spain (special collection of *H. chilense*, Tobes et al. 1995)

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Using and conserving *Avena* genetic resources

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Introduction

The genus *Avena* L. (Poacea) belongs to the tribe *Aveneae* and comprises a polyploid series with a basic chromosome number of $x=7$. Three ploidy levels are recognized: diploids ($2n=2x=14$), tetraploids ($2n=4x=28$) and hexaploids ($2n=6x=42$). All the representative species are annual inbreeders, with the exception of *A. macrostachya*, which is an outbreeding, perennial autotetraploid. The genus contains thirty taxonomic entities which are generally recognized by oat workers, though there is some disagreement over classification of some species/taxon. Historically, one or more of the four basic genomes AA, BB, CC and DD have been allocated to the various taxa based on cytological observations of meiotic chromosome pairing of inter- and intra-specific hybrids and karyotype analyses.

At the diploid level, a number of A genome species/taxa are known but only two species (three taxa) are known to carry the C genome. The A and C genomes are also known at the tetraploid and hexaploid level, but no D genome species is recognized at the diploid or tetraploid level.

It has been suggested (Leggett 1992) that, since the oat genus has been evolving over such a long period of time, the diploid that contributed the DD genome is either extinct, or has diverged to such an extent that it is no longer recognizable as a distinct entity among the known diploid oats. It is also possible that it has not yet been discovered.

Evidence from many disciplines indicates that the BB genome of the tetraploid *A. barbata* complex is so closely related to the AsAs genome of the diploid *A. strigosa* complex that it would be more correctly designated AAA'A' than AABB. Recent evidence from *in situ* hybridization studies indicate that the DD genome of hexaploid oats is very closely related to the AsAs genome at the DNA level (Leggett and Markhand 1995). It is thus possible that a distinct D genome diploid never existed.

Conservation

The cultivated hexaploid oat *Avena sativa* L. ($2n=6x=42$; genomes AACCCDD) is one of the world's major cereal food crops, yet in comparison with wheat, barley, maize and rice, little effort has been made to conserve the wild weedy

forms of oats, which are a vitally important source of genetic variation for crop improvement. To redress the balance, a number of collecting expeditions sponsored by the International Plant Genetic Resources Institute have been undertaken during the last decade, to collect target species of oats identified by the *Avena* Working Group of the European Cooperative Programme for Crop Genetic Resources (ECP/GR) as being poorly represented in world collections, and to assess the degree of genetic erosion due primarily to habitat destruction.

The known distribution of the various wild weedy species of oats centres around the countries bordering the Mediterranean where the majority of species and taxa can be found growing in primary natural habitats. Many of these primary habitats of wild oats are under threat due to man's increasing activities and environmental changes such as desertification. Morocco is unique in that all but three of the wild oat species (*A. canariensis*, *A. ventricosa* and *A. macrostachya*) have been recorded there, and three taxa occur there uniquely. This led Leggett et al. (1992) to speculate that Morocco is a centre of diversity of the oat genus.

Wesenberg et al. (1992) cite over forty working oat germplasm collections worldwide, the most significant being Plant Gene Resources of Canada (Ottawa), the USDA-ARS Small Grains collection (Aberdeen) and the Nordic Gene Bank (Alnarp, Sweden). Many of the accessions held in the various collections are unique and, in many cases, safety duplicates are not held in other collections. Of the smaller collections (not included in the listing mentioned above), unique germplasm held at different institutes in the now separate states of the former Soviet Union appear to be in grave danger of being irretrievably lost (I. Luskutov pers. comm.). Characterization and evaluation data of the bulk of wild *Avena* germplasm collections is limited, and at present there are no core collections of either the cultivated or wild gene pools. The ECP/GR is, however, in the process of compiling such core collections based on European-held *Avena* germplasm.

The gene pools

The species of *Avena* can be grouped into three gene pools – primary, secondary and tertiary (Harlan and De wet 1971) depending on the ease of gene transfer between the different species.

In the primary gene pool [P], there is no restriction on the flow of genes between the wild and cultivated species, and the selected trait(s) can be recovered by a conventional backcrossing programme. Thus, in the case of the hexaploid taxa of *Avena*, the variation is directly available to the breeder.

The secondary gene pool [S] contains only the tetraploids *A. murphyi* and *A. maroccana*. Here, gene flow is only partly restricted, in that F1 hybrids can be readily produced, but are self-sterile. Backcrossing these F1 hybrids to the recurrent parent, however, does produce a small number of seeds, since they are partially female fertile. As the number of backcrosses increases, so fertility levels increase. Both these species, then, are more or less directly available to the breeder.

The tertiary gene pool [T] includes all the diploid and tetraploid species other than *A. maroccana* and *A. murphyi*. Transfers from this gene pool to the

cultivated oat are very restricted, and gene transfers can only be effected by employing complex breeding methodologies. The species in this gene pool are therefore not directly accessible to the breeder.

Aneuploids are an important genetic resource, as evidenced by their use in wheat breeding programmes (Kimber and Sears 1980). The study of aneuploids has enabled genes to be mapped to specific chromosomes, and has accelerated the transfer of genes from species to species or cultivar to cultivar. In oats, aneuploids have been used to a lesser extent, primarily because a complete monosomic series is not yet available. Completion of the series should enable us to locate and manipulate the pairing control mechanism thus removing barriers to recombination between primary, secondary and tertiary gene pools.

Use of *Avena* germplasm

The transfer of resistance or tolerance to pests and diseases has always commanded high priority in oat breeding programmes, to keep yields high and production costs to a minimum. Concern over environmental pollution from toxic fungicides and pesticides is now rightly considered as important as yield potential, thus making genetic resistances of greater importance now than they have ever been.

There are many examples in the literature of the occurrence of genes for resistance/tolerance to pests and diseases in the *Avena*. Examples are resistance to: 1) mildew, from *A. prostrata*, *A. hirtula* and *A. barbata* [T] and *A. sterilis* [P] (see Thomas 1992); 2) crown rust, from *A. abyssinica* (Marshall and Myers 1961), *A. barbata* [T] and *A. sterilis* [P] (Martens et al. 1980) and recently from *A. strigosa* cv. Saia (Aung and Leggett, unpublished data); 3) stem rust, from *A. barbata* [S] and *A. sterilis* [P] (Martens et al. 1980); 4) cereal cyst nematodes, from *A. sterilis* [P] (Rivoal and Cook 1993), *A. maroccana* and *A. murphyi* [S] (P. Hagberg, pers. comm.); 5) BYDV, from *A. sterilis*, *A. occidentalis*, *A. fatua*, *A. hybrida* [P] *A. barbata*, *A. macrostachya* and *A. strigosa* [T] (Comeau 1984).

Genes for characteristics other than pest and pathogen resistances have also been identified, and in some cases transferred to the cultivated crop; for example: 1) high protein content from *A. sterilis* [P] (Takeda and Frey 1977) and *A. maroccana* [S] (Thomas et al. 1980); 2) high oil from *A. fatua* and *A. sterilis* [P] (Luby and Stuthman 1983; Thro and Frey 1985); 3) genes associated with yield from *A. sterilis* [P] (Takeda and Frey 1977); 4) winterhardiness from *A. fatua* [P] (Suneson and Marshall 1967; Rines et al. 1980) *A. macrostachya* [T] (Baum and Rajhathy 1976); 5) dwarfing genes *A. fatua* [P] (Morikawa 1989); 6) high beta-glucan content, from *A. damascena* and *A. atlantica* [T] (Welch and Leggett 1996, in press); 7) herbicide resistance, from *A. fatua* (P.D. Brown, pers. comm.).

Apart from these practical breeding applications, oat species from all sections of the gene pool have been used in scientific studies; for example: isozyme analyses of populations (Allard et al. 1993); mechanisms of resistance to pathogens (Carver and Carr 1977); cytological and evolutionary relationships (Leggett and Thomas 1995), chemical composition (Welch 1995) and so on.

Future uses of *Avena* genetic resources

Increasing numbers of novel products are finding their way onto the market as industrial processors and manufacturers recognize the marketability of naturally occurring and healthy oat or oat-based merchandise. Recently, substantial markets such as talcum powder and other toiletries, oat milk and countless new food stuffs have opened new avenues for growers, millers and processors alike.

As further industrial uses of the oat crop are found, industrial processors are likely to request "specifically designed" oat varieties possessing new or altered characteristics that may not be suitable for other oat-based applications. Genetic variation for such "designer varieties" is undoubtedly present within the genus, but, depending on which gene pool is involved, accessing it could be problematic.

Molecular biological techniques have made an enormous contribution to our knowledge of many biological species, not least our staple food crops. The applications of these new technologies are now coming on stream in the cereals, and successful gene cloning and transgenesis in oats are now a real possibility (Somers et al. 1994). The ability to transfer specific genes in this way will not only make the recalcitrant tertiary *Avena* genepool as accessible as the primary gene pool, but might afford access to genes from any organism in the global genepool.

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Biotechnology and cereal improvement

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Introduction

Since the last meeting of IBGS and IOC five years ago, cereal biotechnology has made tremendous advances, even though it is still in its infancy. Over the next three days we will hear exciting details of the latest developments of biotechnology for cereal improvement. The first transgenic cereals are just being released. In Canada, CIBA has permission to sell a maize hybrid – “Maximizer” – with the Bt gene for insect resistance. My objective in this brief overview lecture is to set a framework for and perspective on developments for this advancing area, keeping in mind that the broad scope of the topic necessitates only a very brief mention of any one topic.

We have heard for a number of years the dire warnings that, at some point in the 21st century, we will not be able to produce sufficient food for our ever-increasing world population. Many authorities have suggested that biotechnology is one of the hopes that we have at our disposal to lessen that danger. This hope depends upon the development and utilization of these newer tools to assist in improving production and protection of our crops in much more precise and rapid approaches than otherwise are possible. Biotechnological tools are rapidly advancing our knowledge of the inheritance of important crop traits and are providing the means of tagging and moving the responsible genes between species.

At the same time, funds for research are decreasing. Governments are facing ever-tightening budgets and must make tough political decisions for survival today, often at the great expense of scientific development and our future food needs. We need to remind them that food shortages can arise very quickly. Diasters in large cereal producing areas could create problems today, as the recent surge in grain futures prices illustrates.

As researchers, we must continue to accelerate the adaptation of new genetic changes while keeping in mind the health and safety of man and his environment. We need to focus on what can be done that is economical and practical as well as on the scientific advances. While we will focus on the science of cereal biotechnology and its applications, we owe it to society to keep safety in our minds.

Progress has come much faster than we would have predicted, particularly in our knowledge of important traits and the evolution and organization of the physical chromosome structure. In the three sessions and posters that follow,

the emphasis will be on 1) describing molecular markers and their applications in understanding the genetic basis of agronomic traits and for marker assisted selection, 2) the physical structure of the chromosomes and their relatedness across species (tissue culture and haploidy tie into these sessions as well as the last session), and 3) gene transformation methods and developments.

Markers and their applications

At the 6th IBGS in 1991, we witnessed an explosion of RFLP mapping studies, along with the very first reports on applications. There have been large collaborative efforts in both barley and oats to develop molecular maps and the molecular tools. In the past five years, molecular mapping has spawned extensive information on the inheritance of agronomic, quality and disease traits, perhaps providing as much gene information as man had gained previously. Now, the molecular marker session will be dominated by applications for quantitative trait loci (QTL) identification and marker assisted selection (MAS) in both barley and oats. Equally important for crop improvement is the further effort needed to develop user-friendly molecular markers, usually based on PCR (such as simple sequence repeats, or SSRs), or those that can be developed much more rapidly (such as AFLP).

The evolution of marker tools is ongoing, and the different marker types each have their applications. RFLPs were the initial marker tool and the basis for most of our molecular maps. A key feature to the rapid development of cereal maps has been the collaboration among scientists and exchange of markers across the various crops. The North American Barley Genome Mapping Project (NABGMP) was the largest but just one of many collaborative projects that gradually meshed as projects developed. Today, the Steptoe by Morex cross of the NABGMP has 509 molecular markers mapped and is still expanding, with the focus on adding genes, AFLP and SSR markers. The markers consist of a wide range of types from RFLP, RAPD, STS, SSR, AFLP, isozymes to genes. We shall hear about the various types of molecular markers and their features. In barley, most mapping progeny were produced as doubled haploids so that they could be preserved indefinitely. Large collaborative projects are also important in oat molecular mapping, where base funding through Quaker Oats has been instrumental in developing a very successful effort. Hexaploid oats is much more complex than diploid barley, but some 561 markers have been placed on 38 linkage groups, many of which will eventually be combined on specific chromosomes. Mapping on diploid *Avena* species is being done to help sort out the homoeologous relationships.

Using RFLP markers is a fairly laborious procedure, and they are not highly polymorphic among the closely related genotypes found in breeding programs. Thus, much effort has gone into PCR procedures. RAPD markers, often through bulked segregant analysis, have been most useful in initial tagging and mapping of important genes, particularly disease resistance genes (Graner, 1996). However, the repeatability of RAPD methods is of concern for tagging genes,

because erroneous results are easily obtained (Penner et al., 1993). Thus, procedures to develop longer and more reliable primers for PCR have been developed, such as STS.

Microsatellites (SSR) are another PCR system of very short tandem repeats that tend to be quite polymorphic but expensive to initially produce. Thus, again collaborative efforts to develop these markers and suitable primers for PCR are in progress. An excellent example of SSR development is in hexaploid wheat, where RFLP and RAPD approaches were not very successful in developing polymorphism. Roeder et al. (1996) have identified 350 polymorphic SSR markers. Sequencing of SSR markers in different genotypes provides additional information for evolutionary and mutation studies.

The AFLP is a very rapid procedure to develop molecular maps due to the large numbers of bands on a single gel. But the number of steps and use of hazardous isotopes are aspects to be improved.

The applications of markers for tagging and selection of genes is an area that has progressed very rapidly in the past five years. There will be many reports detailing the usefulness of molecular markers in both oats and barley. The identification of QTL for many agronomic traits and the selection of these for important quality and disease traits are striking examples of progress. Such tools allow breeders and industry to dream about alternative uses for cereal crops, from the production of specialty products to improving stress tolerance for our variable climates. These new techniques have led to greater collaboration across the various disciplines working to improve cereals.

Maps and chromosomes

We have perhaps received the greatest gift in potential genetic progress from the area of synteny mapping of molecular markers among the different cereal species. In spite of evolutionary changes in basic chromosome numbers and DNA per genome among the *Triticum*, *Hordeum*, *Avena*, *Oryza* and *Zea* genera, it is apparent that sizeable blocks of ancestral DNA have remained together (Moore et al., 1995). The rearrangement of these blocks along with various levels of repetitive sequences allows for the reconstitution of the various chromosomes (Moore et al., 1995; Van Deynze et al., 1995). Understanding this relationship will greatly reduce the effort required to isolate, sequence and tag important genes in many of our crops and, equally important, to gain a better understanding of the inheritance of valuable traits. Since the rice genome has only one-tenth the amount of DNA as wheat or barley chromosomes, group efforts are being made to map and identify genes in the rice genome as a model that can then represent similar gene blocks in other cereal genomes. Some 25,000 rice cDNA clones have been partially sequenced and about 10,000 are thought to represent independent clones (Moore, 1995). Such information will also aid us in understanding the organization of the physical chromosome relative to repetitive sequences and gene distribution.

Mapping is also greatly aided by cytogenetic stocks or procedures such as

chromosome addition lines, chromosomal aberrations, banding procedures and *in situ* hybridization. Studies on the physical chromosome structure are feasible with the molecular mapping of chromosomes. This approach is providing insights into the location of genes and key structures such as centromeres and telomeres. The centromeres have been located on the barley maps using molecular probes on wheat-barley telocentric chromosome addition lines. Similarly, telomere sequences have been mapped to the ends of the barley chromosomes, so that the molecular maps of barley are known to be complete. Chromosomal rearrangements in barley (Kunzel, 1996) and deletions in wheat (Gill et al.) have been very useful in placing molecular probes and genes on the physical chromosomes of these crops. The *in situ* hybridization development of FISH has also been useful for mapping repetitive sequences to the physical chromosomes and, in combination with C or N banding for chromosome identification, assigning them to specific chromosomes. We can expect further refinements in *in situ* hybridization to enable the location of smaller molecular probes on chromosomes or YAC libraries.

At Guelph, we have used GISH to identify segments of *H. bulbosum* chromosomes added to barley which confer powdery mildew resistance. These segments have been tagged with molecular markers in order to follow the resistance through crosses. Relative to mildew resistance, the molecular marker segregation allows us to distinguish homozygous from heterozygous plants. GISH will also be very useful in locating common areas of chromosomes in different cereal species and species hybrids.

Cell and tissue culture have been very important tools in cereal improvement through biotechnology. Examples are haploid/doubled haploid production systems, somaclonal variation and as regenerable targets for gene transformation. Wide hybridization, chromosome elimination and embryo rescue led to the first usable system for doubled haploid cereal breeding (Kasha & Kao, 1970; Choo et al., 1985). The chromosome elimination and embryo rescue system has also been developed for wheat and oats using maize pollen, and it is rapidly gaining use for wheat breeding and research. The work on oats has also led to the potential for the transfer of chromosome material from maize into oats (Rines, 1996). The use of doubled haploids has been widely used and is important in the production of inbred lines for molecular mapping of the barley genome, and it will be for wheat also.

Anther culture, and more recently isolated microspore culture, have also been useful haploid procedures for new cultivar production. Devaux (1996) has determined that at least 59 cultivars of barley have been produced by haploidy procedures practised on the F1 of crosses. All of these, except for five recent ones by anther culture, have been produced by the Bulbosum Method. Recent breakthroughs in isolated microspore culture are likely to make this the method of choice for a haploid system for breeding in the future. Thousands of green plants can be regenerated from a single 10-cm petri plate. We have counted up to 20,000 embryo-like structures developing in one plate, or roughly 1 structure from every 40 to 50 microspores cultured. Breeding programs exist that can

produce up to 100,000 DH lines per year across a large number of different crosses (Jaiser, pers. comm.).

Gene transformation

The development of transformation procedures in cereals has been slow because of poor regenerability of cell culture systems, which were an essential part of the early systems. The most widely used system for cereal transformation at present is microprojectile bombardment of immature embryos (scutellum) and rapid regeneration through somatic embryos (Wan & Lemaux, 1994). Success in oats has been through bombarded embryogenic cell cultures (Somers et al., 1992; Torbert et al., 1995).

Other successful systems for cereals are: direct DNA uptake using PEG or electroporation; silicon carbide fibers; and, most recently, *Agrobacterium* (Table 1). Direct DNA uptake has relied upon the ability to produce and regenerate plants from protoplasts. This has required long-term embryogenic cell cultures and often the plants regenerated have been few and sterile. However, DNA uptake using organized tissues such as immature embryos has also succeeded (D'Halluin et al., 1992), although this system has a complicated selection process due to chimerism. Silicon (Whisker) fibers are a relatively simple system for getting the DNA into the cells (Kaeppler et al., 1992), but again, a regenerable culture system and chimerism are of concern.

Interest in and efforts on *Agrobacterium* transformation of monocots have been extensive (Smith and Hood, 1995). Recent success with *Agrobacterium* in rice (Chan et al., 1993; Hiei et al., 1994), maize, wheat (Cheng et al., 1996; Brettell, pers. comm.) and barley (Tingay, pers. comm.) indicate that this system may develop into the method of choice. *Agrobacterium* is thought to provide a more systematic and stable incorporation of DNA into the host DNA system, but this remains to be proven in cereals. All these procedures require the use of tissue culture, but efforts are being made to deliver plasmid DNA into meristems

Table 1. Chronology of success with gene transformation procedures in cereals. Year and first two letters of first author's name are used for identification in references.

Transformation Method	Crop Species				
	Maize	Rice	Wheat	Barley	Oat
1. Direct DNA uptake (electroporation or PEG)	1988 Rh	1988 To, Zh	1994 He	1995 Fu	
2. Microprojectiles*	1990 Fr	1991 Ch	1992 Va	1994 Wa	1992 So
3. <i>Agrobacterium</i>	1996***	1993 Ch	1996 Ch	1996**	
4. Silicon fibers	1994 Fr				

* Other cereals transformed by microprojectiles are sorghum (1993 Ca), rye (1994 Ca) and triticale (1995 Zi). ** Sonia Rigalo, CSIRO, Canberra, Australia, pers. comm. *** Pers. comm.

that could regenerate without the need for cell or tissue culture (Sautter et al. 1995).

Haploids could be ideal targets for obtaining homozygous transformants. Such transformations have been obtained in barley after particle bombardment using both immature isolated microspores and haploid immature embryos as the source of regenerable cultures (Jähne et al., 1994; Salmenkallio-Marttila et al., 1995; Yao et al., 1996). The use of microspores as targets in other cereals is presently not feasible due to the lack of a highly efficient microspore culture system and the need to improve transformation frequencies, which currently run about one per 1×10^7 microspores

The development of efficient methods for cereal transformation is still in the early stages. Little is known about the process of DNA incorporation into chromosomes. Problems include multiple insertions, breakup of gene constructs during incorporation, control and loss of gene expression, and stability of insertions. However, in spite of such problems, efforts are being made to incorporate useful traits in crops. Pest resistance and quality traits are the primary targets. In barley, a major area of interest is in enhancing or blocking the production of enzymes involved in malting barley (McElroy & Jacobsen, 1995). In other cereals, the major interest is in improving nutritional aspects such as digestibility, essential amino acid balance, oil and protein balance, and quality. The potential for changes to develop alternative uses for crops and for drug production are also under consideration.

In summary, we have seen the very rapid advances in cereal biotechnology in the past five years, with the hope for even speedier developments in the future. The tools of biotechnology are being applied in breeding methodology to more precisely and rapidly incorporate desired genes into improved cultivars. However, since the identification and isolation of the important genes is just in its infancy, a great collaborative effort will be needed. The hope that cereal biotechnology will help delay the impending world shortage of food has a solid basis.

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Practical application of marker assisted selection

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Introduction

The applications of molecular markers in plant breeding are now well known. The most common application is direct marker assisted selection but other applications include accelerated back-crossing, pyramiding genes, analysis and selection of quantitative traits, identification of hybrids, multiple trait screening, selection for resistance to pests and pathogens not present in the immediate environment ("quarantine" traits) and the analysis of alien chromosome segments. Molecular markers have also found application in varietal identification through DNA fingerprinting.

As a tool for more fundamental studies, markers have proved invaluable. These range from improving our understanding of genome structure and behaviour to the isolation of agronomically important genes via map based cloning.

The molecular marker development has not yet reached the stage where all these techniques are available for use. However, the areas where we do have useful markers are in monitoring specific traits, notably disease resistance, in the screening for quarantine traits, in genetic troubleshooting and in delineating the scope of some breeding strategies.

The crucial question in all aspects of marker implementation is cost. The ramifications of this question are the value of trait or traits being monitored, the cost, availability and speed of alternative screening techniques, and the likely segregation pattern of the trait. It is not feasible to try to use markers to select for complex traits that may be determined by five or six genes unless special strategies are introduced to help keep the population sizes small. The efficient implementation of markers will depend upon devising new breeding strategies. The role of molecular markers does not lie in simply replacing existing screening procedures. Rather than reiterate the now well known applications of molecular

markers, this paper will attempt to describe the general breeding strategies that can now be implemented through the use of molecular markers.

Strategies for marker implementation

Basic marker screening

In determining the value of molecular markers there are several important considerations:

- cost and reliability of molecular marker relative to traditional or direct screen
- closeness of linkage
- speed of result
- absence of alternative strategy

These points can be illustrated through examples from the Australian breeding programs.

Cereal cyst nematode (CCN) is regarded as the most serious disease of winter cereals in Southern Australia, but good resistance has been identified from several sources. It appears that all sources (eight have been localized) represent one or more alleles at a single locus. Traditional screening involves a tube bioassay where seedlings are inoculated with nematodes. In order to obtain reliable results, usually five to ten seedlings from each line must be screened. The bioassay takes about three months and involves scoring the number of cysts that develop on roots. This time delay makes crossing onto resistant plants difficult. Further, plants coming through the screen are poorly developed and difficult to use in subsequent crossing.

The *Ha2* gene conferring resistance has been mapped to the long arm of 2H, and RFLP markers are available, located about 5 cM on each side (AWBMA21, 6 cM proximal and PSR901, 4 cM distal) (Kretschmer et al., in preparation). Attempts to convert the RFLP markers to a PCR-based marker system have been only partially successful. Nonetheless, the RFLP markers offer several advantages over the bioassay: results are obtained within four days compared to three months for the bioassay; they identify heterozygotes (*Ha 2* is dominant); and only one plant from each family needs to be screened, compared to five to ten for bioassay.

A similar situation applies to a marker for *Yd2*, a gene conferring resistance to Barley Yellow Dwarf Virus. Initially, an RFLP marker (WG889) was identified that co-segregated with *Yd2* (Collins et al. 1996). Now a PCR based marker has been developed (Paltridge et al., in preparation). This marker is less than 1 cM from the *Yd2*. The bioassay for the resistance is time-consuming, it must be highly replicated and the genetic background of the plant will affect the disease symptoms.

In the above examples, molecular markers offer a clear advantage over bioassays, and these markers are being rapidly incorporated in the Australian breeding programs.

There are other areas where markers will gain regular use because no alternative selection strategy is available. This is the case for pyramiding resistance genes

to a single pathogen. In barley, a major objective will be to apply this technique to scald resistance breeding. The main problem at present is that relatively few sources of resistance with linked molecular markers are available. However, this is likely to become less of a problem as mapping work worldwide progresses. In Australia, a major effort to map scald resistance loci from *H. spontaneum* has been undertaken by Tony Brown's group at CSIRO in Canberra.

In Australia, we have a further important application of molecular markers. Due to our relative isolation, many diseases and pathogen races that are prevalent in Europe and America have not arrived. However, it is probable that they will ultimately get through the quarantine barriers currently in place. Indeed, we have seen several serious failures in our quarantine system in recent years. The increased movement of grain between countries is likely to exacerbate this problem.

In the absence of a particular disease or pest in this country and with quarantine requirements making "shuttle" breeding impractical, it is difficult to breed and select for resistance. Molecular markers offer a means for incorporating such resistances into Australian germplasm, although ultimately the material must be sent overseas for the bioassay. An important example is resistance to Russian Wheat Aphid. This pest is not in Australia, but breeding for resistance has been possible due to the use of linked markers (Nietolopez and Blake 1994).

Defect elimination

In contrast to wheat, where varieties of similar quality are binned together, malting varieties of barley are usually binned separately because maltsters strongly prefer uniform, single-variety parcels of grain. Once a high quality and agronomically acceptable line has been produced, one approach to breeding aims to extend the range and reliability of production of the variety. The process for achieving this objective can be termed "defect elimination." Classically, this would be attempted via back-crossing using bioassay screening systems, with attendant problems including handling recessive genes and recovering the recurrent parent phenotype. In the ideal situation, a variety would be made available in various forms but with essentially the same quality traits, hence minimizing the number of grain segregations. For example, tolerance to high soil boron is required for some production areas of Australia but not in others. A high quality barley variety could be produced as a normal and boron-tolerant variant but with nearly identical quality characteristics. Molecular markers have the potential to greatly accelerate back-crossing. Once a defect in an elite variety has been identified, the gene(s) that would correct the defect can be introduced and the elite background re-established by back-crossing and doubled haploid production. If the loci being transferred have been tagged with a molecular marker, this can also be monitored during back-crossing. Desired recombination events in the area of the target gene can be identified. In the South Australian breeding program, this approach is being used to transfer boron tolerance, manganese efficiency, semi-dwarfism, and resistance to CCN, common root rot and leaf scald. Each of the traits is initially in a single breeding stream. MAS

will be especially useful when the independent streams are merged and multiple trait selection is required.

Parent building

The identification of molecular markers linked to agronomically important traits is now a relatively straightforward process. Mapping programs are identifying markers linked to a number of important traits. As the number of useful tagged genes increases, the complexity of the screening process increases and, more importantly, the sizes of the populations needed to identify the rare individual with the appropriate gene combinations increases to unmanageable proportions. One approach to addressing this problem is described below in strategies for combining molecular markers with doubled haploid generation. However, most breeding programs will be restricted to screening for only a few segregating markers in a selection program. It must be remembered that the breeders will usually have a set of traits that will be monitored using existing procedures; if we combine three or four such traits with the two or three molecular markers, the breeders may well be monitoring five to seven independent traits. The size of the conventional F_2 population needed to obtain the desired combination approaches 10,000 if six genes are to be combined. The problem is particularly apparent where the objective may be to pyramid four or more genes, and this may be the only effective method of long-term control of diseases such as scald.

Lines that contain a collection of such genes will become valuable sources of germplasm. The specific construction of these lines represents the objective of parent building. One commences with a well adapted variety and sequentially introduces the desired genes by back-crossing. The sequential addition can be accelerated by introducing one or more new genes at each generation. The strategy is optimized when each of the genes is tagged by a molecular marker. Figure 1 illustrates the sequence of crosses needed to combine four genes in a single line.

By using molecular markers, the heterozygotes can be identified after the second and third crosses. The final product is heterozygous at each of the loci. After each cross, the desired heterozygotes will be recovered at a frequency of 0.25 (except the first cross, where they will represent 100% of the progeny). At any stage, the heterozygotes can be used to recover homozygotes, most efficiently through the production of doubled haploids. The background can then be improved through accelerated back-crossing. Since each of the desired loci is tagged with a molecular marker, the screening is relatively simple.

This strategy can be expanded to include a large number of loci. The resultant plants can then serve as a source for a large battery of desirable genes. However, in many cases the loci of interest will be linked. If the desired genes are linked in repulsion, this will reduce the frequency of recovery of the desired combination in relation to the closeness of linkage. For such loci, large populations must be screened and the value of the technique will decline.

Parent selection

An Australian Barley Genome Program has been established to develop molecular markers for loci controlling aspects of malting quality and agronomic performance. A further component of this program has been the development of a database of molecular markers of Australian barley varieties. A total of 96 lines comprising all major commercial varieties grown in Australia, a selection of varieties from all barley growing regions of the world, and varieties that have provided useful germplasm or are thought to be of value in the breeding programs have been included. This collection is currently being screened with RFLP probes. The objective will be to have about 1000 polymorphic bands scored for the varieties.

In simple back-crossing programs, where one or more genes are to be transferred into an elite line, a molecular database can be used to help select the recurrent parent and the donor source of the new gene (where a choice is available). This allows an analysis of the molecular relationship of various parts of the plant genome. Several such molecular comparisons have already been performed. By selecting two lines that match for as many loci as possible, the time needed for back-crossing can be greatly reduced. Conversely, where the objective is to generate a new pool of variation for selection, for example for improved quality, a breeder can use the molecular database to identify varieties that are as diverse as possible at the molecular level but show similar quality characteristics. This approach allows breeders to optimise the chances of producing transgressive segregants and novel genetic combinations.

A	B	C	B	D	B	E	B	
R1R1 x	r1r1	r1r1	x	r1r1	x	r1r1	x	r1r1
r2r2		r2r2	R2R2		r2r2	r2r2		r2r2
r3r3		r3r3	r3r3		R3R3	r3r3		r3r3
r4r4		r4r4	r4r4		r4r4	R4R4		r4r4
F1	R1r1	x	r1r1	r1r1	x	r1r1	r1r1	
	r2r2		R2r2	r2r2		r2r2	r2r2	
	r3r3		r3r3	R3r3		r3r3	r3r3	
	r4r4		r4r4	r4r4		R4r4	R4r4	
TC1F1	R1r1		x	r1r1		r1r1		
MAS	R2r2			r2r2		r2r2		
	r3r3			R3r3		R3r3		
	r4r4			R4r4		R4r4		
TC2F1			R1r1	R1r1				
MAS			R2r2	R2r2				
			R3r3	R3r3				
			R4r4	R4r4				

Figure 1. Scheme for combining four desired genes in a single elite background.

Markers and doubled haploids

The generation of doubled haploids has now become routine in many barley breeding programs. This procedure offers significant benefits by generating perfectly homozygous plants, allowing breeders to yield test pure lines and proceed toward release without expensive reselection. However, the coupling of doubled haploid production with MAS offers further benefits. A disadvantage of using F1- or F2-derived DH lines is that the background genotype is still averaging 50% of the favoured parent, P2. A more effective strategy would be to backcross to the recurrent parent (P2) and use MAS to select appropriate donor plants for DH production (Figure 2). The average background genotype of the BC1-derived DH lines will now be 75% of the recurrent parent.

In a further scenario, and in the interests of maintaining recombinants in the background genotype, the BC2F1 population can be intercrossed at random or, preferentially, selected lines can be intercrossed so that a proportion of their progeny will yield AABB if used as DH donor parents. A summary of the consequences of the different strategies is given in Table I. The strategy involves using MAS to choose the appropriate parents before intercrossing and/or for selecting DH donor plants from amongst the progeny after crossing. Since there are 10 possible parental genotypes, then random intercrossing and no MAS will yield 6.3% of the desired DH AABB. Selecting any of the progeny of the intercross with an A-B- genotype as a donor will yield 32.6% AABB, while selecting AABB, AABb or AaBB donor parents will give a frequency of 53%.

Devising recombinational strategies

The barley genome is comprised of only seven pairs of chromosomes. Consequently, in breeding strategies involving several loci, it is usual for the breeder to be seeking to recombine linked loci. In the absence of reliable genetic data, attempts to isolate recombinants can prove fruitless if the linkage is close and/or small populations are screened. A classic example has been the early attempts to break the linkage between the six row allele and resistance to cereal

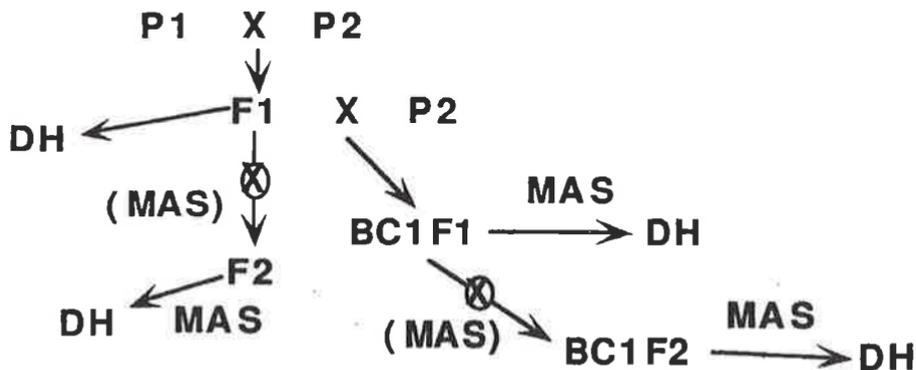


Figure 2. Alternative breeding schemes for utilizing both marker assisted selection (MAS) and doubled haploid production (DH).

Table 1. Recovery of the desired genotype, AABB, in doubled haploids following MAS or no MAS. (Note that these results would also apply to the strategy outlined in Figure 1.)

Total No. Intercrosses	Cross selection Proportion of selected intercrosses	No MAS	Pre-MAS AABB & A-B-	Pre-MAS AABB;AABb & AaBB	Pre-MAS AABB only
10	16/16	6.3%	32.6%	53.8%	100%
5	9/16	11.1%	32.6%	53.8%	100%
3	5/16	15.0%	35.4%	53.8%	100%
1	1/16	100.0%	100%	100%	100%

cyst nematode in the variety Marocaine. We now know that these loci lie about 10 cM from each other. With this information it would have been practicable to design a selection strategy to maximize the chances of identifying the rare recombinants.

The problem of recombining linked loci is likely to become increasingly important as more agronomic traits are mapped, particularly complex traits controlled by several loci. This is particularly well illustrated by chromosome 2H. The map constructed through the Australian Barley Mapping Program has identified 28 loci of agronomic importance; many within only a few cM of each other.

How can MAS help design selection strategies to identify the desired recombinant genotypes? If we assume that a breeder has observed problems in obtaining the desired genetic combination, the first step is to estimate the genetic distance between the traits of interest. This may be possible by using existing genetic data. For example, the two traits may have been mapped previously in different populations. By referral to consensus maps (Langridge et al. 1995) or by tracing common markers, an approximate localization may be possible. In some situations, it may be worthwhile to construct a mapping population and locate the desired genes. This would apply if the recombination is likely to present a problem in several crosses or if three or more loci are to be recombined.

Armed with the genetic linkage data, it is now possible to calculate the probability of obtaining the desired recombinants. The size of the populations to be screened can be judged and the closest markers can be identified for MAS. It will also be possible to devise an optimal screening procedure for identifying the desired recombinants. This will involve identifying readily screenable markers that flank the loci of interest. Reliable PCR-based markers would be used in preference to RFLP markers. These can then be used to identify plants showing a recombination between the flanking markers. These plants can be further analyzed with markers to define more precisely where the recombinational breakpoint lies.

Molecular markers help in these situations in two ways: firstly, by defining the problem and localizing the loci of interest and secondly by assisting in the identification of plants carrying a recombination in the desired area.

Characterization of genetic potential using QTL analysis

Demands for improved varieties from the barley industry and growers have increased in recent years. There is a perception that new technologies, including MAS, will permit breeders to select for complex traits of low heritability and traits with negative genotypic correlations, for example various aspects of end-use quality and tolerance to environmental stresses, such as drought. Such phenotypes are determined by the complex interaction of the environment and the genetic make-up of the plant. The determination of the relative significance of the environment versus genetic composition has been difficult in the past and was based largely upon the calculation of heritability of the trait. More recently, a large number of field trials and complex GxE statistical models have improved our understanding. Neither approach has permitted the definition or localization of specific loci, so, the significance of specific loci could not be identified. Molecular markers can change this and now, through the development of detailed linkage maps, we can envisage the definition and quantification of genetic loci affecting complex traits influenced by the environment. This will strengthen available predictions on what can be expected of a breeding program, potentially to the extent of determining how far crossing and selection can take the trait. It will also permit the definition of the genetic potential for the trait of interest in varieties or other germplasm sources, and markers can be developed that will permit the major loci to be monitored in segregating populations in the same way as other traits.

It should be emphasized that much of the current analysis of such traits is likely to indicate that the trait is either too complex for it to be usefully manipulated in breeding programs, or that the environmental component is so large that little progress can be made through breeding. Whatever the outcome of these types of studies, it will be crucial to determining where breeding effort can be most usefully expended and where we should be concentrating on improved agronomic practice.

There are new statistical techniques that can be used to analyze cross-prediction trials and multi-location trials that provide estimates (Best Linear Unbiased Predictors, "BLUPs") of the Estimated Breeding Values (EBVs) and Estimated Specific Values (ESVs), of varieties should complement the QTL analyses we have discussed here, resulting in a greatly improved ability to manipulate quantitative traits.

Conclusions

Despite the extensive work with molecular markers over the past ten years, and the identification of markers linked to many agronomically important traits, barley breeders have been slow in applying this new technology. This has been partially due to the technical difficulty in screening for RFLP markers, but also to the absence of clear and tested implementation strategies. Many of the strategies described above are yet to be proven, but several, such as direct marker

selection and MAS linked with the use of doubled haploids, have found ready application and are becoming routine in barley breeding. The development of reliable markers for QTLs, the intensive mapping of disease resistance loci and the potential of PCR-based screening techniques (especially the use of microsatellite primers) will provide the next major impetus to marker application. As the density of markers linked to important traits expands, pressure on the development of efficient implementation strategies will also increase. It is already hard to conceive of a barley breeding program in ten years' time that would not use MAS as a central feature of its selection strategies.

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The identification, localization and utilization of molecular markers for rust resistance genes in oat

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Introduction

Together, crown rust (*Puccinia coronata* Cda. f. sp. *avenae* Eriks.) and stem rust (*Puccinia graminis* Pers. f. sp. *avenae* Eriks. & Henn.) are probably the most destructive and widely distributed diseases affecting cultivated oat. The race populations of the pathogens causing these diseases are ever-changing, and it is a constant challenge for breeders to respond to this threat. Different resistance genes within cultivated oat have been identified and used in breeding programs, but more and more sources of resistance have been sought in related species, such as *Avena sterilis* L., as well as diploid and tetraploid *Avena* species (Martens and Dyck, 1989).

One of the breeding strategies for responding to the changing race populations of the stem and crown rust pathogens has been to develop germplasm carrying combinations of several effective resistance genes (Chong et al., 1994). Seventeen genes (*Pg1* to *17*) that confer resistance to different races of the stem rust pathogen have been identified to date (Simons et al., 1978; Harder et al., 1990), and in excess of 75 crown rust resistance genes have been reported (Martens and Dyck, 1989). Combining several resistance genes is not an easy task because of the elaborate rust testing involved and, for some combinations, can not be achieved for lack of available rust races to distinguish the simultaneous presence of different genes. In order to facilitate such breeding objectives, the search for markers to rust resistance genes has been intensified in recent years.

Identification

Near isogenic lines (NIL) carrying single resistance genes have been developed over the years for the characterization of the different rust races or to transfer new sources of resistance from unadapted germplasm (Fleischmann and Baker, 1971; Martens et al., 1979). The availability of such lines has greatly facilitated the identification of markers to rust resistance genes in oats. DNA from NILs differing for the presence of the resistance allele at a given locus are surveyed for polymorphisms at the DNA level. The linkage between the gene and the

putative polymorphic marker is established in a segregating population from a cross between the two NILs.

Protein and Restriction Fragment Length Polymorphisms (RFLP) markers to the two stem rust resistance genes *Pg9* and *Pg13* (Howes et al., 1992; Chong et al., 1994; O'Donoghue et al., 1996) were identified using NILs in a Rodney background that were originally developed for the stem rust differential set (Martens et al., 1979). Similarly, Penner et al. (1993a) identified 2 Random Amplified Polymorphic DNA (RAPD) markers for *Pg3* by screening for polymorphic DNA fragments between Rodney-0 and Rodney-*Pg3*. The Rodney NILs were also used to identify an esterase marker for *Pg1* (James Chong, personal communication). More recently, Pendek background NILs (Fleischmann and Baker, 1971) were used to identify markers for the crown rust resistance genes *Pc38*, *Pc39* and *Pc48* (O'Donoghue et al., unpublished). Rooney et al. (1994), used backcross derived lines to study the crown rust resistance genes present in Amagalon and Obee/Midsouth, two hexaploid oat lines that had been derived through interspecific hybridization. Two genes, *Pc91* (from Amagalon) and *Pc92* (from Obee/Midsouth), and associated RFLP markers were identified in this study. Bush et al. (1994) used paired NILs (with the same gene in two different backgrounds) to identify RFLP markers for three crown rust resistance genes originally transferred to cultivated oat from *A. sterilis*.

The method of bulked segregant analysis (Michelmore et al., 1991) was also successfully used to identify markers for rust resistance genes in oat. This method uses, for polymorphism surveys, a pair of bulked DNA samples created from segregants that differ for the rust resistance gene. Bulk segregant analysis was used by Penner et al. (1993b) to identify a RAPD marker for *Pc68* and by O'Donoghue et al. (1996) to identify RAPD markers for *Pg9* and *Pg13*.

Localization

The development of molecular linkage maps for cultivated oat (O'Donoghue et al., 1995) and diploid oat (O'Donoghue et al., 1992; Rayapati et al., 1994a) is now adding a new dimension to the identification of markers to rust resistance genes in oat, namely localization. Rust resistance genes can be mapped within the existing framework, or markers for rust resistance that have been identified in other populations can be used to locate genes by comparative mapping.

Localization on an existing map gives access to a larger collection of linked markers. This point is especially important in oat because the relatively high level of polymorphism at the DNA level (O'Donoghue et al., 1994) implies that a marker allele shown to be linked to a resistance allele in one background may be monomorphic or associated with the susceptible allele in a different background. Wight et al. (1994) demonstrated that a given RAPD marker allele for daylength insensitivity in oat identified in one cross could not be assumed to be found in all germplasm carrying the same daylength insensitivity allele, nor only in daylength insensitive lines. Because of this, breeders should have access to several marker loci linked to a given resistance gene in order to find one

which will be useful for the particular breeding program they wish to monitor.

Localization also allows us to gain a better understanding of how these numerous resistance genes are organized within the oat genome. This information can be useful to devise breeding strategies and perhaps can provide insight into the evolution of new resistance genes and alleles. It is already known that some of the rust resistance genes are associated in the oat genome. One such group consists of the stem rust resistance genes *Pg3* and *Pg9* and the crown rust resistance genes *Pc44*, *Pc46*, *Pc50*, *Pc68*, *Pc95* and *PcX* (Wong et al., 1983; Chong et al., 1994).

O'Donoughue et al. (1996) used clones that had been previously mapped in cultivated oat (Kanota x Ogle) (O'Donoughue et al., 1995) to survey the NILs used to identify markers for *Pg9*. This allowed comparative mapping between the populations used to establish linkage of the markers for *Pg9* and the cultivated oat map. Using this method, they localized *Pg9*, and consequently the other crown and stem rust resistance genes listed above, to group 4 of the cultivated oat map (Figure 1). Interestingly, Bush and Wise (1996), who studied the inheritance of resistance to crown rust races *Pc54* and *Pc59* in the Kanota x Ogle population, found that resistance to these races was conferred by at least two genes. One of these genes was most likely located on group 4 and the other on group 13.

The *Pg13* stem rust resistance gene was localized to group 3 by O'Donoughue et al. (1996) using a similar comparative mapping approach (Figure 1). Interestingly, both groups 3 and 4 of the Kanota x Ogle map show homoeology to group A of a diploid, *A. atlantica* x *A. hirtula*, map (O'Donoughue et al., 1992). However, the regions carrying the *Pg9* and *Pg13* genes are homoeologous to opposite ends of the diploid group A (Figure 1). This indicates that both arms of homoeologous group A in *Avena* can carry rust resistance genes. Similarly, Rayapati et al. (1994a) mapped crown rust resistance genes in a diploid cross of *A. strigosa* x *A. wiestii*, and found these to be clustered at the end of linkage group A. Comparative mapping of markers for crown rust resistance to race 203 in hexaploid oat with the diploid *A. strigosa* x *A. wiestii* map locates this gene to a region homoeologous to group A, but in an area quite distant from the diploid crown rust resistance genes (Yu et al., 1996). The association of an esterase locus with *Pg1* (James Chong, personal communication) provides evidence that this gene is also located on an homoeologous group A chromosome. Four esterase loci were mapped in cultivated oat. Three of these loci mapped to regions homoeologous to the diploid group A (O'Donoughue et al., 1995). Comparative mapping between diploid and hexaploid oat also revealed reduced recombination in the group 3 region carrying *Pg13* resistance for all three hexaploid oat crosses investigated (Figure 1, O'Donoughue et al., 1996). This type of information provided by mapping can be useful in devising strategies to combine several resistance genes, because it may be more difficult to reduce linkage drag, break linkages and transfer rust resistance genes located in such an area of reduced recombination.

The *Pg9* and *Pg13* stem rust resistance genes have been shown to be associated with endosperm proteins (Howes et al., 1992, Chong et al., 1994). Avenin loci have been mapped to homoeologous group A in cultivated oat (O'Donoughue et al., 1995)

and diploid oat (Rayapati et al., 1994b). Also, O'Donoghue et al. (1996) identified an RFLP marker to *Pg13* using a globulin cDNA clone. This clone identified two RFLP loci in the Kanota x Ogle map, both of which mapped to regions homoeologous to group A (O'Donoghue et al., 1996). The association of endosperm storage proteins

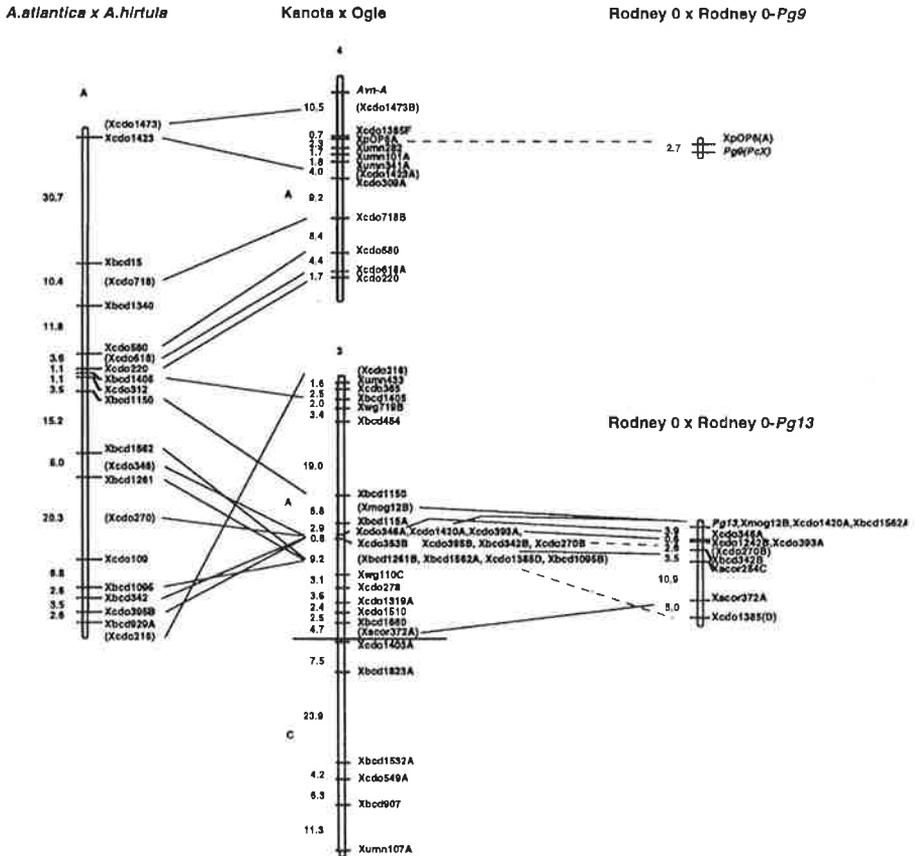


Figure 1. Comparative maps of the rust resistance regions identified in, Rodney O x Rodney O-*Pg9* and Rodney O x Rodney O-*Pg13* with linkage groups 4 and 3 from the linkage map of cultivated oat based on the cross Kanota x Ogle and linkage group A from the *A. atlantica* x *A. hirtula* diploid oat map. Lines between the diploid and hexaploid oat map linkage groups indicate orthologous loci. Solid lines between the hexaploid oat maps indicate the same loci as determined by identical allelic fragments whereas dashed lines represent loci which may be the same based on map position only (localization of *Pg9* and *Pg13* to group 4 and group 3 was confirmed by comparative mapping with a second cross, OT328 x Dumont, (O'Donoghue et al., 1996)). Letters to the left of the Kanota x Ogle linkage groups refer to homoeologies with the linkage groups of the *A. atlantica* x *A. hirtula* map. Map distances are given in centi-Morgans (Kosambi function). Loci in parentheses have been assigned to intervals only (LOD < 2).

with disease resistance loci appears to be conserved across grass species; it has been noted on the homoeologous group I of the Triticeae: in wheat (Howes, 1986), rye (Singh et al., 1990) and barley (Yu et al., 1996). Furthermore, clones that identified markers to crown rust resistance in hexaploid oat (Bush et al., 1994) were shown by Yu et al. (1996) to identify loci linking to disease resistance loci on three maize linkage groups. The conservation in the organization of genomic areas carrying disease resistance genes implies that marker work done in wheat, barley or even maize may help identify markers to resistance genes in oat.

Though homoeologous group A appears to be important for rust resistance loci in oat, it is definitely not the only homoeologous group where rust resistance loci can be found. The area on group 13 identified by Bush et al. (1996) as being involved with crown rust resistance to races PC54 and PC59 in Kanota x Ogle is homoeologous to group C (O'Donoghue et al., 1995). Also, the markers identified by Bush et al. (1994) to the crown rust resistance locus R345 in hexaploid oat map to group C of the *A. strigosa* x *A. wiestii* map. Preliminary data on the localizations of *Pc38*, *Pc39* and *Pc48* indicate that these loci are located in areas homoeologous to groups D, E and C respectively (O'Donoghue et al., unpublished). Two other rust resistance loci and associated markers have been localized using aneuploid analysis. Rooney et al. (1994) located *Pc92* to Kanota monosomic 21 (Jellen's 1993, group 18) and Bush et al. (1994) located the crown rust resistance locus R264B to Kanota monosomic 7 or 13 (Jellen's 1993, group 14). It is not known how these aneuploid lines relate to the molecular linkage maps since the integration of the monosomic series, the molecular linkage map and the C-banded karyotype remains to be completed.

Utilization

To implement marker assisted selection (MAS) for rust resistance in oats, three main requirements need to be met first. In the past five years, we have met the first two of these requirements. First, markers needed to be identified to the different rust resistance genes. To date, markers to thirteen rust resistance genes have been identified, and no doubt markers to more genes will be identified in the near future.

Secondly, because of the level of polymorphism in oat, several potential markers to any given gene needed to be identified in order to realistically provide markers that will be useful in any breeding program. The localization of several of these resistance genes with respect to the existing linkage maps is now giving access to a large number of linked markers.

The third requirement is to provide more user-friendly and cross-applicable markers. Though RFLP and RAPD markers can be used for MAS, the cost and labour-intensive nature of the former and the limited cross-applicability of the latter prevent their widespread use. Several of the RAPD and RFLP markers that have been identified to rust resistance in oats are currently being converted to the more cross-applicable PCR based SCARs (Sequence Characterized Amplified Region) and STS (Sequence Tagged Site) type markers.

Thus, the monitoring of several rust resistance genes in a breeding program

using MAS is now becoming a reality and should be ready for widespread use in the near future.

Acknowledgments

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Molecular mapping of genes conferring disease resistance: The present state and future aspects

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Introduction

It was not until the rediscovery of the Mendelian Laws that genetics turned from mysticism and became a serious science, which for more than eight decades has been the basis for successful barley breeding. Grain yield has been the pivotal breeding goal throughout this period of time, and tremendous success has been achieved by applying conventional cross breeding strategies. Since high yields and good quality require healthy plants, disease resistance was rapidly recognized as an important yield component, and specific attempts to breed for resistance can be traced back to the early 1930s (Straub, 1977). Since then, a large number of genes conferring complete or partial resistance have been introgressed into and combined within cultivated barley, leading to a steady increase in the level of resistance against most major diseases. However, the rapid adaptation to its host entails a permanent race of the breeder against the evolutionary clock of the pathogen. Thus, breeding for disease resistance is becoming an increasingly complex task, and the identification of the desired recombinants based on mere selection on the phenotypic level has already reached the limits of manageability.

The development of molecular markers provides the possibility to select on the genotypic rather than on the phenotypic level. In terms of disease resistance, the availability of selectable markers will allow for a more efficient manipulation of genes without extensive field trials and glasshouse tests, and will finally accelerate the entire breeding process. However, the successful application of marker-assisted selection strategies depends heavily on the availability of appropriate markers tightly linked to genes of agronomic interest. In this context, a number of comprehensive molecular marker maps have been developed during the last five years. Together, these maps contain more than 1000 different RFLP markers, and form a solid basis for the accurate genetic localization of almost any given gene. This paper will summarize the present state of molecular mapping of genes conferring resistance to major barley pathogens, and provide a short outlook regarding future applications and developments in this rapidly evolving field.

Molecular mapping

With respect to fungal pathogens, resistance to powdery mildew (*Erysiphe graminis*) attracts major interest. As can be seen from Table 1, a series of resistance genes, most of which confer race specific resistance, has been identified and tagged by molecular markers. In addition to cultivated barley, *H. spontaneum* has been increasingly used as a donor for mildew resistance genes. Here, the use of linked markers will facilitate the rapid and efficient introgression of the

Table 1. Major resistance genes that have been tagged by molecular markers

Pathogen	Gene	Chrom.	Reference
<i>Erysiphe graminis</i>	<i>mlt</i>	1S	Schönfeld et al., 1996
	<i>Mlf</i>	1L	Schönfeld et al., 1996
	<i>MlHb</i>	2S	Graner et al., unpublished
	<i>MlLa</i>	2L	Hilbers et al., 1992
			Giese et al., 1993
	<i>Mlg</i>	4L	Görg et al., 1993
	<i>mlo</i>	4L	Hinze et al., 1991
	<i>Mla</i>	5S	Schüller et al., 1992
	<i>Mli</i>	5L	Jahoor, pers. comm.
	<i>Mlh</i>	6S	Hilbers, pers. comm.
	<i>Mlj</i>	7L	Schönfeld et al., 1996
<i>Puccinia graminis</i>	<i>Rpg1</i>	1S	Kilian et al., 1994
	<i>rpg4</i>	7L	Borovkova et al., 1995
<i>Puccinia hordei</i>	<i>Rph2</i>	7*	Steffenson, pers. comm.
<i>Rhynchosporium secalis</i>	<i>Rh2</i>	1S	Schweizer et al., 1995
	<i>Rh</i>	3L	Graner and Tekauz, 1996
	<i>Rhy</i>	3L	Barua et al., 1993
	<i>Rh13</i>	6S	Abbott et al., 1995
<i>Pyrenophora teres</i>	<i>Pt.,a</i>	3L	Graner et al., 1996a
<i>Cochliobolus sativus</i>	<i>Rcs5</i>	1S	Steffenson et al., 1996
<i>Typhula incarnata</i>	<i>Ti</i>	5S	Graner et al., 1996b
<i>Barley yellow dwarf virus</i>	<i>Yd2</i>	3L	Collins et al., 1996
<i>Barley stripe mosaic virus</i>	<i>Rsm</i>	1S	Edwards and Steffenson, 1995
<i>Barley yellow mosaic virus complex</i>	<i>ym4</i>	3L	Graner and Bauer, 1993
	<i>ym5</i>	3L	Graner et al., 1995
	<i>ym6</i>	3L	Iida and Konishi, 1994
	<i>ym8</i>	4L	Graner et al., 1995
	<i>ym9</i>	4L	Graner et al., 1995
	<i>ym11</i>	4L	Bauer et al., unpublished
	<i>ym7</i>	5S	Graner et al., 1995
<i>Heterodera avenae</i>	<i>Ha</i>	2L	Langridge, pers. comm.

* Assignment to chromosome arm not possible due to a proximal position of the gene.

corresponding genes into adapted germplasm, and thus will further promote the use of this source of resistance in breeding programs. Also, a race-specific gene derived from *H. bulbosum* (*MIHb*) has been mapped in a translocation stock to the short arm of chromosome 2, where it is tagged by several closely linked markers. Regarding the introgression of alien chromatin, RFLP markers will help to precisely map translocated chromosome segments within the genome of *H. vulgare* and to monitor their inheritance. However, it remains to be seen to what extent recombination around chromosomal segments introgressed from *H. bulbosum* is suppressed. Although high levels of quantitative resistance against mildew are desirable, only two QTL on chromosome 1 and 7 have been identified, together explaining about 20% of the observed variance (Table 2). In a diallel study, Saghai-Marouf et al. (1994) detected quantitative effects for all of those chromosomal regions where major genes are located. This coincidence lends strength to the hypothesis that quantitative differences may exist at loci that are considered to carry major genes.

Cereal rusts form a second group of obligate biotrophic pathogens of worldwide importance. Although the genetic basis of resistance to these pathogens has been thoroughly investigated by classical genetics, only a few genes have been tagged by RFLP markers to date. These include *Rpg1* on chromosome 1S and *rpg4* on chromosome 7L, conditioning resistance to stem rust (*Puccinia graminis*), and *Rph2* in the centromeric portion of chromosome 7, conferring resistance to leaf rust (*P. hordei*). Regarding *P. striiformis*, two QTL have been detected on chromosomes 7L and 4L. Together, these account for 61% of the genetic variation. However, more than 90% of the explained variation was attributable to the major QTL on chromosome 7L.

A series of agronomically important diseases is incited by a group of systematically related fungi, including *Rhynchosporium secalis* (scald), *Pyrenophora teres* (net blotch), *Pyrenophora graminea* (leaf stripe) and *Cochliobolus sativus* (spot blotch). As to scald, several resistance genes have

Table 2. Mapped QTL for disease resistance

Pathogen	Chrom.	r ² *	Reference
<i>Erysiphe graminis</i>	1S, 7L	0.19	Heun, 1992
<i>Puccinia striiformis</i>	7L, 4L	0.61	Chen et al., 1994
<i>Rhynchosporium secalis</i>	2L	0.31	Backes et al., 1995
<i>Pyrenophora teres</i> ¹	4 [†] , 6S, 6L	0.47	Steffenson et al., 1996
<i>Pyrenophora teres</i> ²	1S, 2S, 3S, 3L, 4 [†] , 6S, 7S	0.68	Steffenson et al., 1996
<i>Cochliobolus sativus</i>	1S, 5S	0.70	Steffenson et al., 1996
<i>Pyrenophora graminea</i>	1L, 2S, 4S	0.77	Pecchioni et al., 1996

* Presented r² values apply to multilocus models. [†] Assignment to chromosome arm not possible due to the proximal location of the QTL. ¹ Seedling resistance. ² Adult plant resistance.

been mapped on chromosomes 1, 3 and 6. In addition to these, a major QTL, explaining 32% of the genetic variance, was located on chromosome 2L. Resistance to net blotch is more complex, since seedling and adult plant resistance seem to be, at least in part, conditioned by different sets of genes (Steffenson et al., 1996).

Regarding seedling resistance to net blotch, a major gene has been mapped in the proximal region of chromosome 3L, and 3 QTL were identified on chromosomes 4, 6S and 6L, respectively. Seven QTL for adult plant resistance were identified on the short arms of chromosomes 1, 2, 3, 6 and 7 as well as on the long arm of chromosome 3. Another, minor QTL on chromosome 4 has been located in the same interval as the major QTL for seedling resistance. A major gene for seedling resistance to spot blotch has been mapped on chromosome 1S, and two QTL for adult plant resistance were located on the short arms of chromosomes 5 and 1, respectively. The latter might be identical to the major gene for seedling resistance. Similarly, four QTL conferring resistance to barley leaf stripe were identified on chromosomes 1L(2), 2S and 4S. Together, these explained 77% of the genetic variation, with the largest QTL on 1L already accounting for nearly 60%.

Finally, a gene conferring resistance to *Typhula* blight incited by *Typhula incarnata* has been identified and mapped in the telomeric region of chromosome 5S. In this case, the availability of a molecular marker facilitates breeding for resistance to a pathogen in winter barley, which usually baffles systematic selection efforts since its irregular occurrence is highly dependent on environmental conditions during the winter season.

Barley yellow dwarf virus (BYDV) is characterized by its worldwide occurrence and its large host range, comprising more than 100 grass species, including all major cereals. Incorporation of the *Yd2* gene, located in the centromeric region of chromosome 3L, represents the only way to generate resistant germplasm, although *senso stricto* the gene confers tolerance only. The availability of selectable markers greatly facilitates the identification of tolerant plants since the expression of this trait is highly influenced by both environmental factors and genetic background. In contrast to BYDV, the seed-borne barley stripe mosaic virus (BSMV) has only regional importance in North America and some European locations. Recently, a single gene was mapped to the proximal region of chromosome 1S, and several closely linked markers were identified that might be useful for marker assisted selection.

The barley yellow mosaic virus complex comprises two different virus strains: barley mild mosaic virus (BaMMV) and barley yellow mosaic virus (BaYMV). Of the latter, two serologically related types have been described (BaYMV-1, BaYMV-2). As yet, the occurrence of the BaMMV/BaYMV complex seems to be confined to Central Europe and Japan, where large areas are infested. More than half a dozen genes conferring either partial resistance or immunity to the virus(es) have been mapped on chromosomes 3L, 4L and 5S. The available markers allow a rapid incorporation of these recessive genes without field and/or greenhouse tests.

In addition to resistance genes against fungal and viral pathogens, a single dominant gene conferring resistance to the cereal cyst nematode *Heterodera avenae* has been tagged on chromosome 2L.

Genomic organization of resistance genes

Although knowledge about the number and the genetic localization of disease resistance genes is still far from being complete, the genetic data that have been gathered until now allow a first glimpse of the genomic organization of the corresponding genes (Figure 1). Evidently, they are not evenly distributed along the seven barley chromosomes, but tend to form clusters. These are either composed of genes of different specificity (heterospecific) or of genes that condition resistance against a single pathogen only (homospecific). The presence of heterospecific gene clusters have been described for tomato and wheat (cf. Ellis et al., 1995). In barley, conspicuous clusters are present in the distal portion of chromosome 1S (*Rh2*, *Rpg1*, *mlt*) and in the centromeric portion of chromosome 3L (*Rh*, *Rhy*, *Pt*, *Yd2*). Since different genes were mapped in different progenies, there is only indirect evidence for linkage. Therefore, it is

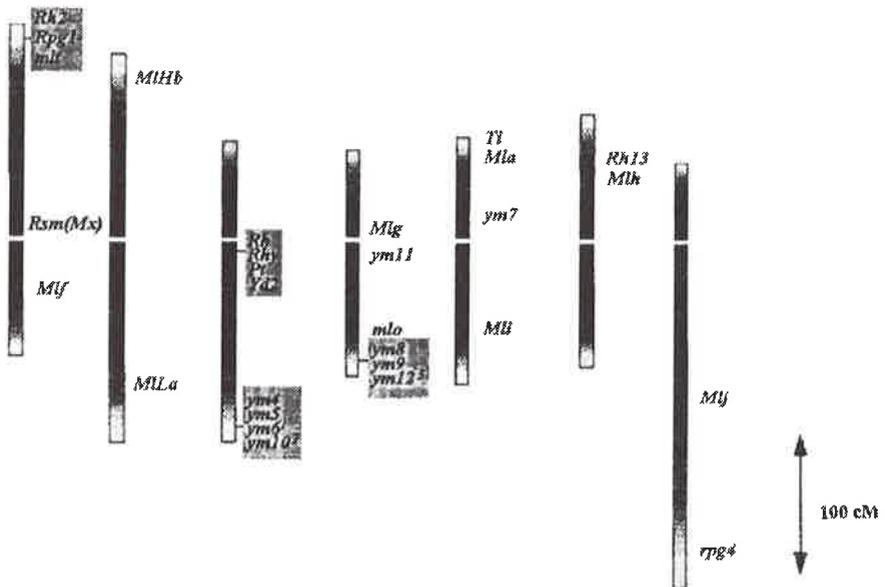


Figure 1. Resistance genes that have been tagged by molecular markers. Individual chromosomes are sized according to the relative genetic distances on the Igri x Franka map (Graner et al., 1991). The short arm is directed to the top. Complex loci are marked by a shaded background. ¹ Located based on linkage to *Est 1,2,4*. ² Baumer, personal communication. ³ Graner et al., unpublished.

not possible at this stage to differentiate between random effects and functional relationships. Homospecific gene clusters are a common feature of the genomes of higher plants (Mahadevappa et al., 1994). In the barley genome, such clusters are present in the distal regions of chromosome 5S (*Mla*) and of chromosomes 3L and 4L (*ym*). While the *Mla* locus represents an extreme example of multiple allelism (cf. Jahoor et al., 1993), the two *ym* complexes seem to consist of closely linked genes. Examples of complex resistance genes have been studied in detail in flax. Here, the *L* locus that confers resistance to rust exhibits multiple allelism. The physical analysis of the gene sequence has revealed that its 3' region consists of a stretch of tandemly repeated motifs, the repeat number of which differed in the two alleles analyzed (Ellis et al., 1995). Hence, it seems that variability in repeat length results in the generation of a new alleles with altered specificity. It is tempting to speculate that the genetic variability of the *Mla* locus is accounted for by a similar mechanism.

In contrast to the *L* locus, the *M* locus in flax as well as the *Rp1* locus in maize consist of an array of closely linked genes, which might be the result of the amplification of an ancestral gene by unequal crossover events mediated by flanking repetitive elements (Ellis et al., 1995). Further analyses will show whether similar structures apply to the different *ym* genes. Since recombination genetics can not provide a final answer to this question, attempts are underway to isolate these genes by a map based cloning approach.

Gene isolation

As a first step toward this goal, high resolution maps have been constructed for the *Mla*, the *mlo*, the *Rpg1* and the *ym4* regions (Hinze et al., 1991; DeScenzo et al., 1994; Kilian et al., 1995; Bauer and Graner, 1995). Next, the corresponding chromosomal regions are being saturated with additional markers. The most closely linked markers will be used to select homologous clones from large insert libraries, which in turn will be used to construct physical maps around these genes. Though theoretically conclusive, a major obstacle for this approach is the large size of the barley genome (ca. 5.5×10^3 Mbp) and its high amount of repetitive DNA. This makes difficult the generation of a comprehensive YAC library and the generation of YAC contigs by tiling up overlapping clones. A possible solution to this problem might, however, consist of resorting to the rice genome, which comprises only about 4×10^2 Mbp. Besides comprehensive molecular marker maps, large insert libraries have been constructed for this species. Despite the different chromosome numbers of rice and barley, large stretches showing identical marker orders have been identified. Regarding the chromosomal region carrying the *Rpg1* gene, it could be even demonstrated that genetic synteny also extends to the physical level (Kilian et al., 1995). Nevertheless, the general usefulness of the rice genome as a platform for map-based cloning of cereal genes critically depends on an identical gene repertoire in both species. This is of particular relevance for the isolation of resistance genes, because these are frequently turned on as the result of a highly specific

interaction between the pathogen and the host plant.

An alternative strategy for the isolation of disease resistance genes exploits the observation that many resistance genes that have been isolated in other plant species share similar sequence motifs, such as leucine-rich repeats and nucleotide binding sites, or represent members of comprehensive and widespread gene families, such as protein kinases (for review see Staskawicz et al., 1995). Isolation and mapping of homologous clones may lead to the identification of "positional candidates," which have to be further tested by genetic analysis. In the long run, this non-specific approach might become superior to map-based gene isolation.

Conclusions

The first phase of genome analysis was mainly characterized by joint efforts to construct comprehensive maps. Now, during the second phase, research activities focus on the utilization of these maps for the genetic localization of agronomic traits and the identification and the development of selectable markers. With respect to disease resistance, more than forty major genes and QTL have been identified so far. With respect to the latter, there is preliminary evidence that some QTL might represent less effective alleles of major genes because (i) many QTL coincide with the genetic position of major genes and (ii) a series of QTL studies revealed one major QTL accounting for most of the genetic variation explained by markers.

The physical isolation of resistance genes is a prerequisite to expansion of our knowledge on the evolution of disease resistance and the functional analysis of the corresponding genes. Together with progress in barley transformation, gene isolation might quickly gain importance for practical breeding. Therefore, attempts are being made to isolate selected genes by a map-based cloning strategy. However, the methodology used is still complex and far from becoming routine. Novel approaches for gene isolation in barley might be based on analysis of the much smaller rice genome, on homology to genes that have been cloned already in other plant species, or on the establishment of a functional transposon tagging system for barley.

In the near future, the use of selectable markers will allow a more efficient manipulation of resistance genes during the breeding process. Thus, traits with a low heritability can be selected more accurately and with less requirements for time and labour, as has been the case in conventional breeding programs. Regarding the realization of gene pyramiding concepts, marker-assisted selection can replace extensive virulence tests, which require the maintenance of large pathotype collections. Even more, the use of molecular markers will facilitate the combination of resistance genes, which, due to the lack of appropriate virulences, can not be differentiated based on their phenotype. This applies to the combination of mildew resistance genes that are effective against all physiologic races of the pathogen or to the combination of resistance genes against facultative saprophytes such as *R. secalis* and *P. teres*. Also, the

availability of selectable markers will further promote the use of *H. spontaneum* as a source for disease resistance by helping to efficiently minimize the linkage drag around introgressed DNA segments.

Undoubtedly, molecular markers represent a useful tool for the barley breeder. Their integration into the breeding process will require, however, a revision of existing concepts, and not only with respect to disease resistance. Although the final version of the optimized concept will vary from breeder to breeder, it is clear that the application of molecular markers can not replace established crossbreeding methodologies. However, their implementation may help to meet the various goals barley breeders have to cope with in the future and, on the scientific level, will promote our understanding of the molecular basis of disease resistance.

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Marker assisted selection for quality in barley and oat

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Introduction

Quality assessment in the breeding of barley and oats requires evaluation of many complex processing characteristics. The use of barley for the production of beer by malting and brewing involves many steps (Figure 1) in which the genetic characteristics of the barley can be important.

Almost all of the traits associated with quality in barley and oat are classified as quantitative or multigenic. These quantitative trait loci (QTL) are also influenced, to varying degrees, by the environment. The progress of genome mapping and the improvement of molecular techniques provide the prospect of

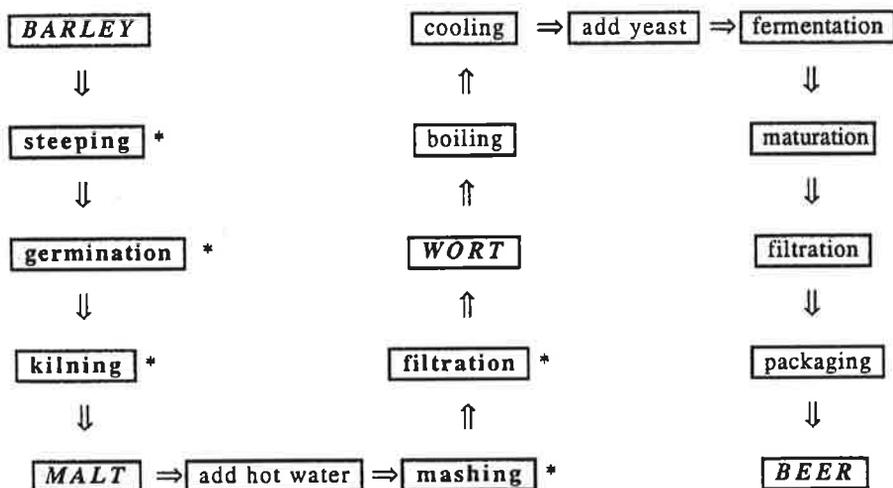


Figure 1. Production of beer from barley. The steps marked* may be influenced greatly by barley genotype. Marker assisted selection for traits expressed at these stages in processing may allow development of barley cultivars with improved processing quality.

understanding the genetics of these complex traits and the application of marker assisted selection for quality in breeding programs.

Advantages and limitations of marker assisted selection for quality

The increasing knowledge of the genomes of cereals is enabling the evaluation of marker assisted selection for a wide range of traits. Marker assisted selection may allow screening and selection for a trait more efficiently. This is of considerable importance in relation to quality traits because of the great difficulty of assessing many of the complex processing characters on large populations. Attempts at marker assisted selection may also result in a better understanding of the genetic components of many economically important quality traits.

A potential problem is the relatively low level of DNA polymorphism among the genotypes used in breeding programs. The polymorphism levels in breeding populations are generally much lower than those found in the populations used to generate the genetic maps. Molecular markers for quality traits identified in wide crosses may not be polymorphic in breeding lines.

At present, one quality character may be associated with many large blocks in the genetic map containing the QTLs influencing the trait. The integration of all or most of these QTLs into lines in breeding programs presents the breeders and molecular biologists with a daunting task. Innovative breeding strategies may be required in the future. The cost may be another obstacle in the use of molecular markers for marker assisted selection. The cost and effort associated with the monitoring of the many QTLs associated with quality in a breeding programs must be compared with the alternative of conventional evaluation of the trait.

Populations for mapping quality traits

The genotypes chosen for the identification of molecular markers for quality must exhibit useful variation in the trait being studied. Parents for mapping populations need to represent the range of the trait likely to be selected in breeding. For example, many genes may control malt extract. However, the genes of most value are those that will determine the level of malt extract in the target environment and at the upper range of malt extract levels. Genes that influence malt extract at low levels (e.g., distinguish poor from very poor genotypes) are not of value in barley improvement.

Double haploid populations offer many advantages in working with quality traits. Disease resistance may be assessed on F₂ plants, allowing identification of molecular markers for resistance genes in segregating populations. However, the evaluation of quality traits usually requires much larger samples than those available from a single plant, and often requires assessment in different sites and seasons. This makes the development of double haploid populations almost essential for the identification of markers for quality traits.

Quality assessment for identification of linkages

The choice of traits for analysis using molecular markers may be influenced

by several factors. The ease of measuring the trait may be important. Traits that are difficult or expensive to measure or are subject to large environmental variation are attractive targets for marker analysis.

The analysis of populations for the establishment of linkages should employ the most reliable method available, rather than the test that might be used in routine selection in breeding. Any errors in measurement of the trait will make the identification of linked markers difficult if not impossible. This consideration makes the identification of markers a relatively expensive task, further emphasizing the need to target the most important traits, where the biggest gains in breeding efficiency are possible.

Some major quality targets for marker assisted selection are listed in Table 1.

Types of molecular marker available

Many different types of markers are available for use in marker assisted selection in oat and barley. Early work has involved RFLP markers, but an increasing array of PCR-based markers are now available. Random amplified polymorphic DNA (RAPD), amplification fragment length polymorphism (AFLP) and microsatellite or short sequence repeat (SSR) markers are among the options. RAPD markers (William et al., 1990) have been linked to disease resistance genes in barley (Poulsen et al., 1995). Random and semi-random primers have been employed successfully in the analysis of barley germplasm

Table 1. Major targets for marker assisted selection for barley quality

Trait	Conventional analysis method
Grain analyses	
Grain size	sieving
Grain protein content	chemical, NIR
Beta-glucan	enzymatic
Malt analyses (these require micro-malting of the sample)	
Malt extract	small scale mashing and specific gravity measurement extract viscosity
Malt enzymes—	colorimetric
alpha-amylase	
beta-amylase	
limit dextrinase	
beta-glucanase	
proteases	
Malt modification	Kolbach index
	Friability
	Malt beta-glucan

(Weining and Henry, 1995) and may prove more generally useful in marker analysis. AFLP markers have been used successfully in the identification of tightly linked markers in other species (Thomas et al., 1995), and should be valuable in oat and barley. PCR of specific genes associated with quality traits may allow markers for quality to be identified. PCR of barley alpha-amylase genes has revealed polymorphisms in the coding region (Ko and Henry, 1994) and in promoters (Weining and Henry, 1994).

Linkage of quality traits to molecular markers

Two main approaches for linkage of quality traits to molecular markers are analysis of linkage between the trait and markers in a segregating population, and bulked segregant analysis (Michelmore et al., 1991), involving identification of markers polymorphic between bulks of DNA made from the population on the basis of analysis of the trait.

There are a few extensive RFLP maps available in barley and oat (Kleinhofs et al., 1993; O'Donoghue et al., 1995). The development of microsatellite maps for barley is also underway in different laboratories. Due to the good coverage of the whole genome and the availability of resources of large genome mapping programs, analysis of quality traits based on RFLPs has been the most widely adopted approach to date.

The molecular markers display conservation of order (collinearity) in different cereal species (Devos et al., 1992). This synteny has been observed not only over large chromosomal regions but also at a micro level as shown by Kilian et al. (1995) and Dunford et al. (1995). Based on the synteny, convergent domestication was reported with comparative QTL mapping in cereals (Paterson et al., 1995). This approach would allow the use markers across species for corresponding traits.

Application in breeding programs

Application of molecular markers in barley and oat improvement requires the development of simple, routine screening procedures. The marker assessment must be made cost competitive with alternative traditional methods of screening for the quality trait. Simple and rapid DNA extraction protocols (Thomson and Henry, 1995) are essential for efficient screening. PCR based methods may also offer advantages. This may require the conversion of RFLP markers to sequence tagged sites (STSs). Further advantages may be gained by eliminating electrophoresis by using an ELISA or fluorescence method to detect the PCR product (Holland et al., 1991).

Examples of quality traits mapped in barley

Table 2 lists some of the quality traits mapped in barley by European and North American labs. The same trait may be mapped to different chromosomes or regions by different labs. This could be due to environmental factors as well as the different mapping populations used for the QTL analysis. Another reason could be the difference in the methods of quality assessment adopted. These

results demonstrate some of the difficulties and complexity faced by molecular biologists and breeders in marker assisted selection for quality traits.

Future trends

One of the major goals of the genome projects is to produce high-resolution genetic maps. Microsatellite maps in humans have superseded RFLP maps in recent years. This trend is likely to emerge in barley and oat as well. Combined with high resolution maps, fine mapping will be accelerated to reduce the size of the regions associated with QTLs.

Synteny in cereals, as revealed by comparative mapping, provides a framework for the comparative analysis of quality traits between different species. The most

Table 2. Quality traits for which markers have been identified in barley*

Trait	Population**	Type of marker	Location of marker	Reference
Grain protein	SxM	RFLP	2, 4, 6,7	Han and Ullrich
	HxT	RFLP	1,2, 4, 6,7	Tinker and Mather
	DxM(winter)	RFLP	4,7	Oziel et al.
	DxM(spring)	RFLP	5,6,7	Oziel et al.
Kernel weight	SxM	RFLP	2,3,4,5,6,7	Han and Ullrich
	HxT	RFLP	1,6,7	Tinker and Mather
Plump grains	SxM	RFLP	2,3,4,5,7	Han and Ullrich
	HxT	RFLP	2,4,5,6,7	Tinker and Mather
Kernel length	IxD	RFLP	4,7	Backes et al.
Keenel thickness	IxD	RFLP	7	Backes et al.
Wort protein	SxM	RFLP	1,2,3,4,5,6,7	Han and Ullrich
	HxT	RFLP	1,2,4,5,7	Tinker and Mather
Beta-glucan	SxM	RFLP	1,2,3,4,5,7	Han and Ullrich
	HxT	RFLP	3,7	Tinker and Mather
Alpha-Amylase	SxM	RFLP	1,2,4,5,7	Han and Ullrich
	HxT	RFLP	6,7	Tinker and Mather
	DxM(winter)	RFLP	1,7	Oziel et al.
	DxM(spring)	RFLP	1,7	Oziel et al.
Diastatic power	SxM	RFLP	1,2,4,5,7	Han and Ullrich
	HxT	RFLP	2,5,6,7	Tinker and Mather
Malt extract	SxM	RFLP	1,2,4,5,7	Han and Ullrich
	DxM(winter)	RFLP	2,3	Oziel et al.
	DxM(spring)	RFLP	7	Oziel et al.
Milling energy	RAPD	BSA	Chalmers et al.	

* Adopted from Backes et al. (1994), Han and Ullrich (1994), Chalmers et al. (1993) and Oziel et al. (1996). Not all of the results are listed here.

** SxM: Steptoe x Morex; HxT: Harrington x TR306; IxD: Igri x Danilo; DxM: Dicktoo x Morex.

attractive QTLs to be targeted will be the quality traits involved convergent selection. The cereal species with smaller genomes and lower repeat content, such as rice, provide an alternative system for gene analysis and cloning.

Even after their map position is known, there may be considerable further research required before we understand the molecular mechanism of the quality traits. The expression of some traits is likely to be the results of cascading and amplifying effects on gene regulation. Alpha-amylase level is an important component of malting quality. Undoubtedly, studies of alpha-amylase gene regulation will help the analysis and mapping of this component of malting quality. On the other hand, QTL mapping may reveal elements associated with gene regulation that are not apparent in normal molecular analysis.

Studies of regulatory genes and/or gene families may contribute to our understanding of the genetic basis of plant development. Quality is often associated with morphological and reproductive traits. One example is the MADS box genes, which play important roles in flower development in various plants (Coen and Meyerowitz, 1991). These genes may have a role in genetic manipulation of agronomically and economically important quality traits (Chung et al., 1994).

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Single locus and multi-locus molecular assays for barley breeding and research

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The spectrum of technologies available for genetic mapping and DNA profiling of plants has never been greater. This provides new opportunities, but also places greater emphasis on choosing appropriate methods of detecting polymorphism. The purpose of this article is to review current molecular marker technologies and identify prospects for their deployment in barley research and breeding.

Five main technologies have been utilized in barley (Powell et al., 1994), and their major features are compared in Table 1. Four of the methods involve amplification via PCR and one, RFLP, is based on hybridization. The development of PCR (Mullis et al., 1986) has therefore revolutionized approaches to detecting polymorphism. Methods for detecting polymorphism based on PCR fall into two main categories: those using defined primers that require sequence information from the species under study, and those using generic primers that can be used essentially on any DNA template.

Defined primers

Sequence tagged site (STS) markers based on the PCR amplification of pairs of primers flanking defined sequences provide a series of co-dominant, transferrable markers that may be used to select indirectly for gene(s) of interest. Progress towards the development of STS markers has been made on a number of fronts. RFLP or RAPD markers linked mainly to genes conferring resistance to a pest or pathogen have been converted to STS markers by either sequencing a closely linked RFLP probe, designing primers for PCR amplification and identifying a diagnostic post-amplification restriction endonuclease digestion profile (e.g., Penner et al. (1995) for *rpg1*; Graner (1996) for Rh3/4 and net blotch; Bauer & Graner (1995) for Ym4; Kilian et al. (1994) for *rpg1*) or by cloning a linked diagnostic RAPD allele, then following the same process of

Table 1. Comparison of molecular marker systems.

	RFLP	RAPD	SSR	AFLP	SAMPL
Principle of assay	Endonuclease digestion and hybridisation	Amplification with random primers	Amplification of SSRs	Amplification of DNA fragments limited by random nucleotides	Amplification of DNA fragments limited by random nucleotides and compound SSRs
Type of polymorphism detected	Single base insertions or deletions	Single base insertions or deletions	Repeat length	Single Base insertions or deletions	Repeat length
Dominance	Co-dominant	Dominant	Co-dominant	Dominant	Co-dominant and dominant
Amount of DNA required	2-10 µg	10-25 ng	25-50 ng	2-50 µg	2-5 µg
DNA sequence required	No	No	Yes	No	No
Radioactive detection	Yes/No	No	No	Yes	Yes

primer design and PCR-RFLP development (G. Penner, personal communication). This approach has been necessary in the past due to a paucity of mapped STS information. However, this has been simplified enormously by the deposition of more than 300 cereal specific PCR-primer sequences (principally from Tom Blake, Luther Talbot, Marion Roeder, Andy Kleinhofs and Mark Sorrells) in the GrainGenes database managed by Cornell (<http://wheat.pw.usda.gov>). These have been derived from sequencing the corresponding genetically mapped RFLP probes (prefixed ABA, ABC, ABG, ABR, AC, AG, BCD, CDO, KSU, MWG, PSR, WG, WMS) and can theoretically be used to develop allele-specific PCR-RFLPs at almost any locus across the barley genome. A more detailed description of probe, primers, populations on which they were mapped, and auxiliary information can be found in GrainGenes by searching for the probe of interest.

While the development of STS markers via the PCR-RFLP route has a number of potential practical applications, it remains far from ideal because of the low level of polymorphism frequently detected in the PCR-derived amplicons and the need for a post-amplification processing step to detect polymorphism. The latest addition to the STS tools available for barley are simple sequence repeats (SSR) or microsatellites. These are genomic sequences that consist of mono-, di-, tri- or tetranucleotide repeats that are both abundant and common throughout most eukaryote genomes (Tautz & Renz, 1984). Variation in the length of the repeat motifs between individuals is revealed by amplifying genomic DNA with two unique oligonucleotide primers that flank and hence define the microsatellite

locus. The utility of microsatellites arise from two main factors: high information content (which is a feature of the number and frequency of alleles detected) and ease of genotyping. Microsatellites are particularly attractive for distinguishing between cultivars, since the level of variation detected at SSR loci is higher than that detected by any other molecular assay (Saghai-Marooft et al., 1994; Becker & Heun, 1995; Powell et al., 1996). In barley, 37 primer pairs that amplify polymorphic SSR distributed across the seven linkage groups (Liu et al., 1996) are available commercially as Barley MapPairs™ from Research Genetics, Inc.

Access to microsatellite primer sequences through a public database would enhance the use of this technology in barley research and breeding. To this end we have been developing methods to create genomic libraries of barley enriched for dinucleotide repeats. Various enrichment methods are available (Rafalski et al., 1996), but we have concentrated on a pre-cloning enrichment procedure. This has involved: endonuclease digestion, size fractionation (300–600 bp) and the creation of DNA fragments with defined sequences at both ends by adaptor ligation. Microsatellite containing fragments are enriched by hybridization to a biotinylated SSR oligonucleotide and subsequent selection on streptavidin-coated magnetic beads. Hybridizing fragments are eluted and rendered double stranded by PCR amplification using primers homologous to the adaptor sequences. Following restriction enzyme digestion, the microsatellite-containing fragments are cloned into a suitable vector such as λ Zap II. To date, enriched $(CA)_n$ and $(GA)_n$ libraries have been created. After plating at low density, clones containing SSR sequences are identified by plaque hybridization with the appropriate SSR oligonucleotide.

Results from our $(CA)_n$ library screening are presented in Table 2. Data from 76 sequenced clones are presented and several categories of repeats (Weber, 1990) are evident. Perfect repeat sequences predominate, both as simple and compound structures of the 33 compound (perfect) repeats identified; 27 are associated with $(AT)_n$ repeats e.g. $(AC)_{19}(AT)_{11}$. For such compound repeats, it will be important to determine which component of the compound repeat is responsible for generating the polymorphism.

To our knowledge, no reports of the identification of SSR markers linked to mono- or polygenic traits in barley have appeared in the literature. Further work is required to examine the distribution of SSR across barley genomes and create index maps. The multi-allelic nature of SSR will allow this class of marker to have greater utility in different barley genepools (e.g. Russell et al., this volume).

Table 2. Numbers of $(AC)_n$ repeats in different sequence categories obtained from an enriched genomic library.

Category	Number	Percentage
Simple (perfect), e.g. $(AC)_n$	29	38
Simple (imperfect), e.g. $(AC)_{11}G(AC)_{10}$	8	11
Compound (perfect), e.g. $(AC)_{21}(AT)_7$	33	43
Compound (imperfect), e.g. $(AT)_7AAA(AC)_{29}$	6	8

Opportunities also exist for the deployment of SSR markers to retrospectively examine the development of new barley cultivars. An analysis of genotypes that have contributed to the lineage of a given cultivar may be analyzed with SSR. This will allow an analysis of the transmission of alleles (and linked chromosomal regions) between generations to be assessed, providing new insights into the consequences and effectiveness of selection on plant phenotype. Graphics software is currently available that allows molecular-marker-based data to be integrated with pedigree information (Boutin et al., 1995).

Generic primers

Randomly amplified polymorphic DNA (RAPD) and related techniques, AP-PCR (arbitrarily primed PCR; Welsh & McClelland, 1990) and DAF (DNA amplification fingerprinting; Caetano-Anolles et al., 1991) have been used extensively because of their simplicity, non-radioactive detection, easy access to primers and their relatively low cost. Numerous reviews have been published (e.g., Tingey & del Tufo, 1993; Waugh & Powell, 1992) and the technology will not be considered here. The main applications of RAPD to barley have been in the area of "targeted polymorphism," using near isogenic lines (NIL) and various pooling strategies (e.g., Barua et al., 1993). This has allowed in certain cases the RAPD products to be converted to STS markers (see earlier section). Despite these achievements, RAPD markers suffer from several disadvantages, relatively low levels of polymorphism detected in barley cultivars, dominance, sensitivity to DNA concentration and amplification conditions. The latter two issues have hindered the wider applicability of RAPD markers to barley breeding and associated research.

Amplified fragment length polymorphism (AFLP) was developed by Zabeau & Vos (1993) and is based on the detection of restriction site variation in conjunction with sequence polymorphism in adjacent sites. Details of the procedures are given in the patent (EP534858), and the articles by Rafalski et al. (1996) and Vos et al. (1996). Commercially produced kits are also available (e.g., the AFLP™ plant mapping kit from Perkin Elmer), at a cost that improves flexibility.

The main advantage of AFLP markers is their high multiplex ratio (Powell et al., 1996), which means that a large number of amplification products can be assayed in a single reaction. Resulting polymorphisms are mainly dominant, but a proportion (approximately 5% in barley) are co-dominant. This technology has wide applicability in cultivar fingerprinting, linkage mapping, saturating regions of the genome (using pooling strategies or NILs), map-based cloning and backcross conversions.

The availability of doubled haploids (DH) for barley linkage map construction provides an ideal resource for integrating AFLP markers with other marker types. Becker et al. (1995) have added 118 AFLP markers to the Proctor x Nudinka DH population, and Hayes et al. (this meeting) have integrated AFLP markers onto the Dicktoo x Morex linkage map. The present status of our own linkage map construction based on AFLP markers is summarized in Table 3. In

this case we are particularly interested in establishing if AFLP alleles are transferrable between different crosses (see Waugh et al., this meeting). These results, together with that reported by Becker et al. (1995), were based on *EcoRI* and *MseI* digests and associated procedures. An important issue is to establish the efficiency of different primer combinations and restriction enzymes in detecting polymorphism. Template DNA has been prepared using *EcoRI/MseI* and *PstI/MseI* digests and tested with a range of primer pairs across four barley crosses (Table 4). These results demonstrate that there are significant differences ($P < 0.001$) between crosses and between restriction enzymes ($P < 0.001$) in terms of the number and proportion of polymorphic products detected. Based on these data, *PstI/MseI* restriction enzyme digests are more efficient than *EcoRI/MseI* in generating informative markers in barley.

Backcrossing programmes designed to introgress gene(s) from a donor parent into elite germplasm are likely to be the first examples of the use of molecular markers in barley improvement. Two complementary aspects need to be considered: use of molecular markers to monitor and select for introgressed gene(s) and to identify recombinant individuals that have genome compositions significantly better than would be predicted from theoretical expectations. High multiplex assays such as AFLP are particularly useful for recurrent or donor parent analysis. This is illustrated in Figure 1 for a BC1DH population (supplied by P. Hayes, Oregon State University) that has been genotyped with 62

Table 3. Number of markers used to construct genetic maps in four barley doubled haploid populations.

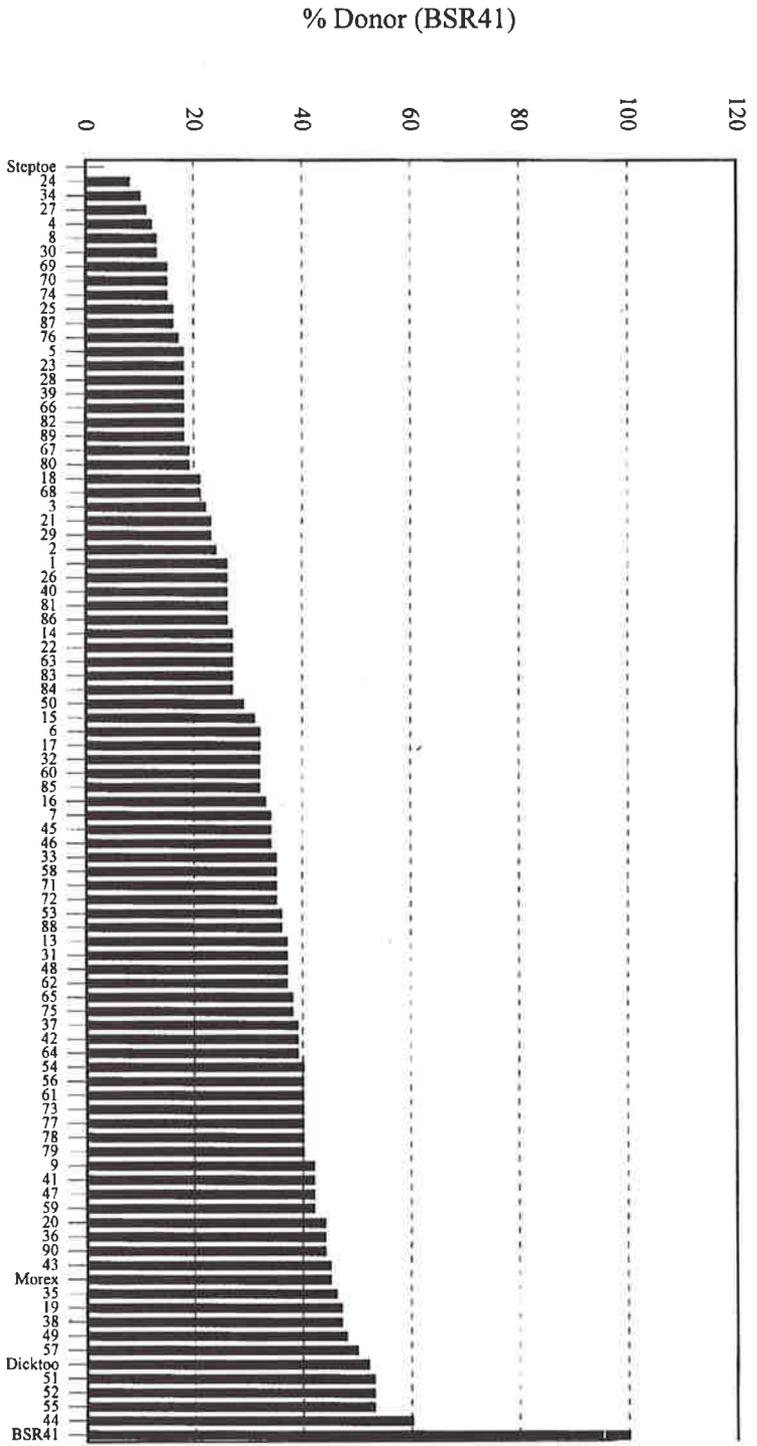
	AFLP*	Other markers	Total
Blenheim x E224/3	329	144	473
Dicktoo x Morex ^a	249	78	327
Igri x Franka ^b	202	469	671
Lina x <i>H. spontaneum</i> ^c	580	460	1043

* Amplification fragment length polymorphism. Populations supplied by ^aP. Hayes, Oregon State University, US; ^bA. Graner, Grünbach, Germany; and ^cS. Tuveesson, Svalöf Weibull, Svalöv, Sweden.

Table 4. A comparison of the number of polymorphic products and the proportion of polymorphic products produced with different template DNA from four different crosses.

	Number of polymorphic products		Proportion of polymorphic products	
	<i>EcoRI/MseI</i>	<i>PstI/MseI</i>	<i>EcoRI/MseI</i>	<i>PstI/MseI</i>
Blenheim x E224/3	8.5±1.03	13.7±1.59	0.092±0.0049	0.226±0.0109
Dicktoo x Morex	21.9±1.05	23.6±1.70	0.213±0.0067	0.226±0.0109
Igri x Franka	10.8±1.03	11.5±1.70	0.119±0.0055	0.138±0.0102
Lina x <i>H. spontaneum</i>	22.4±1.06	25.8±1.76	0.248±0.0076	0.283±0.0131

Figure 1. Percentage donor DNA revealed by AFLP analysis (Stephoe x BSR41 BC DH population)



polymorphic AFLP products. The percentage donor DNA composition varies from 8% to 60%, which illustrates the spectrum of genotypic values obtained and emphasizes the benefits of applying a genetic analysis to introgression programmes. Thus, selection of genotype 24 with 8% donor DNA would be equivalent to advancing this population to a BC₃ generation. Furthermore, reduction in the number of individuals processed in a backcross programme would reduce the cost associated with further testing and evaluation. The advantages of AFLP (high multiplex ratio) and SSR (high information content) have been combined in a new assay, termed selective amplification of polymorphic loci (SAMPL). This method was first reported by Vogel and Morgante at Plant Genome III (abstract P245) and is based on the use of a perfect, compound simple sequence repeat that is self-anchoring. This primer is used in conjunction with an AFLP primer to reveal polymorphism in genomic regions that contain SSR. In addition to improving the information content of AFLP, this technique may also provide a means of isolating microsatellite repeats without the need for creating genomic libraries.

Conclusions

The choice and utilization of any molecular marker system will be influenced by several criteria. These include technical complexity, use of radioactivity, amount of purity of genomic DNA, available resources and the goals and magnitude of the breeding objective. It is also important to recognize that polymorphism detected with various molecular assays reflects different mutational mechanisms. Thus, AFLP and RFLP markers detect restriction site changes, RAPD detect insertions/deletions/point mutations and inversions, whereas SSR-based polymorphism is due to polymerase slippage. These factors need to be borne in mind when different marker systems are compared. Further effort is required to develop and implement automation technologies (Rafalski et al., 1993; Mazur & Tingey, 1995) together with efficient data analysis and retrieval procedures. In addition, the economic returns of molecular breeding need to be fully evaluated.

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Using QTL in barley

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Many traits important to barley improvement – yield, quality and some types of resistance to biotic and abiotic stresses – can be described as effects of quantitative trait loci (QTL). Increasingly complex statistical models have been developed to account for multiple unlinked loci, to distinguish between linkage and pleiotropy, and to quantify QTL x QTL and QTL x environment interactions.¹ At this point, QTLs are statistical rather than genetic entities. How many genes, and what kinds of genes, determine a QTL? Quantitatively inherited traits may be controlled by many genes, with each gene having a small effect highly subject to environmental influences.² However, in studies of North American mapping populations, quantitatively inherited traits were determined by few QTLs, and QTL x environment interaction was the exception rather than the rule.^{3,4} This may well be a function of the QTL detection procedures and population sizes employed. The challenge posed by trait complexity, in terms of phenotyping, is not necessarily related to the number of QTLs detected. For example, low temperature tolerance and heading date are, respectively, difficult and straightforward phenotypes to quantify, yet one QTL was found to determine low temperature tolerance and six QTL to determine heading date in the Dicktoo x Morex population.⁵ This may have a biological basis, or it may be due to the precision with which phenotypes are measured. QTL may have a simple genetic basis: they may result from alleles at loci where the action of other alleles with more dramatic effects can be explained by monofactorial models.⁶ We should soon be able to test this hypothesis in barley. For example, integration of morphological markers with comprehensive molecular marker maps will allow for alignment of QTL in adapted germplasm with extreme phenotypes in genetic stocks. Likewise, quantitative resistance genes can be comparatively mapped with those conferring hypersensitive responses.

The distinct end uses of barley – malt and feed – are excellent challenges for QTL analysis. Extensive efforts to characterize the physiology and biochemistry of the malting and brewing processes have generally provided descriptive rather than predictive tools and, as a consequence, have not been extensively integrated into variety development. QTL analysis may provide a solution: it can quantify discrete effects of, and interactions among, the genes determining quality in malting and brewing. Selection for yield in feed barley has been quite effective, and yet quantitative genetics have made few direct contributions to these gains. Allard⁷ articulated the limitations of biometrical procedures that provide estimates

of population genetic variances. Via QTL analysis, both populations and individual genotypes can be described. By characterizing the location and effects of the genetic determinants of trait expression and by providing markers for selection, QTL strategies should provide opportunities to unify and apply physiology, biochemistry and basic genetics to barley improvement.

QTL procedures have already been used to locate determinants of a number of economically important phenotypes in barley, including malting quality and yield. These efforts are detailed elsewhere in these proceedings and reviewed by Hayes et al.⁸ The issues, in terms of utility, are selection response and more effective and interesting breeding. A number of applications of QTL to plant breeding were enumerated by Paterson et al.⁹ This review will focus on preliminary data from two experiments: one uses QTL information to develop ideal genotypes and the second attempts to develop a useful classification of germplasm.

Ideal genotypes with all possible favourable QTL alleles for grain yield and malt extract were derived from the Steptoe x Morex population. An example of QTL main effects and QTL x environment interaction for grain yield, as detected

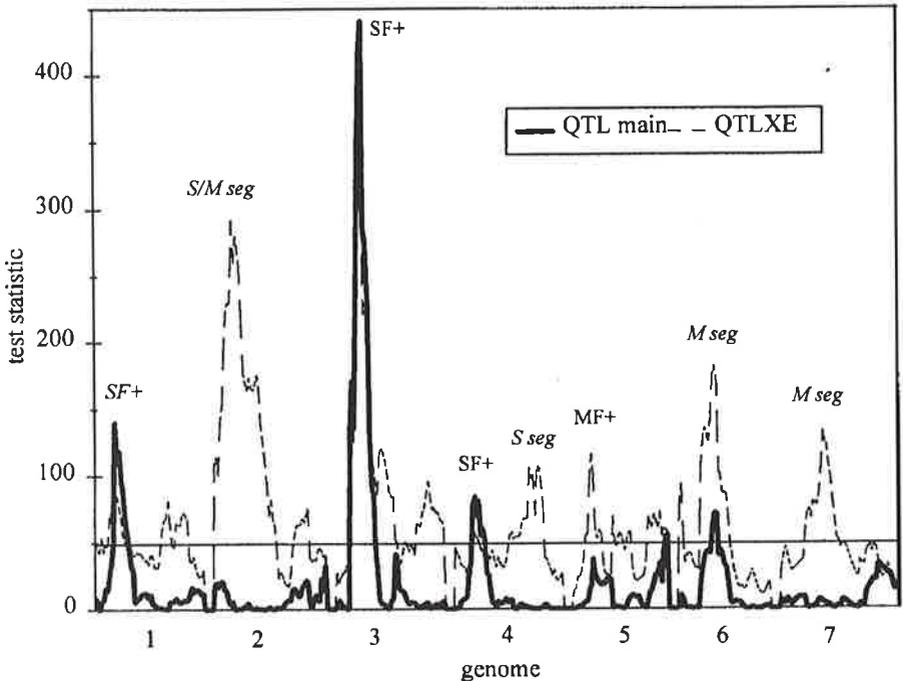


Figure 1. QTL and QTL x E interaction for grain yield in the Steptoe x Morex mapping population. S and M indicate if Steptoe or Morex contributed the larger value allele, respectively. Seg and F indicate if the QTL was segregating or fixed in the SM73 x SM145 cross. F+ and F- indicate fixation of favorable and unfavorable alleles, respectively.

by the simplified Composite Interval Mapping (sCIM) procedures of MQTL,¹⁰ is shown in Figure 1. We designed a mating (SM73 x SM145) that would produce an ideal genotype (IG) population containing lines with combinations of these favourable-yield QTL alleles with those for malt extract. In Figure 1, “F” or “seg” indicate if the QTL detected in the base mapping population is fixed or segregating in the IG population. For fixed QTL, “+” or “-” indicate favourable or unfavourable alleles. “S” or “M” indicate the parent (Steptoe or Morex) contributing the favourable allele. The IG population of 100 doubled haploid (DH) lines was genotyped for polymorphic QTL regions, and one environment of data is available from the ongoing phenotyping of a subset of lines.

The preliminary results are intriguing. Most large-effect QTL were fixed in this mating, and the selected IG genotypes represent a substantial improvement in both grain yield and malt extract (Table 1). Morex and Steptoe showed the expected phenotypic combinations of high extract:low yield and high yield:low extract. SM73 and SM145 were inferior for both characters, but two-character transgressive segregant progenies were derived via QTL-based selection. These genotypes, derived after one cycle of QTL-based selection, compare favourably with the best phenotypic selections from the base mapping population. With the major QTL fixed, only smaller-effect QTL are segregating. Significant effects, as estimated by regression, were detected at these loci and are comparable to those detected in the base population. The QTL selections have unacceptably high wort beta-glucan values, attributable to the fixation of Steptoe QTL alleles on chromosome 7 near the *Ale* locus. Additional genotypes from the IG population that carry Morex QTL alleles in this region are currently in field trials. These initial data validate QTL detected in the base population and confirm that genetic advance can be achieved with QTL-based selection. They also serve as a reminder that traits in addition to grain yield and malt extract are required in a malting variety.

The genotype-specificity of many QTL limits straightforward application of results from one population to another. For example, there are no apparently orthologous grain yield or malt extract QTL in the Steptoe x Morex and Harrington x TR-306 populations.¹¹ This is, in fact, grounds for optimism: not all favourable QTL are fixed in elite North American germplasm. An alternative to attempting to extend QTL results from one population to another is to integrate information from a range of germplasm to identify *regions* of the genome that *may* affect trait expression. This information can be used to classify germplasm and design matings that will maximize the probability of accumulating favourable alleles. An example of this approach is shown in Figure 2. QTL determining growth habit (measured as heading date or maturity date) and components of malting quality were positioned on the Harrington x Morex skeleton map. QTL positions were obtained from published reports from a range of mapping populations. The relationships of growth habit QTL to previously-described Mendelian genes was reviewed by Pan et al.⁵ Cloned genes with a role in starch hydrolysis (*Agl*, *Amy1*, *Amy2*, *Bmy1*, and *Bmy2*)¹² were then added to this map at their interpolated positions. Of the 22 QTL regions identified, 19 were clusters

of two or more characters, 11 were clusters of 4 or more characters, and growth habit QTL were present in 6 of these clusters. QTL clusters were found in the vicinity of the *Amy1*, *Amy2*, *Bmy1*, and *Bmy2* loci. These are obvious candidate determinants of malting quality QTL. The summary does not do justice to regions of complexity, however, such as that for multiple malting quality traits coincident and distal to *Amy2* on chromosome 1 detected in the Steptoe x Morex population. No QTL for enzymes directly involved in starch hydrolysis were detected in the vicinity of *Agl* (an alpha-glucosidase locus) on chromosome 1, despite the importance of this enzyme in starch breakdown.¹² In the Steptoe x Morex and Dicktoo x Morex populations, the feed parents contributed favourable QTL alleles for diastatic power in the vicinity of the *Bmy1* locus, supporting the contention of Tanksley et al.¹³ that favourable alleles may be found in unprepossessing germplasm. Coincident QTL for a range of malting quality traits near the centromere of chromosome 7 may be due to alleles at grain dormancy QTL.¹⁴ The presence of growth habit QTL in many of these clusters underscores the complexity of trait expression and may provide breeders with tools for tailoring varieties to specific environments.

To this base map and summary of QTL, we added information on markers used for clustering genotypes based on Amplified Fragment Length Polymorphisms (AFLPs). Over 300 AFLPs were screened on 42 parents of mapping populations for a genetic diversity analysis.¹⁵ Seventy-four of the 332 AFLPs were also mapped in Harrington x Morex. Based on the assumption that amplified fragments are allelic, the locations of the mapped AFLPs are shown as

Table 1. Grain yield and malting quality characters for parental stocks, Ideal Genotype lines and phenotypic selection from the Steptoe x Morex mapping population (Klamath Falls, Oregon, USA, 1995).

Genotypes	Grain Yield (kg / ha)	Grain Protein (%)	Malt Extract (%)	Alpha Amylase (20° units)	Diastatic Power (° units)	Beta Glucan (ppm)
<i>Parental stocks</i>						
Steptoe	6055	10.4	75.9	31.9	61	900
Morex	5270	13.5	80.0	59.2	153	90
SM73	5547	11.9	77.6	39.7	66	381
SM145	5195	12.7	76.2	41.4	113	750
<i>QTL selections</i>						
9	6205	10.9	78.8	36.3	84	590
11	6794	11.8	79.0	33.4	64	780
19	6325	11.6	78.5	37.6	78	236
<i>Phenotypic selections</i>						
31	6401	11.1	80.7	43.6	101	374
34	6292	11.6	80.8	52.0	98	290
35	6790	11.3	79.7	50.6	103	482

hatch marks in Figure 2. In a principal component analysis of the 74 AFLPs on the 42 genotypes, three components explained 43% of the total variance. From the analysis, the 12 AFLPs with eigenvector coefficients $\geq 80\%$ were identified, and these are labelled in Figure 2. These mapped AFLPs coincide with three of the multi-QTL clusters (chromosomes 1, 3, and 7). Clustering of QTL on linkage

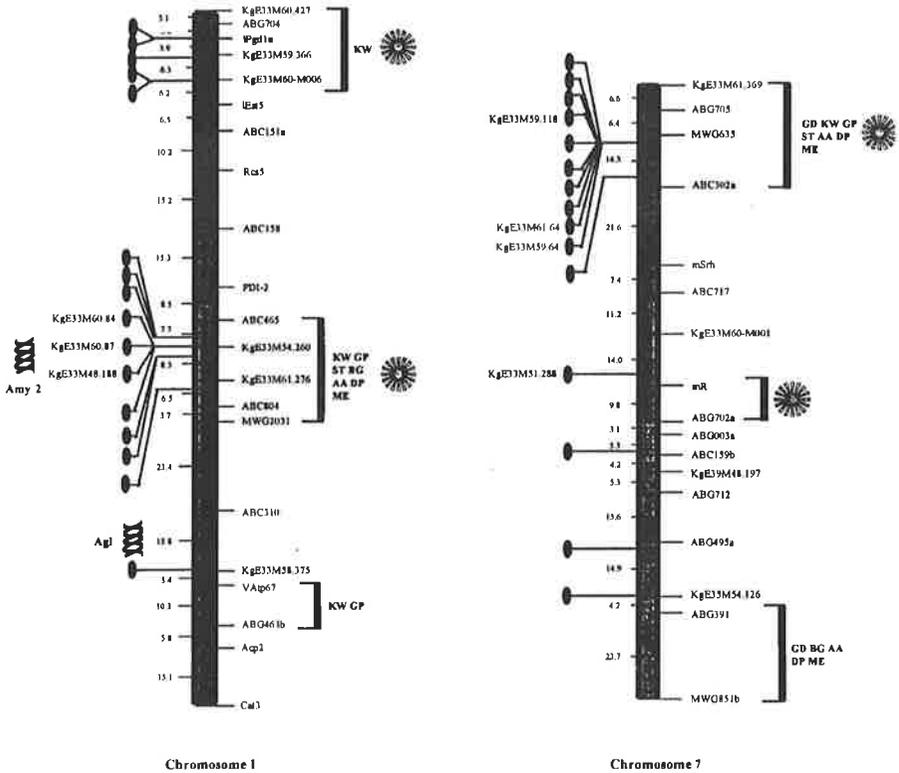


Figure 2. QTL determining components of malting quality, QTL for growth habit and cloned genes involved in starch degradation positioned on chromosomes 1 and 7 of the Harrington x Morex base map. KW = kernel weight, GP = grain protein, ST = soluble to total protein ratio, BG = wort beta-glucan, AA = alpha amylase, DP = diastatic power and ME = malt extract. The icon indicates heading date or maturity date QTL. *Amy2* and *Agl* are beta-amylase and alpha-glucosidase loci mapped as RFLPs. Data contributing to this summary were derived from: Kjaer, B. et al. 1991. Plant Breeding 106: 261–274; Powell, W. et al. 1992. Genetics 130: 187–194, Hackett, C.A. et al. 1992. Theor. Appl. Genet. 85: 120–126; Chalmers, K.J. et al. 1993. Theor Appl. Genet. 87: 314–320 and references 3, 4, 5, 8, 11, and 14 from this report. Linkage map distances are in cM. Left-side hatch marks and AFLP loci are described in the text.

maps may be due to pleiotropy, to multi-locus clusters or to the reduced recombination in regions of the genome that, on a linkage map, increases marker density. In terms of genome organization, evolution and genetic dissection, these possibilities have very different implications. In terms of designing matings and selection strategies, the net effect is for multiple phenotypes to be inherited as a block. The availability of markers that define such key regions should be of great utility in classifying germplasm and designing breeding strategies.

In summary, QTL analyses are a useful tool for unravelling phenotypic complexity. When it comes to using QTL, expectations from QTL-based selection should be realistic. The QTL are the principal, not sole, determinants of trait expression. Continued improvement in DNA-level polymorphism technology and in QTL analysis software will simplify QTL detection and manipulation. This will place detection of polymorphism and analysis of effects in perspective: they are not ends in themselves but tools to make barley breeding more interesting, effective and profitable.

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Comparative genetics of wheat, barley, oat, rice and maize

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Introduction

Comparative mapping based on DNA markers detecting restriction fragment length polymorphism (RFLP) has documented the genetic conservation of linkage relationships among cultivated species of both mono- and dicotyledonous plants. Recent comparative mapping efforts in the grasses have focused on the identification of a set of cDNA probes from rice, oat, barley, and maize that detect "anchored" loci, linkage relationships, and homoeologous chromosome segments. Selected probes have demonstrated their efficacy for comparative mapping by hybridizing to DNA from seven grass genera (Van Deynze et al., 1995a, 1995b, 1995c). The anchor probes were used to extend the existing RFLP linkage maps of rice (Causse et al., 1994), maize (Gardiner et al., 1993), wheat (Nelson et al., 1995a, 1995b, 1995c), and oat (O'Donoghue et al., 1992, 1995) and to compare map structure between these crops (Van Deynze et al., 1995a, 1995b, 1995c).

By comparing linkage relationships among anchored loci, intervals of segmental homoeology have been defined across *Oryza*, *Zea*, *Triticum*, *Hordeum*, and *Avena* (Ahn et al., 1993; Kurata et al., 1994; Moore et al., 1995; Van Deynze et al., 1995a, 1995b, 1995c), as well as other inter- and intra-generic map comparisons. The identification of conserved linkage relationships throughout the grass family provides perspectives on the genetic relationships remaining in these diverged genera, and increases the understanding of these agronomically important species, both individually and in relation to each other.

The ability to cross-reference related loci via RFLP linkage maps will facilitate the identification of related genes and the transfer of information across species. Recently, several quantitative trait loci associated with seed size, reduced shattering, daylength insensitivity, and osmotic adjustment were located on homoeologous chromosome segments in several grasses (Paterson et al., 1995). Correspondence of these QTL with homoeologous chromosome segments and putatively orthologous loci across genera indicates that the genes underlying many of these traits may be identical by descent.

Review of comparative mapping

Wheat and oat relationships

A convenient organization of molecular linkage maps that contain anchored loci is a "consensus" map. Compilation of anchored loci from three or more maps simplifies subsequent comparisons by condensing probes contributing pertinent information for the comparison. For example, chromosome 1 linkage maps of *Triticum aestivum*, *T. tauschii*, and *T. monococcum* were compared to existing barley and rye maps to develop a consensus map for Triticeae species (Van Deynze et al., 1995a). A Triticeae group 1 consensus map was developed that consisted of 14 agronomically important genes, 17 DNA markers that were derived from known-function clones, and 76 DNA markers derived from anonymous clones. This consensus map allowed comparison of the Triticeae group 1 chromosome consensus map with linkage maps of rice, oat, and maize.

Subsequently, a consensus map of the basic genome of the Triticeae tribe (*Triticum aestivum*, *T. tauschii* and *Hordeum* spp.) was produced and served as the basis for comparing the Triticeae to rice, maize, and oat (Van Deynze et al., 1995b; Nelson et al., 1995a, 1995b). The orders of markers detected by probes mapped in rice, maize, and oat were conserved for 93%, 92%, and 94% of the length of Triticeae consensus maps, respectively. The duplicated chromosome segments within the maize genome were identified by homoeology of segments from two maize chromosomes to regions of one Triticeae chromosome. Homoeologous segments conserved across Triticeae species, rice, maize, and oat were identified for each Triticeae chromosome.

To include a representative oat genome in comparative mapping, a diploid oat map (*A. atlantica* x *A. hirtula*) was expanded by anchoring loci to the rice, maize, and wheat maps (Van Deynze et al., 1995c). By examining 92 to 99% of the length of the oat genome map with probes common to Triticeae species, rice, or maize, it was determined that 84%, 79%, and 71%, respectively, was conserved between these species and oat. Generally, the order of loci among chromosomes homoeologous to oat chromosomes A and D was the most conserved, and those of chromosomes homoeologous to oat chromosome G were the least conserved. The degree of conservation ranged from whole chromosomes 101 cm long to small segments involving two loci 2.5 cm long.

By comparing the homoeologous segments of the Triticeae, rice, maize, and oat, it is evident that many regions have been maintained in all four species. The relative positions of major genes governing traits such as seed storage proteins and resistance to leaf rusts have been conserved between cultivated oat and Triticeae species. Comparing homoeologous segments and anchored loci is an increasingly common approach to identify putatively orthologous genes and transfer information across the grasses.

Consensus map of the Poaceae

Unfortunately, the anchoring of all loci across all species is not feasible. Occasional complications, including the inability to detect diverged loci using

heterologous probes and the reliance on RFLP to map loci, can complicate traversal of species-specific and two-species comparative maps. A simple organization of anchor loci into a consensus map, consisting of homoeologous chromosome segments common to all grass species, aids in cross-genera comparisons and provides evolutionary perspectives on commonality and diversity of the grass genomes.

Anchor loci on RFLP linkage maps of wheat (Nelson et al., 1995a, 1995b, 1995c), rice (Causse et al., 1994), diploid oat (Van Deynze et al., 1995c), and maize (Ahn et al., 1995), and comparative maps (Van Deynze et al., 1995a, 1995b, 1995c) have been compared to produce a consensus map of domesticated grasses. **The grass consensus map was constructed based on (1) twelve foundation segments defined in rice and the discrete duplication of ten of these segments in the maize genome, (2) inclusion of probes detecting anchored loci on homoeologous linkage segments in at least two genera, (3) inclusion of probes detecting incongruent loci in a single species if they fit criterion 2, and (4) omission of probes detecting loci mapped in only oat and wheat to ensure that the consensus was representative of the domesticated grasses (Wilson et al., manuscript in preparation). A Pooid consensus map was constructed including probes meeting the aforementioned criteria (except 4) for the Triticeae and oat. Order was inferred by relative positions of adjacent markers across species. A consensus map approach incorporates the accumulated map information and enhances the linkage relationships delimited by anchored loci.**

The genome of rice contains the basic set of grass segments and has the least complex genomic structure. As the least complex, it corresponds to the foundation segments of the Poaceae consensus map and has been the "core" species in cross-genera comparative maps (Ahn and Tanksley, 1993; Ahn et al., 1993; Moore et al., 1995; Kurata et al., 1994; Van Deynze et al., 1995b, 1995c).

Of 129 of anchor probes mapped in maize, 77 (60%) detected polymorphic duplicate loci in the maize genome (Ahn et al., 1995). Evaluation of probes detecting duplicate loci revealed that the resultant markers often exhibited duplicated linkage relationships. The map positions of the anchor markers has supported and refined the duplication previously reported for maize segments (Helentjaris et al., 1988; Ahn et al., 1993).

All of the foundation segments are represented in the wheat, barley, and oat genomes. Segment 12 is the most difficult to identify due to a limited number of anchored loci on this segment. It is apparent that a portion of Triticeae 5 (Van Deynze et al., 1995b) and oat F (Van Deynze et al., 1995c) are the homoeologous segment 12. Across the grasses, rearrangement and duplication of the foundation segments may represent divergence points among and within the three major clades, and within the major tribes, genera, and species. The consensus map of the Poaceae will facilitate (1) comparison of genomic structure across the grasses based on a basic set of homoeologous chromosome segments and (2) the transfer of information across species.

Current application of comparative genetics to plant breeding

The ability to relate genetic maps, genes, and emergent phenotypes across genera can be expected to provide a foundation for the merging of scientific disciplines that will enhance plant breeding efforts. The scientific study of the grasses has been largely dependent upon agronomic interests, and the ability to study the specific pathways, molecules, and genes underlying traits of interest. Across the domesticated grasses, agronomic interests differ, and, frequently, a well-characterized trait in one crop has received minor attention in others.

However, some traits that are fundamental to plant development and structure have been investigated in several domesticated grasses. Grain dormancy affects the basic survival, cultivation, and processing of grains. Plant breeders seek to control dormancy to prevent preharvest sprouting (PHS) of mature grain under conditions of high moisture and temperature, but retain the ability to break dormancy after harvest. Variation for PHS resistance is evident in red and white wheat, but red wheat generally exhibits higher levels of dormancy. In barley (Ullrich et al., 1993) and white wheat (Anderson et al., 1993; Sorrells and Anderson, 1996), grain dormancy is complex and has been evaluated as a quantitative trait.

Association of agronomic traits with QTL and biochemical pathways: The relationship between grain dormancy and carotenoid biosynthesis in cereals

In wheat, quantitative trait loci for PHS resistance were identified in a population of 78 recombinant inbred (RI) lines from the cross NY6432-18 (NY18) x Clark's Cream (CC) grown in 13 environments over a period of 5 years (Sorrells and Anderson, 1996). Map positions of significant loci in this study were located relative to linked loci on the wheat consensus map. The loci identified in white wheat were compared to the Poaceae consensus map to identify the foundation segment(s) and anchored loci in rice and maize (Sorrells and Anderson, 1996). Comparison of linkage relationships in regions containing a significant marker were used to delineate the smallest interval that could be compared across species. Candidate loci were identified based on map positions reported in maize and rice.

Prior to comparative maps, information accumulated in maize was difficult to relate to the genetics of wheat, barley, rice, and oat. Comparative maps facilitate the identification of loci in maize that may be orthologous and allow transfer of genetic information that will enhance studies in oat, wheat, and barley. In maize, a number of viviparous mutants are characterized by exhibiting precocious germination (Robertson, 1975). We propose that QTL affecting grain dormancy in wheat, barley, and rice correspond to the genes that generate the *vivipary* (*vp*) mutants in maize.

The gene generating *Vivipary 1* mutants has been isolated from maize and was determined to be a transcriptional regulator that is necessary for the induction

of ABA responsive genes (McCarty et al., 1991). Expression of the *Colored aleurone (C)* gene in maize is directly related to *Vp1*. The *C* gene acts in concert with the *Pericarp color 1 (red pericarp)* gene to regulate the *anthocyaninless 1* gene product (common to the both the anthocyanin and phlobaphene pathway) and with the *R* gene to regulate the *Bz1* gene (anthocyanin pathway) (Dooner et al., 1991). Dependence upon the *Vp1* transcriptional activator for proper expression of the *C* gene indicates a common regulatory mechanism controlling not only the ABA responsive genes, but the genes controlling anthocyanin and phlobaphene biosynthesis as well. This hierarchical regulatory mechanism explains the pleiotropic effects of *Vp1* on germination and pigment production in the aleurone and the pericarp in maize. Comparative maps indicate that the *Red grain color (R)* locus on wheat group 3 chromosomes may be orthologous to *Vp1* in maize. We propose that the *R* gene may exert pleiotropic effects on grain color and dormancy in wheat, and may underlie the relationship between higher levels of resistance to PHS and red grain color observed by Nilsson-Ehle (1914).

The remaining *vivipary* mutants are divided into two classes: carotenoid deficient and ABA deficient. These mutants exhibit precocious germination due to reduced levels of embryonic ABA due to blockage in the carotenoid biosynthetic pathway of which ABA is a final derivative (Smith and Fong, 1993). Map positions orthologous to *vp2=vp7*, *vp8*, and *w3* underlie the QTL intervals we have identified in white wheat populations (Table 1).

Near the end of the short arm of chromosome 1 of wheat we have mapped a QTL using BCD1434 that corresponds to a QTL reported on homoeologous chromosome 5 of barley reported by Ullrich et al. (1993). Using the consensus map of anchored loci, the predicted position of a locus orthologous to BCD1434 would be located on homoeologous segments of maize chromosomes 6 and 8

Table 1. QTL associated with PHS in white wheat and putative orthologous loci in maize involved in transcriptional regulation of ABA responsive genes or carotenoid biosynthesis (updated from Sorrells and Anderson, 1996).

PHS Locus	Chromosomal location			Candidate Locus	Gene product
	wheat	barley	maize		
<i>Red Grain</i>	3L	3L	3	<i>Vp1</i> <i>Red pericarp</i> <i>anthocyanin-activator</i>	transcription regulator unknown (rice) unknown (rice)
CDO431	1S	—	5S	<i>vp2=vp7</i>	phytoene C11'-12 desaturase
BCD1874	5L	7L	2,7	<i>w3, vp9</i>	phytofluene 11'-12 dehydr.
CDO795	4L	4L	1,5	<i>vp8</i>	unknown
BCD1434	1S	5S	6,8	<i>Y1?</i>	phytoene synthase

(candidate locus may be *Y1*). The QTL we identified with CDO431 mapped near the centromere of wheat chromosome 1. The homoeologous region in maize is duplicated on chromosomes 1 and 5 (Poaceae segment 10) and contains *vp2* and *pink scutellum* (*vp7*). CDO795 is close to *vp8* of maize and a QTL in barley. On wheat chromosome 2, using CDO64, we mapped a QTL on the short arm. Comparative identification of the homoeologous segment in maize is complicated due to the detection of incongruent loci by CDO64 across genera. While this locus is on the same arm as a locus hybridizing to the clone (pBS128) for ABA response (Cadle et al., 1994), it does not appear to be close enough to be orthologous. Further evaluation of probes detecting linked loci will be necessary to identify candidate loci. Another QTL, identified using BCD1874, mapped to wheat chromosome 5 in a interval homoeologous to portions of maize chromosomes 2 and 7 (Poaceae 9) and is putatively orthologous to the *white3* mutant.

We propose that *vp* mutants of genes involved in carotenoid biosynthesis may represent loci that are orthologous to wheat genes that affect PHS in white wheat. White wheat may contain an altered gene(s) at the *Red grain color* locus (with pleiotropic effects on dormancy inferred by comparison the orthologous *Vp1* locus in maize). From this perspective, the *R* locus may condition a strong type of dormancy, and when altered in white wheat, alleles at secondary loci explain the genetic variation in white wheat populations.

By mapping QTL associated with the same complex phenotype in related species, as well as in different genetic backgrounds within a genera, the use of cross-genera comparisons is likely to provide valuable clues about the precise map location of the underlying genes. After mapping genes that comprise a single metabolic pathway, we may predict associations between these genes and previously identified QTL.

Comparative mapping can provide independent verification of the relationships by providing evidence of conserved map position, and when examined as an entire pathway across the domesticated grasses, the association of independent genes with QTL positions may support such relationships. While additional mapping research is needed to confirm these results, it is clear that the location and function of some of the loci affecting grain dormancy have been conserved in these genera.

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Physical mapping of cereal chromosomes, with special emphasis on barley

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Introduction

The molecular linkage maps of cereals are improving rapidly by adding new types of markers, by merging of different species-specific maps and by comparative mapping of markers between related genomes. Since recombination frequency is not constant along chromosomes, the efficient use of the resulting dense maps requires a detailed understanding of the non-linear relationship between genetic and physical distances. In cereals, various methods are available to tackle this task at different levels of resolution. Molecular techniques can be applied to small regions of the genome in order to convert genetic distances between closely linked loci into accurate physical distances. This is necessary for map-based cloning and includes, for example, long-range restriction mapping using pulsed-field gel electrophoresis or assembly of overlapping large-insert DNA clones (cosmids, YACs, BACs) into contigs for "walking" towards a gene of interest. In general, molecular techniques have good chances for small genomes like rice (Umehara et al. 1995) but are difficult to apply to large genomes (Tanksley et al. 1995).

In the *Triticeae* cereals the success of positional gene cloning depends on the possibility of defining genomic regions where physical distances between linked loci are short enough to be bridged by molecular techniques. So far, respective conclusions based on well-founded results are only available for wheat, which has the largest genome of all cereals. Using an elegant system for producing great numbers of stable terminal deletions (Endo 1995), so-called cytogenetic ladder-maps have been constructed for nearly all of the 21 wheat chromosomes (reviewed by Gill and Gill 1994; Mickelson-Young et al. 1995). These maps provided detailed insights into the relationship between physical and genetic distances along the entire chromosome. Besides the general feature of suppressed recombination in the proximal and frequent recombination in the distal chromosome regions, local hot spots for recombination could be identified (Gill et al. 1993).

Colinearity of RFLP and gene order between wheat, rye and barley is well-documented (e.g., Devos et al. 1993). Reduced recombination frequency within the pericentric regions of chromosomes is also a phenomenon in rye and barley (e.g., Heslop-Harrison 1991; Pedersen et al. 1995). Therefore, variable

recombination intensity as observed in wheat is assumed to be valid for rye and barley, too. However, in diploid cereals, systematic studies of the relationship between physical and genetic distances were lacking because of the difficulty to localize genetically mapped DNA probes *in situ*.

Recent results obtained in barley with a PCR-mediated technique for mapping RFLP markers to micro-isolated translocation chromosomes revealed an unexpectedly heterogeneous distribution of recombination along the length of the chromosomes. Substantial variation was observed even within such chromosome regions that show, on average, high recombination rates. These data may provide useful information for positional gene cloning approaches in barley, as demonstrated in the following.

Physical mapping of barley chromosomes

The method requires: (1) a stock of lines with well-defined reciprocal translocations, (2) a technique that enables micro-isolation of individual chromosomes, and (3) single-copy RFLP probes with known genetic localization. Then, the experimental route is: (1) partial sequencing of the RFLP probes and synthesis of probe-specific PCR primers, (2) micro-isolation of suitable translocation chromosomes involving the studied linkage group, and (3) PCR with probe-specific primers and chromosome-specific template DNA to assign the RFLP probe to one of the two interchanged chromosomes (Figure 1). The data obtained provide the basis for integration of the genetic maps between physical landmarks on chromosomes (Sorokin et al. 1994; Künzel et al. 1995).

Utilization of translocations for mapping purposes has a long tradition in barley, going back more than 40 years (Burnham and Hagberg 1956). At present, about 1000 translocation lines are available (Künzel 1993). Since chromosome-specific painting by suppressive *in situ* hybridization is not yet possible in plants (Fuchs et al. 1996), at present the most precise localization of breakpoints is obtained by analysis of Giemsa-banded chromosomes (Linde-Laursen 1988). At the beginning, translocations with well-defined breakpoints were selected for physical mapping (Marthe and Künzel 1994). Later on, less precisely defined breakpoints could also be included. This is possible when the breakpoints are located between the linearly ordered markers in different regions of the genetic map. In this case, the physical distances between loci flanking the breakpoints can be approximated by logical considerations. Consequently, the positions of breakpoints can be delimited in many cases. By mapping both breakpoints of a given translocation between the markers within the corresponding linkage maps, a further delimitation of the breakpoint positions is possible since each breakpoint defines the physical position of the other. Mutual consideration of breakpoint positions for many of the studied translocations yielded remarkably refined breakpoint positions as a result of the high degree of self-improvement of the entire system. Therefore, precise physical positions of both translocation breakpoints and genetic markers are to be expected when the integration of breakpoints and genetic markers is completed for all chromosomes.

By this method, a total of 109 breakpoints have exactly been localized between

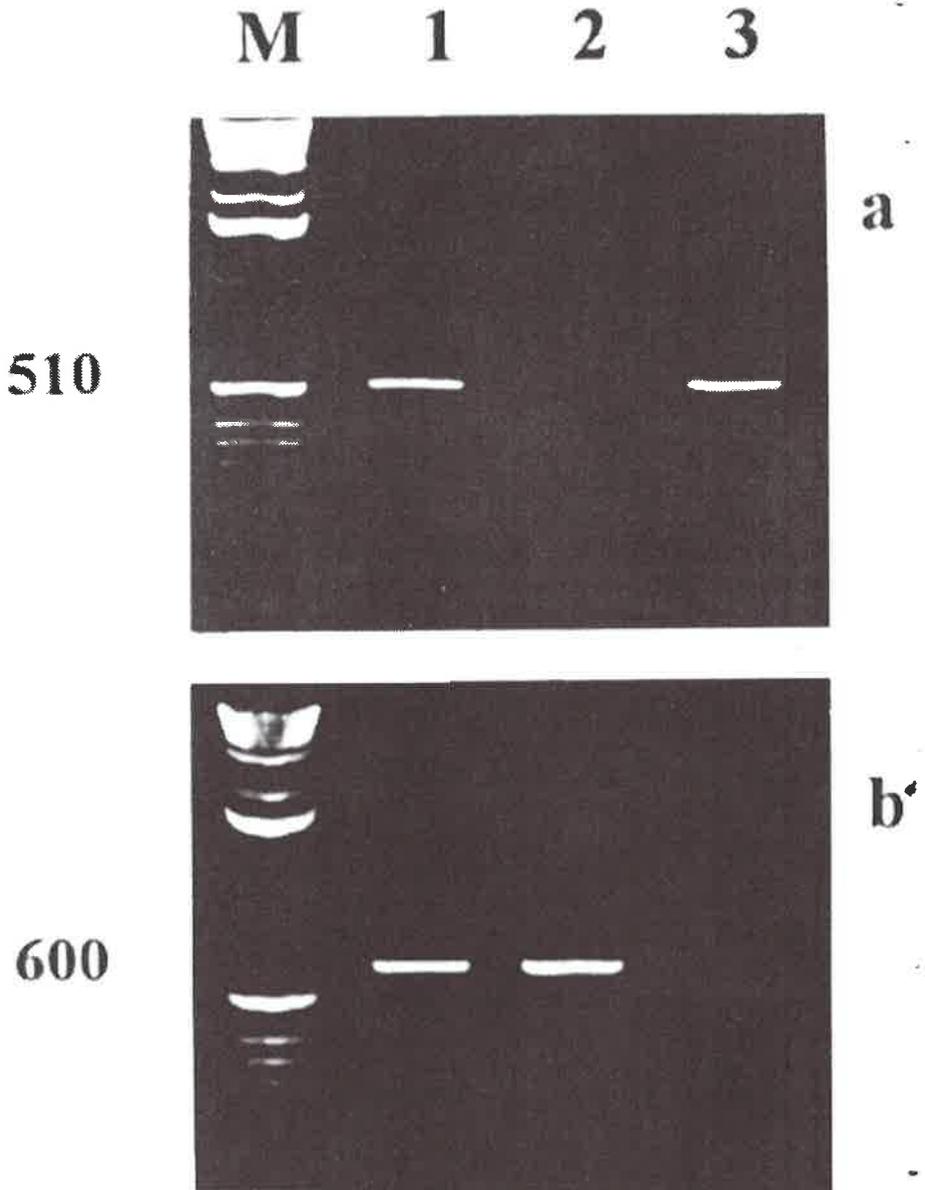


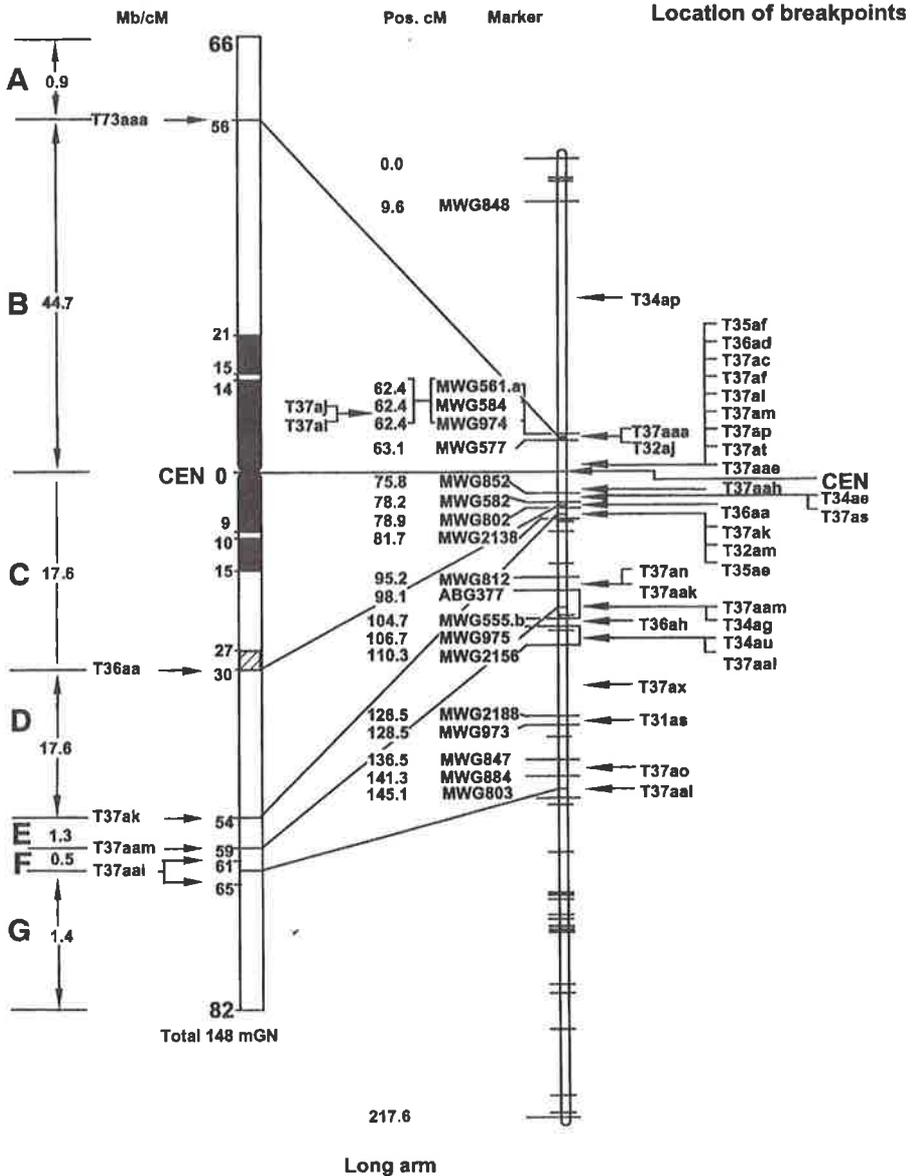
Figure 1. PCR products obtained with RFLP probe-specific primers and template DNA from micro-isolated nuclei and chromosomes of translocation line T4-5ai. M, DNA size markers (1 kb ladder, BRL). Lane 1, 5 prophase nuclei; lane 2, 5 chromosomes T45ai; lane 3, 5 chromosomes T54ai. a. Primers according to MWG800. b. Primers according to MWG913.

the markers of the Igri/Franka derived RFLP maps for chromosomes 3, 5 and 7 (Sorokin et al. 1995, Korzun and Künzel 1996a). More than the half of these breakpoints occur within the extended median chromosome regions that show low rates of recombination. This holds true for 18 of the 32 breakpoints in chromosome 3 (Figure 2), 31 of the 38 breakpoints in chromosome 5 (Figure 3) and 19 of the 39 breakpoints in chromosome 7 (Figure 3). The breakpoints located outside these regions tend to be clustered at specific areas of the genetic maps. This is particularly evident for chromosome 7. Beyond that, each chromosome arm has its own characteristic feature. For example, in the short arm of chromosome 7, the two breakpoints localized distally to MWG502 indicate that this outermost locus of the map does not represent the terminus of this arm (Figure 4); while in the long arm of chromosome 3, no breakpoint was found within the distal part of the genetic map that contains many markers and spans more than 70 cM (Figure 2).

Well-defined breakpoints were used for most informative positioning of markers within the chromosome arms to relate the physical and genetic maps. In conformity with other studies (e.g., Pedersen et al. 1995), suppressed recombination in the proximal regions was found to be a general feature, but with distinct modifications for individual chromosomes. For example, severely suppressed recombination distinguished a proximal part of 74% length of chromosome 3, but only of 57% and 56% of the proximal length of chromosomes 5 and 7. Furthermore, suppressed recombination comprised larger areas of the short arms (85%, 70% and 73% of the arm lengths) than of the long arms (66%, 49% and 46%) for chromosomes 3, 5 and 7, respectively. Even more important, substantial variation in the distribution of recombination events was observed within the distal chromosome regions showing a generally high frequency of recombination. While in the short arms the highest frequency of recombination was observed in the most terminal segments, small physical regions of very high recombination rates were identified clearly apart from the ends of the long arms in chromosomes 3 and 7. The physical correspondence of genetic distance of defined subregions varied between 0.5 to 44.7 Mb/cM in chromosome 3 (Figure 2), 1.6 to 50.2 Mb/cM in chromosome 5 (Figure 3) and 0.2 to 118.0 Mb/cM in chromosome 7 (Figure 4).

Using the same PCR technique and the knowledge of the homoeologous relationship between wheat/rice and wheat/barley (e.g., Ahn et al. 1993; Kurata et al. 1994; Moore et al. 1995), RFLP probes mapped on rice chromosomes 5 and 10 were allocated to cytologically defined regions of barley chromosome 5 (Korzun and Künzel 1996b). These results are illustrated in Figure 5. Besides a barley-specific shift of the CDO105-locus from the long to the short arm, it was found that the rice linkage group 5 of about 135 cM maps into two separate parts that relate to the distal portions of both the short and long arms of the barley chromosome. Thus, the markers of rice map 5 were localized to those regions of the barley chromosome that show high recombinations rates. In contrast, the four markers of rice map 10, about equidistantly distributed over nearly the complete map-length of about 75 cM, were assigned to an interstitial segment of

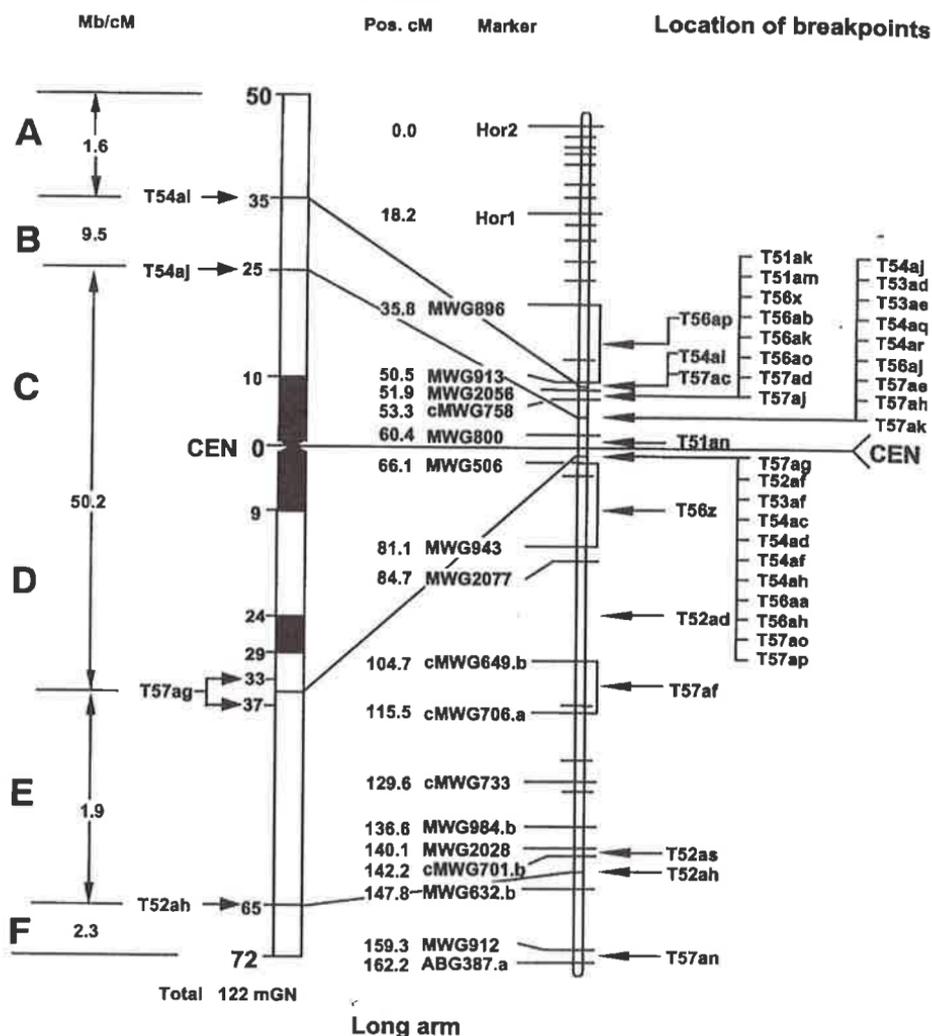
CHROMOSOME 3 (3H)
Short arm



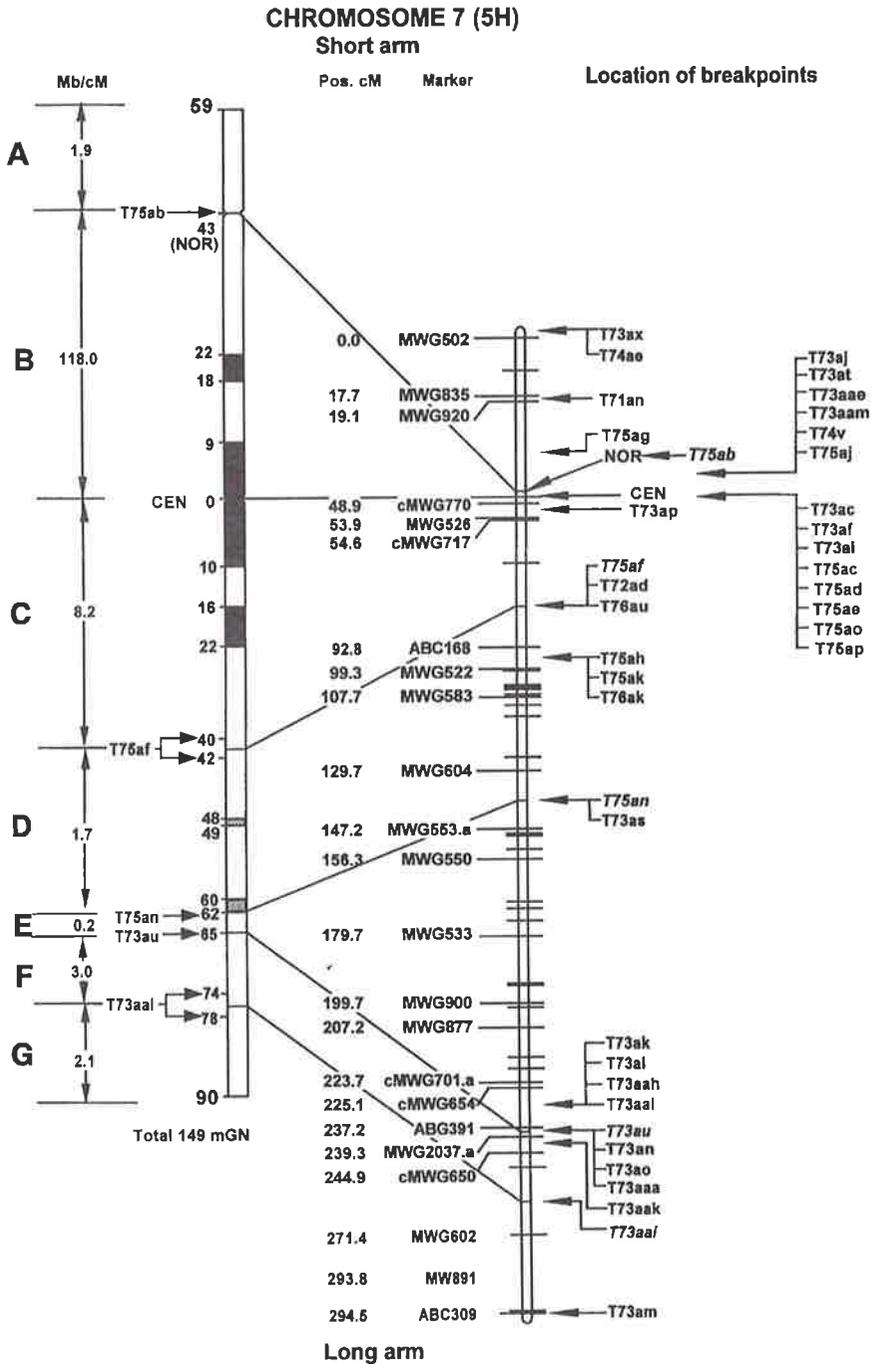
Figures 2 to 4. Comparison between the physical and genetic maps of barley chromosomes 3, 5 and 7. **Left:** Idiograms based on N-bands according to Marthe and Künzel (1994) and measurements in milliGeNomes (mGN) according to Jensen and Linde Laursen (1992). Prominent N-bands are shown in black and rare N-bands are hatched. Translocation breakpoints used for map construction are shown on the left of the idiograms: single arrow indicate precisely located

CHROMOSOME 5 (1H)

Short arm



breakpoints, linked arrows indicate regions to which breakpoint are assigned. The estimated mean distances in megabases (Mb) per one centiMorgan (cM) for the subregions shown on the far left are based on the value of 5350 Mb for the size of the haploid (2C) barley genome (Laurie et al. 1992). **Right:** Linkage maps according to Graner et al. (1994). The marker positions are shown by bars; those used for PCR are named. The location of breakpoints within the linear array of markers is indicated by arrows. Corresponding regions of the genetic and physical maps are connected by lines.



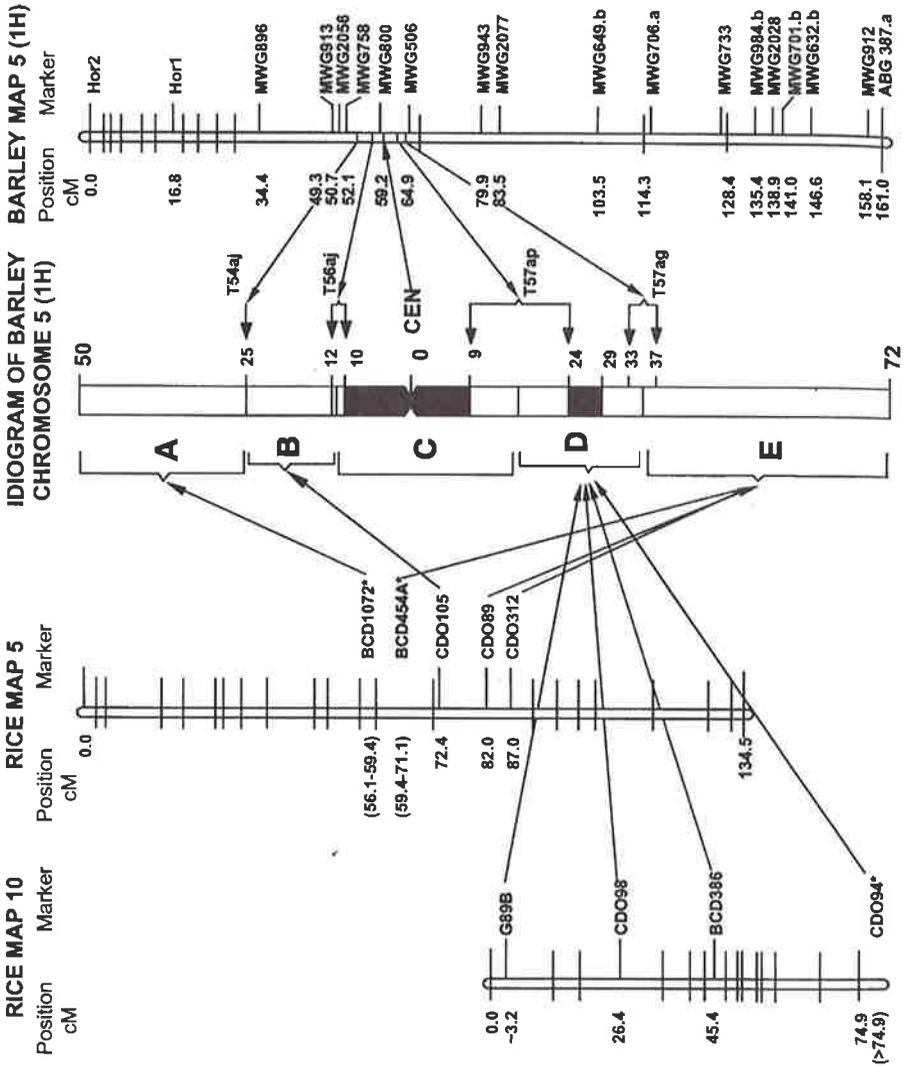


Figure 5. Comparison between barley chromosome 5 (1H) and the linkage maps of rice chromosomes 5 and 10 according to Causse et al. (1994). Probes marked by asterisks (*) on the rice maps have been assigned to centiMorgan (cM) intervals (shown on the left in brackets) with a LOD score (2.5 (cf. Causse et al. 1994). The position of G89B was included by inference based on the rice map 10 published by Inoue et al. (1994). For details regarding the barley maps see legend for Figures 2 to 4. Corresponding regions among the maps are indicated by arrows.

the long arm of the barley chromosome. This segment of about 26% arm length represents a region of highly suppressed recombination and relates to an interval of only about 3 cM without any marker in the Igri/Franka map.

Conclusions

PCR-assisted mapping of single-copy RFLP probes on micro-isolated translocation chromosomes of barley proved to be of high efficiency in relating genetic to physical distances. Similar to wheat, small regions of barley chromosomes have been identified that show very high recombination rates. From these regions, characterized by about 0.5 megabases per centiMorgan on average, positional gene cloning seems possible. For other regions of the barley genome with a low recombination frequency, the small rice genome offers a more suitable tool for map-based gene cloning. This is especially true for the extended interstitial segment of the long arm of barley chromosome 5, which corresponds to rice chromosome 10.

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Wide crosses for haploids

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Introduction

Wide hybridization, defined as crosses between two biological species, has been used in cereal crop breeding both as a means to introgress traits (genes) from a related species and as a means of generating haploid plants for genetic analysis and breeding purposes. Haploid production in certain wide crosses occurs as a result of differential loss of chromosomes of one of the donor species, usually the pollen donor species. The objective of this paper will be to discuss findings on haploid production via wide crosses in cereals, with an emphasis on the very wide crosses (across subfamilies) and in particular on some novel and exciting results we have obtained from crosses of oat by maize.

Triticeae species haploids from interspecific and intergeneric crosses

The initial consistent production of cereal haploids via wide hybridization was from an interspecific cross with barley (*Hordeum vulgare* L.) haploid plant recovery following crosses with *H. bulbosum* L. (Kasha and Kao, 1970). A variety of factors influence the frequency of haploid plant recovery, including genotype of parents, growth conditions, post-pollination treatments of florets with gibberellic acid and other growth hormones, embryo rescue media, and finally growth and colchicine treatments of rescued plantlets. Analyses of these aspects all contributed to the development of a system enabling the introduction of the scheme into breeding programs and the release of doubled haploid lines as cultivars (Choo et al., 1985). Because of limitations on doubled haploids, including technical resources required and reduced opportunity for recombination and selection in line development, the approach has not been widely adapted by barley breeders; however, the bulbosum scheme has proven extremely valuable in developing pure-breeding doubled haploid populations for molecular marker and quantitative trait loci mapping (Kleinhofs et al., 1993; Hayes et al., 1993).

The discovery that *H. bulbosum* pollinations of certain wheat (*Triticum aestivum* L.) cultivars permitted the production of wheat haploids by chromosome elimination raised hopes for increased use of doubled haploids in wheat breeding (Barclay, 1975). Subsequent work, however, revealed that most wheat cultivars,

especially those grown in Europe and North America, contain *Kr-1* or *Kr-2* alleles, which severely restrict pollen penetration and seed set in the intergeneric cross with *H. bulbosum* (Snape et al., 1979; Falk and Kasha, 1981). Thus, the bulbosum scheme for doubled haploid wheat breeding has had limited use.

Triticeae species haploids from inter-subfamily crosses

An exciting alternative to *H. bulbosum* as a pollen donor for wheat haploid production came from the description by Laurie and Bennett (1986) of haploid wheat embryos being produced from wheat x maize (*Zea mays* L.) hybridizations by elimination of the maize chromosomes during the initial cell divisions of the developing embryo. Subsequent recovery of wheat haploid plants was obtained either by ovary culture (Comeau et al., 1988) or spikelet culture (Laurie and Bennett, 1988). Application after pollination of a solution of 2,4-dichlorophenoxyacetic (2,4-D) either directly onto florets (Laurie et al., 1990) or by injection into the upper internode of the wheat spike (Suenaga and Nakajima, 1989) was shown to promote ovary and embryo development in these very wide crosses. An estimated 50% to 85% of embryos initiated survived to time of rescue at about 16 days post-pollination, and 66% of those germinated into healthy green seedlings (Laurie and Reymondie, 1991). Frequencies of embryo recovery and subsequent germination into plants from these wide crosses can be strongly influenced by several factors, including growth conditions of the plants, developmental stage of the floret at pollination, post-pollination hormonal treatments, timing of and media used in embryo rescue, and other yet-undefined factors such that caution is needed in comparing frequencies among experiments or laboratories (Laurie and O'Donoghue, 1994). However, the general conclusion from numerous reports, including specific analysis of *Kr* allele differences, is that, while there may be some variation in frequency of wheat haploid plant recovery with different genotypes of wheat or the maize pollinator, there does not appear to be a genotype restriction to the degree observed in Triticeae interspecific crosses caused by *Kr* allele effects. The potential of wheat x maize crosses for producing wheat doubled haploids for genetic studies and breeding purposes has been recognized by several groups, including a program set up by N. Howes and T. Aung at Agriculture and Agri-Food Canada, Winnipeg. This group is currently producing 500 to 1,000 wheat doubled haploids per month, with 3 to 10 plants recovered per wheat spike emasculated and pollinated (N. Howes, personal communication). Keys to their success include careful timing of emasculation and pollination, light synchronization of maize anthesis, split applications of 2,4-D, and early excision of embryos for rescue before they are fully developed.

Sexual hybridization followed by uniparental chromosome elimination has been found to extend to several members of two subfamilies (Pooideae and Panicoideae) of the Gramineae family (Clayton and Renvoize, 1986) that include wheat and maize, respectively. Haploids of Triticeae species have been produced from crosses of bread wheat x maize, teosinte (*Z. mays* ssp. *mexicana*), eastern

gamagrass (*Tripsacum dactyloides* L.), sorghum (*Sorghum bicolor* L. Moench), and pearl millet (*Pennisetum glaucum* R. Br.), from crosses of durum wheat (*T. turgidum*) x maize, and from crosses of barley x maize (see Riera-Lizarazu et al., 1996 for complete reference list). Barley x maize crosses were of limited interest for barley haploid plant production because of the low frequency of plant recovery (1.5% to 4.7% of florets pollinated) compared with barley x *H. bulbosum*, but were made using maize stocks carrying active transposons to test for possible maize DNA introgression (Chen et al., 1991). In 41 barley plants recovered, no maize introgression was detected either cytologically or by DNA blot hybridizations using maize transposable elements and highly repeated sequences as probes. Similarly, maize transposable element transfers have not been detected in wheat x maize crosses (Laurie and O'Donoghue, 1994). Alien donor chromosome retention in very wide crosses involving wheat also has been found not to occur, or is a very rare event. No evidence of smaller pollen donor chromosomes were detected cytologically among 191 (Laurie and Reymondie, 1991), 450 (Matzk and Mahn, 1994), 118 (Inagaki and Bohorova, 1995), and 377 (Inagaki and Mujeeb-Kazi, 1995) wheat plants recovered from crosses to maize, pearl millet, or sorghum. There are two published reports of alien chromosome retention in widely distant crosses with wheat, one involving wheat and maize (Comeau et al., 1992) and one in wheat and pearl millet (Ahmad and Comeau, 1989), but in neither case was the extra chromosome found to be transmitted to progeny.

Oat haploids from inter-subfamily crosses and alien chromosome retention

Cultivated hexaploid oat (*Avena sativa* L., tribe Aveneae) was found to be like the Gramineae species just discussed in yielding haploid plants through uniparental pollen donor chromosome elimination following inter-subfamily hybridization with maize or pearl millet (Rines and Dahleen, 1990; Rines et al., 1996). Unlike in the crosses of Gramineae species to maize, the post-pollination addition of an auxin was not critical for embryo development and rescue, although application of a low level (10 mg/L) did appear to aid embryo and plant recovery (Rines et al., 1996). A novel feature of the haploid oat plants recovered from the wide crosses was that they may be partially self-fertile through the production of unreduced gametes, with up to 40% seed set (Davis, 1992). Furthermore, about one-fourth of the progeny from self-fertilization in these haploid oat plants are aneuploids, with monosomy being especially common. This rich source of aneuploids has been exploited to develop materials that have been a long sought goal in oat cytogenetics – a complete set of monosomics for all 21 chromosomes (Jellen, Fox, Rines and Phillips, unpublished). These monosomics are being used to locate DNA molecular markers to chromosomes, and thus develop an integrated chromosomal/molecular marker genetic map for oat.

Another novel feature arising from oat x maize hybridization is that, in contrast to the situation described earlier for the inter-subfamily crosses involving

Triticeae species, we have found pollen donor chromosome retention to be relatively common in oat x maize crosses (Riera-Lizarazu et al., 1996). From 90 plants recovered over the course of seven experiments involving the emasculation and maize pollination of about 15,000 oat florets and the excision and plating of over 1,000 embryos, 30 plants were found by root-tip analysis to contain one to four smaller maize chromosomes in addition to a haploid complement of 21 oat chromosomes. Genomic *in situ* hybridization (GISH) of root-tip cells using fluorescent-labeled total maize DNA as probe was used to definitively demonstrate that these smaller chromosomes were of maize origin. Using a maize repeated sequence probe, eight additional plants of the 90 were found to be apparent chromosomal chimeras where some tissues in a plant contained maize sequences while others did not. Maize DNA molecular markers were used to identify the maize chromosome(s) present in the various oat-maize progenies. Eight of the ten different maize chromosomes were found among these plants. Furthermore, some of the plants with only a single maize chromosome present, and in two cases ones with two maize chromosomes present, were partially self-fertile to yield self-fertile maize chromosome addition oat plants. Fertile addition lines were recovered for maize chromosomes 2, 3, 4, 7, 8, and 9. These plants represent novel materials for maize gene mapping and isolation, study of maize gene expression in oat, and potential introgression of maize DNA, or active transposons, into oat.

The recovery of haploid oat plants from crosses of oat x subfamily Panicoideae species has been reported recently by two additional research groups. Machan et al. (1995) from 1,220 maize-pollinated oat florets recovered 50 (4.1%) embryos, and 35 of these (70%) developed into plants. The recovered plants were treated with colchicine for chromosome doubling. Doubled plants were reported to show the same anthesis date and avenin electrophoresis patterns as the oat parent, with no mention of possible retained donor parent DNA or trait modification. Matzk (1996) recovered embryos at frequencies of 2.2%, 1.5% and 9.8% from several thousand oat embryos pollinated with maize, eastern gamagrass, and pearl millet, respectively. Although 17.7%, 2.3% and 26.1% of the respective groups of embryos germinated, their growth in general was abnormal, with often only leaf-like structures or coleoptiles. A total of 43 plantlets died after forming one to four leaves and/or roots. Small extra chromosomes, presumably retained from the pollinator, were present in root tips of some of the nonviable F_1 plants from oat x maize and oat x pearl millet crosses. Only four plants survived, and these were among a portion treated with colchicine. Three of these were fertile. Although progeny of each of the fertile plants had the normal oat chromosome number of 42, one line was variegated in phenotype, leading the author to suggest that genetic information was apparently transferred from maize or activated within the oat genome.

The wide variation among the three published reports in viable plant frequency from excised embryos from oat x maize or pearl millet crosses could be due to differences in genotypes, post-pollination hormone treatments, embryo rescue medium, and other technique differences. We have postulated (Rines et al., 1996)

that one possible reason for our low plant recovery frequency from oat x maize embryos (often less than 10%) versus that of ourselves' and others' reported frequencies for plant recovery from wheat x maize embryos (from 20 to 70%) may be due to detrimental effects of maize chromosome retention in the non-growing oat x maize embryos. It is of interest that the one report with high plant recovery from oat x maize embryos (Machan et al., 1995) contained no evidence of maize chromosome retention in the recovered plants. A technique variable for which there have been no reports in the oat x maize or wheat x maize systems is the growth temperature during early hybrid embryo formation following pollination. We are now exploring possible growth temperature effects because this has been shown to be a critical factor influencing rate and degree of uniparental chromosome elimination in barley x *H. bulbosum* crosses (Pickering, 1984).

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Doubled haploids in barley

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Introduction

The possibility of generating barley haploids both by the interspecific hybridization of barley with *Hordeum bulbosum* (Kasha and Kao 1970) and by anther culture was reported in the early 1970s (Clapham 1973). Scientists and breeders rapidly adopted the method because the doubled haploids (DHs) produced provide several advantages over standard F₂ populations, which have been the subject of several reviews (Kasha and Reinbergs 1975; Choo et al. 1985; Snape 1989). Today more DHs have been produced in barley than in any other crop. These have been used extensively for cultivar release, genetic analyses (for review, see Pickering and Devaux 1992), for mapping the genome with various kinds of markers (Graner et al. 1991; Heun et al. 1991; Tragoonrung et al. 1992; Zivy et al. 1992; Kleinhofs et al. 1993; Giese et al. 1994; Saghai Maroof et al. 1994; Becker and Heun 1995a, 1995b; Becker et al. 1995; Langridge et al. 1995) and for Triticeae comparative mapping analyses (Snape 1996). Although the *H. bulbosum* (*Hb*) and anther culture (AC) methods have been used successfully for DH production, the two techniques differ in several aspects that may influence the nature of the recovered gametes. Firstly, plantlets are obtained through the development of zygotic embryos in the *Hb* but via organogenic and embryogenic structures in AC. Consequently the *in vitro* culture phase is shorter in the *Hb* and does not require the use of growth substances as it does in AC. Secondly, the *Hb*- and AC-derived DHs arise from female and male gametophytes, respectively. To assess possible deviations of *Hb*- and AC-derived DHs as a consequence of differences between the two systems, investigations have been carried out in three areas. We have studied DNA mutation and methylation, comparative mapping and segregation distortions in different DH populations.

DH production

To ensure production of DHs from every possible hybrid in a breeding program, the *Hb* and AC methods have been used in parallel since recalcitrant genotypes to the two techniques have been identified (Foroughi-Wehr et al. 1976; Pickering and Hayes 1976). A preliminary study that had revealed a significant genotype x technique interaction (Devaux 1987) has now been confirmed on a larger scale. Haploid plant production efficiency, i.e. the number of viable green plants per spike processed, was assessed by the two techniques for 958 F₁ hybrids (785 winter and 173 spring types). Of the 785 winter types, 432 (55%) responded better through AC than through *Hb*, while for spring types 132 (76%) were more efficient with the *Hb* method. These results are in accord with those reported by Huang et al. (1984) and Friedt et al. (1987) who used a survey of 3 genotypes and 6 hybrids, respectively. In addition to the genotype by technique interaction, the two techniques employ dissimilar environmental conditions for donor plant growth. In the *Hb* method the pollen donor and recipient plants are grown at temperatures above 18°C to insure good quality of *Hb* pollen and elimination of the *Hb* chromosomes from cells of young embryos (Pickering 1985). The optimal temperature in AC is much lower, ca. 12°C (Foroughi-Wehr and Mix 1979; Lyne et al. 1986). Therefore, the *Hb* DH production can be carried out in the warm season and AC in the cool season with some overlap. As a consequence, energy can be saved and permanent staff employed continuously.

At least 59 DH lines have been released as cultivars worldwide (Table 1). Nearly all of them were produced by the *Hb* method, but five recent releases (Anthere, Henni, Lyric, Tantangara and Tender) were by AC. To our knowledge, all DH lines have been produced from F₁s and there are no advantages to using other generations (Iyamabo and Hayes 1995). Noticeable are several DH cultivars for which the sites where they were generated are very different from the site of selection and cultivation, a potential advantage of using DHs recognized early by Kasha and Reinbergs (1975). For example, Gwylan and Jing Zhuo were produced in Europe but selected and cultivated in New Zealand and China, respectively.

Much progress has been made in isolated microspore culture and regeneration. Recently, Cistué et al. (1995) reported a frequency of over 17 green plants per anther of the cultivar Igri. Also using Igri, Hoekstra et al. (1992) and Davies and Morton (1995) showed that higher frequencies of DHs can be produced by isolated microspore culture than with anther culture. If this could be consistently repeated with a range of genotypes, there is no doubt that this technique would play a more predominant role for DH production.

DNA mutation and methylation

Genetic changes are apparently induced by tissue culture (Larkin et al. 1989; Karp 1991) and a variety of phenotypic alterations have been reported (Ullrich et al. 1991; Baillie et al. 1992). Cytological and morphological aberrations

have been more frequent in AC than in *Hb*-derived DHs (for a review, see Pickering and Devaux 1992). We have studied the level of DNA variation in 30 + 30 phenotypically normal DH plants derived by AC and *Hb* from the cultivar Igri (Devaux et al. 1993). Polymorphism was not found among the DH lines for 273 RFLP and 89 PCR-amplified fragments, indicating absence of deletion/insertion mutations. The number of base pairs screened (401,640) by these two

Table 1. Doubled haploid barley cultivars produced by the *Hbm* and by AC (*)

Company/Institute	Country	Name of cultivars	References
Abed PBS	Denmark	Etna, Give, Loma, Loke, Riga, Rima, Verona, Paloma, Bereta, Aberdeen, Pondus, Perma, Tender*	Rasmussen pers. comm.
Agriculture Canada	Canada	DB202	Choo et al. 1995
Canterbury Malting	New Zealand	Valetta	Pickering pers. comm.
Crop & Food Res	New Zealand	Gwylan	Coles 1986
Florimond Desprez	France	Michka, Lombard, Moka, Anka, Vodka, Gaelic, Gotic, Logic, ZF3642, Jing Zhuo, Douchka, Tattoo, Jerka, Lyric*	Pickering and Devaux 1992 Lefebvre pers. comm.
ICI seeds	UK	Waveney	NIAB 1988
IPG/PBS	Poland	KA7/3	Adamski et al. 1995
IPGG	former USSR	Istok, Odesskill 15, Preria	Choo pers. comm.
NSW Agriculture	Australia	Tantangara*	Read 1995
Saaten-Union	Germany	Anthere*, Henni*	Jäger-Gussen pers. comm.
Semico	Canada	HD87-18.14, HD87-12.1	Choo et al. 1995
WG Thompson	Canada	Mingo, Rodeo, Craig, Winthrop, Lester, Ontario, TB891-6, Prospect, Bronco, Sandrina, Beluga, McGregor, T090-017, T086-156, T081-009, T103-003, H30-11	Ho and Jones 1980 Campbell et al. 1984 Shugar and Etienne Choo et al. 1995 Shugar pers. comm.
WPBS	UK	Doublet, Pipkin	Jones et al. 1985; 1986

classes of molecular markers indicated that the point mutation rate was less than 0.25×10^{-5} . However, when DNA was digested with the methylation-sensitive restriction enzymes *HpaII* and *MspI*, RFLPs were obtained. Interestingly, 96% (49 out of 51) of the total methylation polymorphisms were from AC-derived DHs. These changes were probably induced during the *in vitro* culture phase of AC. The longer period in culture and the use of growth substances for the AC- versus the *Hb*-derived DHs might account for the higher level of methylation polymorphism observed. Müller et al. (1990) reported higher genetic instability of the rice actin genes in plants regenerated from calli maintained for 67 days versus 28 days in culture. However, we could not exclude the possibility that different tissues, i.e. immature zygotic embryos (*Hb*) versus microspores (AC), may have different initial levels of DNA methylation as reported for carrot (Palmgren et al. 1991) and tomato (Messeguer et al. 1991).

Comparative mapping

Meiotic recombination is a crucial phenomenon on which genetic studies and improved organism breeding is based. The process may be affected by various factors, including male vs. female meiosis. The *Hb*- and AC-derived DH lines represent female and male recombinant products, providing an excellent opportunity to compare recombination in male and female meioses of barley. Map distances were determined in 101 *Hb*- and 101 AC-derived DHs obtained from an F_1 (Steptoe x Morex) hybrid using molecular markers covering most of the barley genome (Devaux et al. 1995). The AC-derived population showed an 18% overall increase in the recombination rate. This was observed for every chromosome and for most of the chromosome arms. Eight pair-wise distances between individual loci were significantly higher in the AC population and one in the *Hb* population. Although three of the eight distances compared were centromeric, the two most significantly different distances were located in telomeric regions. The most telomeric intervals from 13 chromosomal arms were 43% longer in the AC than in the *Hb* population, while nontelomeric intervals were only 12.5% longer. Although overall increased and decreased male recombination has been reported in other systems (reviewed in Devaux et al. 1995), increased recombination frequency at the telomeres in male meiosis seems to be emerging as a general phenomenon. This could be a consequence of the pairing process of homologues at meiosis, since interstitial pairing is more frequent in female than in male meiosis (Bojko 1983). Rouyer et al. (1990) hypothesized that more random initiation of pairing could explain the lesser expansion of the female genetic map in the distal regions of chromosomes. Interestingly, Armour (personal communication 1996) found that the frequency of mutations for some minisatellites located in telomeric areas of human chromosomes were 10-fold higher in males than in females and also reported higher recombination frequencies in males than females. There is some evidence that subtelomeric regions of human (Saccone et al. 1992) and cereal (Moore et

al. 1993) genomes are much more gene-rich than proximal regions. Therefore, increased male recombination at the telomeric regions may be selectively advantageous, since it results in an increased number of gene assortments in the more abundant male gametes that are subjected to high selection pressure at the pollination and/or fertilization stages. Based on the above discussion, one could speculate that more variability is likely to be expected among DHs derived from AC than from the *Hb* in the absence of *in vitro* selection.

Segregation distortions

To fully exploit the genetic variability within a cross, DHs must be derived from a random sample of gametes, i.e. no or little selection should occur during the *in vitro* culture phase. Studies using morphological traits and several kinds of markers failed to show any significant deviation from expected segregations in *Hb*-derived DH populations (Kjær et al. 1990; Powell et al. 1990; Schön et al. 1990). Kleinhofs et al. (1993) reported some distorted loci in the Steptoe x Morex *Hb*-derived DH population; however, the magnitude of distortions was much smaller than that of AC-derived DHs from the same F_1 hybrid (Devaux et al. 1995) and the frequency was low enough to be attributed to random chance. Two areas of strong distortion have been found on chromosomes 1H and 5H in AC-derived DHs from the F_1 (Steptoe x Morex) (Devaux et al. 1995). Similarly, distorted loci were reported in AC-derived DH populations from other crosses on chromosomes 1H (Heun et al. 1991; Kintzios et al. 1994; Logue et al. 1995), 5H (Graner et al. 1991; Steffenson et al. 1995) but also on all the other chromosomes, i.e. 4H and 6H (Thompson et al. 1991), 2H and 7H (Graner et al. 1991; Heun et al. 1991; Logue et al. 1995) and 3H (Graner et al. 1991; Devaux et al. 1995). If most of these biased gamete selections result from linkages with genes involved in AC culturability, this would suggest that the character is complex and controlled by many genes dispersed in several genotypes.

Segregation of 28 protein encoding genes was investigated by two dimensional electrophoresis in a population of 62 AC-derived DHs from the F_1 (Kaskade x DH8293) (Zivy et al. 1992). Significant distortions were found at seven loci representing four linkage groups. Anther culturability studies of 50 of these DHs showed that this characteristic was significantly higher for DH lines having the DH8293 alleles at the three loci of one linkage group and at another locus than those having the Kaskade alleles (Devaux and Zivy 1994). Of the other loci revealing polymorphism between Kaskade and DH8293, but not different from a 1:1 ratio in the F_1 -derived DH progeny, two had a significant effect on anther culturability. One of the first-mentioned two markers and one of the latter two were found to be linked to genes involved in both embryoid production and green plant regeneration, while the other two were linked to genes involved only in green plant regeneration. Three of the four most efficient alleles for anther culturability originated from DH8293, which was actually the best AC responder of the two parents.

Conclusion

Barley DH production has found extensive applications in genetic studies and breeding, resulting in many successful cultivars. Most of the DH-derived cultivars have been produced via the *Hb* method, but very recently the first AC-derived DH cultivars have also appeared. The *Hb* and AC techniques are similar, but with some interesting and complementary characteristics. The AC-derived DH lines show more variability than the *Hb*-derived ones, possibly due to increased DNA methylation and higher recombination frequencies. This may be either an asset or a deficiency, depending on the purposes for which the lines are being developed. The AC-derived DH lines also show more segregation distortion than is observed with the *Hb*-derived lines. This is probably an undesirable characteristic in most cases, but may help to identify genes important for anther culturability in barley. Due to the different cultural conditions employed for the two methods, it is possible to exploit both methods for optimum efficiency and minimum cost at different seasons of the year.

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Genetic transformation of barley – Approaches towards molecular barley breeding

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Summary

Different methods have been developed recently to transform barley (*Hordeum vulgare* L.) by direct gene transfer. These include direct DNA uptake into protoplasts, followed by plant regeneration from selected protoplasts. Bombardment of isolated microspores with DNA coated microprojectiles has resulted in transgenic, homozygous barley plants, and bombardment of immature scutella with subsequent regeneration via somatic embryogenesis has given rise to transgenic plants too. All these procedures have specific advantages as well as disadvantages, so even now alternative approaches are being investigated – e.g., *Agrobacterium* mediated gene transfer, microinjection, or other sophisticated techniques. The major goals with transgenic barley plants are to improve disease resistance, to improve tolerance for abiotic stresses, to modify the quality of the raw material for brewing purposes, to change the composition of the storage products – e.g., starch in the kernel – and, last but not least, to improve the growth characteristics of the plant. Today, direct gene transfer can be considered a reliable tool in plant breeding and a valuable supplement for defined, single gene based genetic modifications of barley.

Direct gene transfer to barley protoplasts

Protoplasts represent a useful system for plant genetic manipulations such as somatic hybridization, cytoplasmic recombination or direct gene transfer. For this, an efficient plant regeneration system from isolated protoplasts is a prerequisite.

The major source for protoplasts of cereal crops are embryogenic cell suspensions (review: Krautwig and Lörz 1995). The establishment of suspension cultures producing a high yield of viable protoplasts is very laborious and strongly

genotype-dependent. Additional problems arise during the long period of culture with a decrease in morphogenic capacity and the occurrence of somaclonal variation. Up to now, there have been only a few reports on efficient and reliable regeneration of fertile barley plants from suspension culture-derived protoplasts. This indicates that plant regeneration from barley protoplasts still remains a problem.

As an alternative, the use of primary callus has been suggested as a source for protoplast isolation. In the case of barley, primary callus can be produced from immature embryos, microspores, or anthers. The obvious advantage of this material lies in the shortened tissue culture period and, subsequently, in a reduced risk of somaclonal variation.

We developed two rapid regeneration systems for protoplasts from primary callus derived from either young scutella or anthers of barley (Stödt et al. 1996). Primary callus of barley (*Hordeum vulgare* L.) derived from scutella (cv. 'Dissa') and anthers (cv. 'Igri') was used for protoplast isolation and plant regeneration. The protoplasts were embedded in agarose and cultured with nurse cells. The plating efficiency varied from 0.1% to 0.7%. Shoots regenerated from the developing callus. Plantlets were transferred to soil and cultivated in the greenhouse three to five months after protoplast isolation. All plants were normal in morphology, and most of them flowered and set seeds.

Gene transfer by direct delivery of DNA into protoplasts has been successful via electroporation or chemical treatment of the cells with polyethylene glycol (PEG). Protoplast transformation followed by regeneration of transgenic plants has so far been mainly performed with protoplasts originating from embryogenic cell suspensions. However, although there are protocols for efficient gene transfer to protoplasts, the applicability of these systems for the production of transgenic barley plants is limited due to the difficulties involved in regenerating large numbers of plants from isolated protoplasts.

Biolistic transformation of multicellular target tissue

As an alternative to protoplast transformation, microprojectile-mediated gene transfer has the potential to overcome these limitations. The essence of microprojectile systems for plant genetic transformation is to use high velocity particles to penetrate cell walls and to introduce DNA into intact cells, thus circumventing the host range limitation of *Agrobacterium* and the problems of plant regeneration from protoplasts. The transfer of DNA into cells and tissues with embryogenic capacity takes place with high efficiency. The choice of appropriate target cells is of major importance because there are few tissues and cells capable of plant regeneration. Using embryogenic suspension cells and embryogenic callus cultures, successful transformation and regeneration of cereals, such as maize, rice, wheat, oat and barley, could be achieved. However, the morphogenetic competence of cells is significantly reduced during long term maintenance, and the phenomenon of somaclonal variation limits the suitability of these cells for transformation.

These limitations could be overcome by directly targeting tissues or cells that can be obtained easily and manipulated *in vitro*. In barley, scutellar tissue of immature embryos (Wan and Lemaux 1994) or microspores (Jähne and Lörz 1994) are suitable primary explants for bombardment. The time necessary for preparation of the target cells is comparatively low, and the risk of somaclonal variation is negligible since the period in *in vitro* culture is reduced to a few weeks. Another advantage of microprojectile bombardment of primary explants is that even genotypes that are recalcitrant in protoplast culture can be transformed successfully.

Microprojectile-mediated transformation of isolated microspores

The most obvious aim of microspore culture is the production of homozygous cereals for breeding purposes. At the moment, anther culture is the most suitable tool for providing dihaploid plants because it is a relatively simple and fast method. Techniques for the culture of isolated microspores are much more complicated, and more experimental facilities are needed (review: Jähne and Lörz 1995). However, microspore-derived tissues are very attractive for applications aiming at transformation since they are unicellular, and transgenic, homozygous plants can be produced in a comparatively short time.

A system for the biolistic transformation of barley using freshly isolated microspores as the target tissue has been developed. Independent transformation events led, on average, to the recovery of 1 plant per 1×10^7 microspores. Regenerants could be transferred to soil after a very short time, about 2 months after bombardment. The transferred genes were inherited in all progeny plants, indicating the homozygous nature of primary regenerants. This is an important advantage of the microspore system because transformed plants that have integrated foreign DNA in coding regions necessary for plant metabolism or development will not be selected. The dihaploid state of the regenerants excludes the recovery of plants carrying lethal mutants. The biolistic transformation of microspores appears to be a very promising approach for the transfer of genes of agronomic interest. However, a prerequisite for this transformation system is the availability of a highly efficient regeneration system. Up to now the culture of isolated microspores provides regenerative cells in high amounts with only a few genotypes of barley.

Molecular barley breeding by direct gene transfer

Improved disease resistance is a major goal in all breeding programs. Molecular barley breeding by direct gene transfer is aiming for this too. Other projects are related to brewing characteristics, the modification of plant substances, or growth characteristics of the plant. Some aspects of our research activities related to barley are summarized in Table 1.

Table 1. Present research projects by AMP II, University of Hamburg, aiming for genetic modification of barley by direct gene transfer.

Virus Resistance ¹	method:	particle bombardment of scutella
	strategy:	cross protection
	genes:	BaYMV (mutagenized) coat protein
Fungal Resistance ²	method:	particle bombardment of microspores
	strategy:	growth inhibition
	genes:	stilben synthase, chitinase, RIP
Malting characteristics ³	method:	particle bombardment of scutella
	strategy:	tissue specific expression
	gene:	heat stable beta-glucanase
Modification of Starch ⁴	method:	particle bombardment of scutella
	strategy:	antisense-expression, over-expression
	genes:	GBSS I, GBSS II, branching enzyme, debranching enzyme
Senescence ⁵	method:	particle bombardment of microspores
	strategy:	antisense-expression
	gene:	rpoA
Gene Isolation	methods:	protoplast transformation, particle bombardment of scutella
	strategy:	gene tagging
	genes:	transposable element system Ac/Ds

In cooperation with: ¹ MPI, Köln; ² AgrEvo, Frankfurt; Bayer AG, Monheim; Saatenunion, Hannover; ³ Dr. O. Olsen, Copenhagen; ⁴ Prof. L. Willmitzer, Berlin; ⁵ Prof. K. Krupinska, Köln.

There are numerous other genes that might be of interest for genetic modification and direct transfer to barley, but these cannot be discussed in this article. As an example, we report in some detail on experiments to genetically modify barley by a heat stable beta-glucanase.

Expression of a heat stable beta-glucanase

Throughout the malting process, a prerequisite for efficient digestion of starch by amylase activity is hydrolysis of beta-glucans of endosperm cell walls by (1-3;1-4)-beta-glucanase (EC 3.2.1.73). The problem of heat-inactivation of endogenous beta-glucanases during the kilning and mashing step in the brewing process can be resolved by adding heat-stable beta-glucanases of fungal and bacterial origin or bioengineered yeast as well as by expression of corresponding genes in the endosperm of barley plants during germination.

A hybrid gene "H(A107-M)" (Borriss et al., 1989), consisting of 107 amino acids (AA) from the N-terminal and 107 AA from the C-terminal coding region of (1-3;1-4)-beta-glucanase from *Bacillus amyloliquefaciens* (AMY) and *Bacillus*

macerans (MAC), respectively, was cloned and analysis of enzyme activity was carried out in *E. coli* (Borriss et al., 1989), yeast (Olsen et al., 1991) and barley aleurone protoplasts (Phillipson et al., 1993). Expression of the gene in *E. coli* resulted in full activity after incubation for 1 hour at 65°C, pH 5.5, and the glycosylated enzyme expressed in yeast showed half-life of 100 minutes at 70°C, pH 6.0. In contrast, endogenous barley (1-3;1-4)-beta-glucanase isoenzyme II loses 50% of activity after incubation for 15 minutes at 52°C.

In our experiments, a construct "pEII-alphaH(A107-M)-N" expressing the described hybrid gene under control of the (1-3;1-4)-beta-glucanase isoenzyme II promoter (Wolf, 1992) was used in co-transformation experiments of barley microspores. For targeting of the heat-stable enzyme to the endosperm, the signal peptide of alpha-amylase 2 from barley (Phillipson et al., 1993) was fused to the N-terminus of the hybrid enzyme. A remarkable observation of this co-transformation experiments was the occurrence of "soft" phenotypes in large numbers of calli and low regeneration frequencies of plants. Anyhow, regeneration of 25 phosphinothricin resistant plants was necessary to produce a single barley plant showing insertion of multiple copies of the hybrid gene in the genome by Southern blot analysis. Most of the copies resulted in the expected fragment size of the promoter-gene fusion after restriction, indicating perfect insertion into the genome. In addition, several smaller and larger fragments appeared that can be discussed as partial insertions, polymerization of several gene copies and cytosine methylation. In Southern blot analyses of 15 progeny plants, hybridization signals corresponding to the parental plant were observed in 8 plants. In 5 plants, segregation of a single integration locus was detected, while one progeny plant showed segregation of another transgene locus. One plant lost the integrated loci, altogether.

The methylation status of the inserted gene copies were characterized by restriction of the genomic DNA of the parental plant with the methylation-sensitive isoschizomers *Hpa* II and *Msp* I. Comparison of the two restriction patterns by Southern blot analysis showed a shift to high molecular weight signals in *Hpa* II digested genomic DNA, indicating methylation of C residues in the CCGG restriction sequence.

The phenotype of the transgenic parental plant was unexpected. Leaves of the plant felt soft to the touch and were oriented to the soil. The potential relationship between the observed phenotype of the plant and expression of the hybrid gene in leaves was characterized by Northern blot analysis. A major signal at around 2 kb and additional, less abundant signals corresponding to larger 3.4 kb and 4.1 kb hybridizing fragments were detected in a coarse mRNA preparation. None of the hybridizing fragments showed the expected size of 1.1 kb. This result can be explained by non-activity of the terminator fused to the hybrid gene, transcription of 3' deleted copies, and rearrangements inside the transgenes. Whether or not the detected transcription of the gene was mediated by the promoter of (1-3;1-4)-beta-glucanase isoenzyme II, or could be traced back to position effects remains subject to debate. Subsequent analysis of (1-3;1-4)-beta-glucanase activity in total protein extracts of leaves from the parental plant and 3 progenies for 30

minutes and 3 hours at 56°C, pH 6.0 (Olsen et al., 1991) pointed out that no heat-stable beta-glucanase activity was detectable. Control experiments under standard conditions (30 minutes and 3 hours at 30°C, pH 4.6) resulted in low (1-3;1-4)-beta-glucanase activity. However, enzyme activity in 3 out of 4 transgenic plants was almost twice as high as activity in protein extracts isolated from a non-transformed control plant. Whether or not this level of beta-glucanase activity reflects differences in the age of leaves used for protein extraction or expression of the hybrid enzyme missing a heat-stable phenotype, remains a subject of discussion. However, expression of beta-glucanase to a greater extent might possibly be responsible for the phenotype observed with the transgenic plants.

For analysis of expression of heat-stable beta-glucanase during germination, grains of the transgenic parental plant and a control plant have been germinated for 1-4 and 6 days. Total protein extracts of 3-5 germinated grains were used in beta-glucanase activity tests under standard (30 minutes at 30°C, pH 4.6) and high temperature (30 minutes at 56°C, pH 6) conditions. Under standard conditions, enzyme activity was detectable from the first day of germination and increased during the following 5 days in the transgenic and the control plant. On the second and third day of germination, grains of the transgenic parental plant expressed 2.5 and 1.5 times the level of beta-glucanase activity, respectively, but the following two points of measurement resulted in lower expression of the transgenic plant, compared to the control plant. No beta-glucanase activity was detectable under high temperature conditions in protein extracts of germinated grains from the transgenic or the control plant. Whether absence of heat-stable beta-glucanase activity in germinating grains of the transgenic barley plant can be put down to the fact that tissue and developmental specific transcription of RNA is inefficient, or the transcribed RNA is incorrect and not translated, remains a subject of debate at this time of investigation. In addition, lack of glycosylation or inefficient glycosylation would probably result in reduced thermostability (Olsen et al., 1991) of the bacterial hybrid enzyme expressed in barley.

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Progress in genetic engineering of oat

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Introduction

Oat (*Avena sativa* L.) was first genetically engineered in 1992 (Somers et al., 1992). This advance in oat genetic manipulations resulted from development of the three major components of all plant transformation systems: i.e., tissue cultures with plant regeneration capacity, DNA delivery methods and selectable marker systems. Oat tissue cultures capable of plant regeneration were improved beyond those of the initial reports (Cummings et al., 1976; Lorz et al., 1976) by the identification of genotypes highly responsive to tissue culture and plant regeneration (Rines and Luke, 1985) and development of methods to select and maintain friable, embryogenic callus (Bregitzer et al., 1989). Microprojectile bombardment was established as a routine method for high frequency DNA delivery into plant cells (Klein et al., 1987). Selectable marker genes were isolated and constructed for use in monocot transformation systems (Thompson et al., 1987; Fromm et al., 1990). The initial genetic engineering system for oats used friable, embryogenic callus initiated from a highly culturable genotype. Friable, embryogenic callus was bombarded with the Biolistics PDS-1000 device to deliver DNA carrying the *bar* gene, which encodes phosphinothricin acetyl transferase as the selectable marker. While few fertile plants were produced using this protocol, those plants that did produce seed transmitted the transgene and transgene phenotype to their progeny, demonstrating stable transformation.

Over the past four years, we have made steady progress in improving the oat genetic engineering system. An immediate requirement for developing a system useful for oat improvement was to replace the *bar* gene, which confers resistance to phosphinothricin-based herbicides, with a selectable marker that does not confer herbicide resistance. Cultivated oat and its wild weedy relatives, *A. sterilis* and *A. fatua*, are sexually compatible. Thus, there is a high probability that transgenes will be transferred between the crop and its wild relatives. Transfer of herbicide resistance from the transgenic crop to wild oat may have adverse ecological consequences in agricultural systems where phosphinothricin-based herbicides are used for weed

control. Likewise, other transgenic traits that may increase the competitiveness of wild oat must be evaluated carefully before introduction into the crop. We developed a system using the *npt II* gene encoding neomycin phosphotransferase in combination with the use of paromomycin, a type of neomycin, for selection of transgenic tissue cultures (Torbert et al., 1995). This selectable marker gene is likely to pose reduced ecological risk compared to herbicide resistance. We also have investigated integration, expression and inheritance of the introduced transgene DNA to gain an understanding of the system in terms of the genetic behavior of fertile, transgenic plants (Pawlowski et al., this meeting). In this paper, the current status of the oat genetic engineering system will be reviewed, highlighting some future directions for continued improvement.

The oat genetic engineering system

Torbert et al. (1995) described the paromomycin selection system for isolation of fertile, transgenic oat plants. Embryogenic callus was initiated from immature embryos of the genotype GAF/Park, which is a F_1 selection of a cross of "Garland" by an *A. fatua* accession crossed to "Park" (Rines and Luke, 1985). This line has been further selected and purified, but nevertheless is of limited agronomic value. Routine procedures for obtaining target tissue were to initiate callus from plated immature embryos over a period of 12 or more weeks, followed by bulking up callus for microprojectile bombardment. Often, callus age approached one year before microprojectile bombardment. Following DNA delivery, paromomycin selection of transgenic callus before plant regeneration may take up to an additional 20 weeks. On average, this system produced 3 transgenic tissue cultures per microprojectile bombardment, of which 25% regenerated fertile plants. This low frequency of fertile plants was presumed to result from the long duration of tissue culture of the immature embryo-derived callus.

The use of immature embryos directly for microprojectile bombardment as described for other cereals is an approach to shorten the length of time in tissue culture. However, in contrast to most other cereals where callus arises from the scutellum forming the primary target tissue, callus formation from immature embryos of oat appears to initiate from the mesocotyl (Bregitzer et al., 1995). This tissue provides an exceedingly small source of totipotent cells per primary explant. Torbert et al. (this proceedings) describe a new approach for callus initiation in oat that increases the frequency of fertile, transgenic plants. Mature embryos are used as the explant, and the period from callus initiation to regeneration of transgenic plants is reduced to 6 months. More than 90% of the transgenic tissue cultures produced fertile plants, while plant regeneration frequency is also improved, thus doubling our production of fertile transgenic plants. Towards further improving the system, we are screening other genotypes for their response in this mature embryo callus system. While the mature embryo culture system probably will not completely alleviate the genotype effects on culture response, improved genotypes may be identified for genetic engineering.

Transgene expression and inheritance

Some of the past problems of the oat genetic engineering system appear to be obviated by development of a safer selectable marker and use of an alternate tissue culture system to improve fertility of transgenic plants. The next major challenge stems from our lack of understanding of and inability to manipulate factors that affect transgene expression. Pawlowski et al. (this meeting) generally observed stable inheritance of transgene DNA sequences with segregation ratios that indicated single gene disomic inheritance. In this study, the transgene phenotype, which is beta-glucuronidase (GUS) activity expressed in mature seed, was observed in 23 out of 27 independent transgenic lines tested, which is similar to reports using this plasmid in other species. GUS expression was observed in progeny of all plants regenerated from the same transgenic GUS-positive tissue cultures. GUS activity was stably transmitted to progeny in about half of the lines evaluated. Homozygous GUS-positive progeny were isolated from most of these lines and were stable for two generations of self-pollination. This observation of stable inheritance is encouraging and indicates that the system will be useful for plant improvement. However, the remaining lines exhibited some level of phenotypic nonconcordance with the transgenic genotype, suggesting problems with transgene expression. In most lines, the majority of transgenic progeny exhibited GUS activity. In some lines, however, several transgenic progeny did not exhibit GUS activity. This behavior was consistent over generations in some transgenic lines, and in other lines varied over generations. The lack of transgene expression in transgenic plants is generally categorized as transgene silencing. Genomic integration position effects and interactions between multiple copies of the transgene or with rearranged integrated transgene copies may be the mechanisms underlying these problems in transgene expression (Finnegan and McElroy, 1994; Flavell, 1994; Matzke and Matzke, 1995). Some method of decreasing the numbers of integrated transgenes, which often vary from 1 to 20 or more, may avert some of these silencing events. There also are reported strategies to reduce position effects and increase transgene expression, such as incorporating matrix attachment regions flanking the transgenes in the transformation construct (Allen et al., 1993).

We currently are investigating expression of reporter genes controlled by three different promoters that function constitutively in vegetative tissues. All the three promoters are derived from plant viruses. GUS expression is monitored in the leaf tissue of regenerated plants. Similar to the previous study, GUS expression was detected in about 50% of transgenic callus tested and their regenerated plants. In contrast to the observation of GUS expression in seed of all plants regenerated from the same tissue culture, there is variation in the leaf GUS expression levels (from none to very high levels of GUS activity) among different plants regenerated from the same tissue culture for some of these transgenic cultures. From a total population of 49 independently selected GUS-positive transgenic tissue cultures from which multiple plants were regenerated, 32 regenerated plants that were uniformly GUS positive. The remaining 17

tissue cultures regenerated plants of which at least one exhibited GUS staining, but usually among the plants regenerated from the same tissue culture there were several GUS-negative individuals. We tested these plants for expression of the selectable NPT II marker gene. All plants tested appeared to be transgenic, based on this test, whether they expressed the reporter gene or not. Whether the plants that were GUS-negative in leaves will express GUS in the seed has not yet been tested, but we are anxiously waiting to find out. Furthermore, the stability of leaf GUS expression will be followed over generations.

The mechanism(s) underlying this type of variegated transgene expression are a matter of speculation and must await further extensive analysis. Perhaps they arise because the promoters for vegetative expression are of viral origin. To test this theory will require production of plants transformed with constitutive promoters of plant origin. A few plant promoters are available, but undertaking this experiment will require about one year to arrive at an answer. Other less tractable possibilities exist. Possibly the problem of variegated expression in leaves relates to extensive DNA methylation that occurs in grass leaves during development and that may greatly reduce the frequency of genomic sites capable of transcription in this tissue. Alternatively, variegation may result from a stochastic event occurring in the host chromatin flanking the transgene integration locus. Regardless of the mechanism(s) that controls these transgene expression behaviors and patterns, the observation that only 65% of the transgenic tissue cultures produced regenerated uniformly transgene positive phenotypes represents a substantial multiplier that further reduces the probability of producing the desired transgenic phenotype.

Conclusions

Enthusiasm about future applications of genetic engineering to oat improvement is warranted. Substantial progress has been achieved in developing an efficient, safer selection system for production of transgenic oats, and coupled with a new tissue culture approach, we are able to produce sufficient numbers of transgenic plants to conduct a reasonable level of experimentation and to initiate oat improvement efforts. The genetic engineering system remains based on tissue culture, which is a drawback because of genotype effects and problems associated with tissue-culture-induced genetic variation. The latter problems can be minimized with proper experimental design of transformation experiments, i.e., production of sufficient transgenic plants to select the desired type. Obviating the genotype specificity of the oat tissue culture system will require development of either a new genotype-independent tissue culture system or a non-tissue culture transformation approach. Without these achievements, the focus on a specific genotype is not overly disadvantageous but will require additional time for backcrossing to introgress genetically engineered traits into cultivars. The main problem currently and probably for some time to come is that there is a critical need for better understanding of mechanisms affecting transgene expression for the potential of oat genetic engineering to be realized at the breeding level.

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Recent research on genotype-environmental interaction

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A review of selected recent literature on the subject of genotype-environmental interaction revealed that: a) there is still considerable variation in the definition and perceived importance of stability, b) certain types of stability seem to be heritable and relatively independent of mean performance, c) some researchers are starting to emphasize the need to distinguish crossover from non-crossover interactions, d) the most popular method of analysis is shifting from joint regression analysis to models with additive main effects and multiplicative interactions (AMMI), e) there is a gradual movement toward clustering genotypes into groups with similar responses, and f) there is little information to explain the biological nature and causes of genotype-environmental interaction.

Stability and adaptability

In a recent review paper, Lin and Binns (1994) suggested that adaptability should refer to that part of a genotype's response that relates to differences according to location, while stability should refer to that part of the response that varies over years. Their rationale was that differences between locations are predictable, while differences between years are not. On the basis of their suggestion, adaptability would refer to response to predictable environmental influences, while stability would refer to response to unpredictable environmental influences.

In a comparison of four measures of stability, Lin and Binns (1994) concluded that only those measures that measure variability over all environments or over seasons within locations show evidence of being heritable. These authors indicated that there is little evidence that stable genotypes have been developed through plant breeding efforts. Assertions that stable genotypes have not been developed raises serious doubt about the value of volumes of print that have appeared in the last 30 years reporting on regression coefficients, deviation variances and related statistics.

Federer and Scully (1993) pointed out that "the concept and definition of stability needs considerably more discussion and quantification." With a pair of simple graphs, they made the point that efforts to select stable genotypes would, by some definitions, lead to a choice of genotypes that would be totally unsuitable for commercial production. They also showed that genotypes with similar variability

over environments may differ immensely in their usefulness in commercial production. Their statement that "the most desirable response type would be denoted as unstable by current concepts of stability" must surely raise the question of whether or not stability by itself is a desirable characteristic of new genotypes.

Additive main effects and multiplicative interactions

In a series of publications starting in 1988 and proceeding up to publication of a book (Gauch, 1992), Hugh Gauch and colleagues developed and popularized analysis of two-way genotype-by-environment tables in terms of additive main effects and multiplicative interactions (AMMI). Rather than describing interactions in a two-way table of additive interactions (with possible summary in terms of variances or regressions), AMMI summarizes the interactions in terms of multiplicative components. Often, the interactions can be adequately summarized as the sum of one, two or three multiplicative components, where each component contains a number of terms equal to the number of genotypes plus the number of environments plus one. With large tables, this may result in a significant reduction in the number of terms to be described. Furthermore, it is possible to visualize those parts of the interactions described by the first two multiplicative components in a biplot diagram.

There is debate about the appropriateness of tests for retaining components in the AMMI model. Cornelius (1993) found that a conservative test used by several researchers resulted in far too many significant components and warned AMMI users about the problem. AMMI users will have to verify that any AMMI computer program they use employs a more realistic test of component significance.

In their review of measures of stability, Lin and Binns (1994) questioned the predictability of interactions described in relation to empirical measures of environmental influence. Similar questions may arise in relation to AMMI analysis. It will remain to be proven whether sufficiently large portions of the interaction effects are due to fixed patterns of response that can be identified and used for predictive purposes. It also remains to be seen whether multiplicative models such as AMMI provide better insight into patterns of interaction than the additive models they replace.

Crossover interactions

Cornelius et al. (1993) referred to several authors who have noted that interactions involving rank changes (crossover interactions) are much more important in crop improvement than are interactions that merely reflect differences in scale. In cases where crossover interactions are absent, there is no need to consider any aspect of genotype-environmental interaction; the genotype with the best mean will be as good as or better than all other genotypes throughout the entire range of environmental influence.

Cornelius et al. (1993) proposed a clustering method that would allow classification of genotypes into groups within which there were no crossover interactions. Within such groups, the genotype with the best mean would be best. Their proposal was based on a shifted multiplicative model of genotype-

environmental interaction. They found that all but six of 41 genotypes of wheat could be grouped into nine clusters, within which there was very little evidence of rank change. These authors noted that the Azzalini-Cox test for presence of rank change is highly conservative in that it fails to detect cases of crossover interaction that should be considered significant.

Nature and causes of interaction

Much of the literature on genotype-environmental interaction consists of various statistical descriptions of two-way tables of data. Federer and Scully (1993) argued that research into the nature of genotype-environmental interaction should require experimental control of the environmental component. Their argument implies that descriptions of genotype-environmental interactions are not very useful unless one knows which elements cause the environmental differentiation. Lin and Binns (1994) also emphasized the importance of determining the underlying biological factors that cause some environments to be similar. They suggested that, unless researchers determine the underlying biological factors, any study of genotype-environmental interaction is incomplete.

Van Eeuwijk (1992) discussed statistical methods for incorporating additional information about environmental variables into analysis of genotype-environment two-way tables. The paper described a series of models that may be used to incorporate both qualitative and quantitative information about genotypes and/or environments. Van Eeuwijk applied multiple regression, factorial regression, AMMI analysis and redundancy analysis to data on nitrate concentration in lettuce. The author was able to relate a significant part of the interaction to differences in day length caused by choice of planting date. Since planting date is highly predictable, predictions of genotype-environmental interactions should be useful.

The same author was involved in a study (van Eeuwijk and Elgersma, 1993) of genotype-environmental interaction for seed yield in ryegrass. In this case, temperature, relative humidity and precipitation were used to characterize environments. A series of three related analyses led to the conclusion that much of the observed interaction was due to temperature during critical stages of crop development. The authors did not discuss the possibility that conclusions might be different if the experiment were repeated with a different pattern of precipitation-temperature relationships. Nor did they discuss how predictable temperature patterns and hence interactions would be.

Cluster, superiority and reliability analyses

Rather than use parametric statistics to describe genotype-environmental interactions effects, several authors have proposed techniques that might be more applicable to crop improvement programs. Various types of cluster analysis (e.g., Cornelius et al., 1993) can be used to put genotypes into groups that have similar patterns of response to environmental stress. Within groups, selection could then be based on average performance.

Lin and Binns (1994) reviewed earlier work and proposed use of a superiority

measure to evaluate potential new cultivars. Performance of a potential cultivar is compared to the best performance observed in each trial. The superiority measure is calculated as the average of the squares of differences between performance of the test cultivar and maximum performance in each environment.

Reliability analysis (Eskridge et al., 1993) is similar in principle to the superiority measure of Lin and Binns (1994). Basically, Eskridge et al. (1993) proposed that each cultivar be compared to a defined check cultivar in at least 15 different environments. Test-check differences could then be analyzed and probabilities assigned to differences of various size. If the probabilities of positive differences exceed those of negative differences, the test cultivar would be judged superior to the check. A graphic representation of probabilities for all possible differences is known as a reliability function. Graphs of reliability functions can be used to compare different test cultivars and to detect instances of crossover interaction. The authors showed how reliability analysis is related to various types of stability measures. One of the advantages of this type of analysis is that the concept of probability of outperforming a check cultivar is close to what a producer would perceive as important.

Genotype-environmental interactions: Fixed or random?

In their review, Lin and Binns (1994) pointed out that early analyses of genotype-environmental interaction considered environmental and interaction effects to be random. Information about the sizes of variances of the different types of interaction could be used as a guide for estimating the optimum number of years, locations and replications for future crop improvement work. When interaction effects, particularly genotype-location interactions, are considered fixed, the question becomes one of which locations to test in the future rather than how many.

Much of the literature on the analysis of genotype-environmental interaction fails to clearly indicate whether or not the methodology assumes that interaction effects are fixed or random. There is an almost complete absence of discussion of the implications of unspecified assumptions concerning the interaction effects. From a statistical perspective, the distinction between fixed and random effects is based on whether or not the experiment contains all possible levels of a factor or only a representative sample of all possible levels. In the former case (fixed), inferences are restricted to the levels that were actually used in the experiment. Any inference of future performance must involve one of the levels that have already been assessed. In contrast, with a random factor, only a sample of possible levels are evaluated, and future experiments may experience a level that has yet to be investigated. With random effects, statistical analysis should be limited to estimating variances within the population from which the levels were sampled.

From an applied point of view, it seems useful to consider all factors to be random unless it is possible, and appropriate, to repeat levels at some future time or in some other place. Most plant breeders would consider years and their interactions to be random. There is considerable debate about how locations should be viewed. Conceptually, that portion of the location effect comprised of

the long-term average is predictable by choosing the same location at some future time. In crop improvement, however, one usually makes inferences about future performance at many locations. In this context, it seems most reasonable to consider location effects and their interactions with genotypes as random samples from larger populations.

A large amount of the literature on genotype-environmental interaction describes analyses that implicitly treat at least a large proportion of interactions as fixed effects. These analyses include AMMI (Gauch, 1992), shifted multiplicative models, crossover analysis (Cornelius et al., 1993) and others. If interactions are really random, it is not possible to predict when and where observed interactions will occur in the future. Descriptions of the observed data are useful only if they lead to some biological insight into the nature and causes of the observed interaction. In many cases, they do not!

Conclusions

Superiority measures and reliability analyses represent a major change in the approach to dealing with the crop improvement problems resulting from genotype-environmental interaction. This new approach seems to be relatively insensitive to any debate about whether interaction effects should be viewed as fixed or random. New statistical methods for analyzing two-way genotype-environment tables of data are based largely on the assumption that interaction effects are fixed. Will they be any more fruitful than methods that have been reported on extensively in the past 30 years?

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Recurrent selection in autogamous crops – Past, current and future

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My assignment today is recurrent selection in self-pollinated crops. I will discuss 1) past efforts, 2) several current efforts, some of which address primarily related topics, 3) associated correlated responses generally, 4) in a preliminary way, two ongoing efforts in our group to utilize molecular markers to analyze two quantitative traits, and 5) how these efforts using QTLs to genetically dissect complex traits may provide us with information about the validity of our assumptions.

Because of space limitations, the coverage of all topics will necessarily be condensed. I begin by listing a number of pertinent issues: 1) number of traits to be selected, 2) management of correlated but unselected traits, 3) creation and development of initial population, 4) method of progeny evaluation, including generation of testing, and 5) intermating procedures, including whether a closed or an open system is used and for how long. Certain genetic parameters such as linkage, epistasis, effective population size, and effect of gene size *per se*, as well as possible interactions among them, will also instruct choices about specific aspects of procedures to be employed.

The record of previous efforts on recurrent selection in self-pollinated crops has been covered quite well by Carver and Bruns in a review paper given at the 10th Australian Plant Breeding Conference in April, 1993. Further, I reviewed most of our Minnesota efforts on oats at the Second South American Oat Workers Conference in November, 1993. Thus, here the primary emphasis will be on results obtained since those two reviews were written. Tables 1 and 2, which are modified and updated from those in Carver and Bruns, contain a summary, across different crops and specific selection protocols, of the response to multiple cycles of selection for yield and related traits (Table 1) and for quality traits (Table 2). Carver and Bruns concluded, "A review of genetic gains (ΔG) reported for grain yield and related traits since 1985 indicates that recurrent selection has been equally, if not more, effective than traditional breeding methods."

While most past efforts involve evaluation of early generation progeny, which allows for a shorter cycle time, two efforts have quite successfully evaluated advanced generation, almost homozygous progeny. One of these, Beaver and

Table 1. Genetic gains in grain yield and related characters from two or more cycles of recurrent selection in autogamous crops (abbreviated from Carver and Bruns, 10th Australian Plant Breeding Conference, April, 1993).

<u>Crop</u>	<u>Trait selected</u>	Family unit <u>selected</u> ^A	Last cycle <u>completed</u>	<u>Genetic gain %</u> ^B		<u>Reference</u>
				per cycle	per year	
Barley	grain yld	S_0 sp + S_1	2	14.7	4.9	Marocco et al. (1992)
	spike wt	S_0 sp + S_1	2	7.8–11.5	2.6–3.8	Parlevliet & van Ommeren (1988)
Oat	grain yld	S_1	3	5.4	5.4	Frey et al. (1988)
	grain yld	$S_{3,5}$	5	7.9	2.6	Pomeranke & Stuthman (1992)
Peanut	seed yld	$S_{1,3}$	3	3.2	1.2	Monteverde-Penso & Wynne (1988)
Soybean	seed yld	S_1	4	2.0–4.2	1.0–2.1	Burton et al. (1990)
	seed yld	S_1	5	5.4	2.7	Rose et al. (1992)

^A sp = single plant; HS = half-sib family.

^B Genetic gain expressed as % of base population (C_0) mean. Where multiple populations were evaluated, the range in genetic gain is reported.

Table 2. Genetic gains in quality characters from three or more cycles of recurrent selection in autogamous crops (abbreviated from Carver and Bruns, 10th Australian Plant Breeding Conference, April, 1993).

<u>Crop</u>	<u>Trait selected</u>	Family unit <u>selected</u> ^A	Last cycle <u>completed</u>	<u>Genetic gain %</u> ^B		<u>Reference</u>
				per cycle	per year	
Oat	protein yield	S_1	3	4.3–5.5	4.3–5.5	McFerson & Frey (1991)
	oil content	S_0 sp	6	7.2	7.2	Schipper & Frey (1991)
	test wgt. & grain yield	S_1	3	1.7	1.7	Klein et al. (1993)
Soybean	oleic acid	S_0 sp	3	5.1	5.4	Carver et al. (1986)
Tobacco	total alkaloids	FS	3	2.5	1.3	Matzinger et al. (1989)

^A sp = single plant; HS = half-sib family; FS = full-sib family.

^B Genetic gain expressed as % of base population (C_0) mean. Where multiple populations were evaluated, the range in genetic gain is reported.

Kelly (1994), used recurrent selection on dry beans (*Phaseolus vulgaris* L.) to develop indeterminate red-seeded beans that yielded more than determinate types and had larger seed size. Selecting $F_{2.5}$ material produced much better results than selecting among F_2 plants. The particular combination of all desirable traits did not exist in any of the original parents, and the authors attribute the better results of $F_{2.5}$ selection to greater genetic stability in the generation of selection.

A second exception is the Minnesota effort on oat grain yield. We have used advanced line selection because of the initial indication of relatively large amounts of epistasis. Because the A x A portion of the epistasis can be fixed in advanced generations, we delayed selection to take advantage of it and have made excellent progress per unit of time in spite of the three-year cycle time.

While most recurrent selection efforts require at least one calendar year to complete a cycle of selection, Rooney et al. (1995) reported on a rapid cycle procedure to reduce days-to-flowering in oats in which at least three cycles could be completed in a single year. They practiced selection on S_0 progeny before anthesis and intermated only early flowering plants that were not closely related. Unfortunately, with the increased recombination (maximum possible), aneuploids arising from meiotic failures because of translocation heterozygotes forced us to conclude that at least one generation of selfing between intermating will be necessary to prevent aneuploid formation.

One topic not included in the Carver and Bruns review is recurrent selection for partial disease resistance. In 1988, Parlevliet and van Ommeren summarized their successful efforts to increase the level of partial resistance to barley leaf rust and powdery mildew by recurrently selecting against susceptible types. They were especially successful when no effective major resistant genes were present to obscure partial resistance genes.

The next general topic we want to discuss is that of correlated responses. In summary, the record regarding unwanted correlated responses is very mixed. Most likely, the method of original population creation, whether or not random mating was done before selection, population size, and testing generation all contribute to presence or absence of correlated responses. For now, the issue can be divided into trying to prevent correlated responses and trying to fix them if they occur. The likelihood of unwanted correlated responses is probably directly connected to the amount and nature of repulsion linkages when selection is initiated. There does seem to be some benefit of random mating during population development; however, its benefit has not been demonstrated empirically. If repulsion linkages are present, selection in early generation will probably produce less unwanted correlated responses than advanced generation selection.

The long-term recurrent selection program for grain yield in oat at the University of Minnesota, currently in the seventh cycle, has achieved substantial gains on the primary trait; however, unacceptable increases for plant height and maturity date have been observed (Payne et al., 1986; Pomeranke and Stuthman, 1992). Burton et al. (1990) conducted single trait recurrent selection for grain yield in two soybean populations that segregated for genetic male sterility. Four cycles of selection were effective in increasing the mean grain yield of one of the

populations, while no response was observed in the other. The main unwanted correlated response observed in this study was a significant increase in plant height in the responsive population.

Unwanted correlated response, though common, has not been observed in all recurrent selection programs reported in the literature. Klein et al. (1993) have successfully applied recurrent selection for grain yield and test weight in oat, with no detectable changes observed in plant height and maturity among the best yielding C_3 lines. Rose et al. (1992) conducted successful selection for grain yield in soybeans without negative correlated responses. Similarly, no unwanted correlated responses were observed by Marocco et al. (1992) after two cycles of recurrent selection to increase grain yield in barley.

Negative associations among breeding traits can slow breeding progress regardless of the selection methodology. They are especially critical when closed cycle recurrent selection is being used. Some of the causes for their unfavorable associations are: genetic linkage, pleiotropy, genetic drift, and physiological alteration of the level of traits by the environment, which cause compensatory changes among associated sets of traits (Yan and Wallace, 1995). Negative associations due to genetic linkage can be broken. Random intermating before selection for quantitative traits in self-pollinated crops has been proposed as a way to increase the probability of obtaining useful recombinants (Jensen, 1970). In the previous examples where unwanted correlated responses were not observed, one or more cycles of *random mating* were usually carried out before selection was initiated. Only one cycle of recombination was done once selection was begun. Altman and Busch (1984) and Piper and Fehr (1987), however, concluded that random intermating prior to selection was not a cost effective way to increase gain from selection.

"*Pre-selection*" for adequate levels of traits correlated with the trait of interest may prevent unwanted correlated response. Branson and Frey (1989) carried out two generations of selection for plant height, maturity, plant type, and groat oil content in an oat gene pool obtained by mating interspecific F_1 plants (*A. sativa* x *A. sterilis*) to a group of locally adapted cultivars prior to recurrent selection for groat oil content. An evaluation of the first three cycles of selection detected an increased groat oil content, and some of the high oil oat lines were equivalent to check cultivars for all agronomic traits, suggesting that selection for groat oil content had little effect on those traits.

Seed yield and seed protein content have been found to be negatively correlated in wheat (Davis et al., 1961) and soybean (Shorter et al., 1977), so an increase in one often leads to a decrease in the other. McNeal et al. (1978) and Delzer et al. (1995) increased grain protein percentage in spring wheat with recurrent selection; however, grain yield was also decreased, and consequently the total protein produced per unit area was the same or less than the initial population. *Multiple trait selection* may avoid or minimize such correlated responses. Holbrook et al. (1989) used a restricted index for increased yield while holding seed protein constant in soybean. Two cycles of index selection resulted in measurable increases in yield and total protein, but none in protein concentration. In addition

to maintaining protein content, oil content also did not change. Klein et al. (1993) used multiple trait selection to simultaneously improve grain yield and test weight. Additionally, a restriction was applied so that the means for heading date and plant height of selected lines did not exceed the respective means for the cycle populations from which they were chosen. Marocco et al. (1992) conducted recurrent selection in barley for higher grain yield, but also employed a multiplicative selection index that included lodging resistance, plant height, heading date, and kernel weight, among other agronomic traits. Restriction on plant height and maturity date to the population means was also applied by McFerson and Frey (1992) in selecting for protein yield in oats. In most of these examples where a form of multiple trait selection was applied, unwanted correlated responses were minimal or nonexistent.

Secondary selection for plant height and heading date by alternative parental selection was conducted by Haugerud (1992) on the recurrent selection oat population at the University of Minnesota, with the aim of correcting some of the undesirable correlated response observed while maintaining the yield gains already obtained. This strategy, however, was not effective in obtaining the desired corrections.

A more strict approach to correct the unwanted correlated response was the "*opening*" of the *gene pool* by the introgression of elite germplasm with adequate levels of modified agronomic traits. The cultivars Starter and Ogle were chosen since they are shorter and earlier than the C4 parents. Pomeranke (1990) compared the opened and closed systems for this population and found improvement for the agronomic characteristics of the progeny over those from the closed system. He also observed that the introgression of germplasm did not change grain yield. Further selection efforts in this program include the generation of three subpopulations and the use of multiple trait selection within each subpopulation to achieve additional correction for heading date and plant height, and to increase the grain yield potential of the populations (Dolan, 1994; Sosa and Stuthman, 1995). The initial results are encouraging.

Another topic of interest is a better understanding of the genetic basis for the gains that have been achieved. The analyses that have been done to date are almost totally statistically based because that methodology is about the only approach available. For the mathematics involved to be manageable, it has meant that simplifying assumptions regarding gene effect size, linkage and epistasis had to be made and essentially equal/average values had to be assumed. In some cases, such as the assumption of equal sized gene effects, we know the assumption is not valid. However, analysis without this assumption would be difficult or impossible. With the advent of QTL technology, we have tools to follow individual chromosome regions rather than just deal with averages to elucidate the effect of the invalidity of certain simplifying assumptions. A poster by De Koeper and myself at this meeting describes our preliminary efforts at identifying QTLs for oat grain yield using parents from our recurrent selection program. We argued that any molecular markers increasing in gene frequency in a somewhat parallel fashion to our yield gains over cycles are excellent candidates for yield QTLs. In summary, we have

identified six regions associated with different markers that appear to be QTLs for grain yields. Please check the poster for details. As we verify that we actually have QTLs for grain yield we will be able to follow individual regions from one generation to the next.

In another and related effort, we are developing near-isolines of molecular markers that are associated with a compositional trait. A direct comparison between pairs of such "isolines" will begin to answer the question of differential sizes of gene effects. Evidence of clustering of loci affecting individual traits may provide valuable information about linkage of QTL. The investigation of potential connection between linkage of regions and putative epistatic expressions involving linked regions is really not possible with current methodology. Continued development of QTLs could provide improved procedures to evaluate current commonly made simplifying assumptions and to measure the impact of invalid assumptions. Results from QTL analysis would provide guidance on which generation would be most useful for evaluating random mating, on which population size would be most effective in estimating family means, and on how to prevent genetic drift.

As for the future of plant breeding, the linkage between biotechnology and quantitative genetics may well allow plant breeders to reconsider current standard selection practices generally. As a minimum, such linkage offers the possibility of verifying the heretofore almost unidimensional approach to analyzing the genetics of quantitative multiple loci traits.

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European oat breeding now and in the next century

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Introduction

The main European oat-producing countries are shown in Table 1. The order roughly parallels that for population size. Countries producing noticeably less oats than expected are the United Kingdom, France, Spain and Italy, and those producing more oats than expected are Finland and Sweden. Production has been fairly stable since 1979–81 in the former Soviet states, Finland, the United Kingdom and, to a lesser extent, Sweden, but has fallen significantly in France, Germany and Poland. It remains a major cereal in relation to wheat and barley in Finland and Sweden, of intermediate importance in Russia, Belarus and Poland, and of lesser importance (at a level of about 10% of the wheat crop) in Germany and Ukraine. In total, Europe produces a quarter of the world's oats, more than that produced in North America.

Table 1. Oat production in Europe (top ten countries in descending order) and elsewhere ('000 tonnes).

	1979–81	1992	1993	1994
Russian Fed.	–	11241	11556	10748
Germany	3347	1314	1731	1748
Ukraine	–	1246	1479	1385
Poland	2387	1229	1493	1243
Finland	1183	998	1202	1150
Sweden	1635	807	1295	1024
Belarus	–	723	871	752
France	1850	694	726	685
UK	587	502	477	605
Romania	57	508	554	494
USSR	12812	–	–	–
Europe	14407	8382	10030	9712
North America	10289	7133	6600	7085
World	40958	33933	35647	33735

Source: FAO Yearbook Production 1994, publ. Rome 1995.

Most oats are used for on-farm animal production in their country of origin. Human consumption is also important in the UK (41% of total production in 1993/94) and Germany (9% of total production), but is virtually nil in France. Sweden and Finland have in the past exported up to 30% of their oats for milling to the United States and Europe, but this has been dependant on export subsidies, which may no longer be as generous following these countries' accession to the EU. There is no intervention support for oats in the EU but the crop is protected by import tariffs and eligible for area aid. Naked oats are grown in several European countries, but are still in their infancy. Lacking major trading outlets, oat production and breeding is on a much reduced scale compared to that for wheat, barley, maize, oilseed-rape and other crops. Accordingly, the investment in breeding and research in the crop is relatively low. Most has been carried out by private breeders, and recently many of them (e.g., Cebeco in Holland and Lochow-Petkus in Germany), have ceased breeding oats.

Fortunately, the view in the UK has been that, while the major crops are able to generate enough royalties to sustain large private breeding programmes, the breeding of certain minor crops with high potential, such as oats, can only be fully supported with the aid of public funds. In the case of oats, this involves a partnership between the Ministry of Agriculture Fisheries and Food (MAFF) that funds the strategic research, farmers' levies channelled through the Home-Grown Cereals Authority (HGCA) that exploits partly developed material and the seed industry (Semundo) that funds the official testing and seed production of lines.

The genetic base of the European oat crop

Oats arose as a secondary domesticate of wheat and barley crops in the Near East, and were taken to northern Europe during the Neolithic agricultural revolution. The Romans regarded them as a food for horses and northern barbarians.

Most of the spring oats now grown in Europe trace back to Probsteir, an old German land race, from which the Swedish breeder Hjulmar Nilsson derived Victory (released in 1908). Victory was exclusively used throughout Europe and was one of the ancestral genetic sources of the North American gene pool. Popular varieties that contain significant portions of their genes from Victory have been extensively used in breeding programmes throughout the continent.

Studies of breeding progress are of interest. An analysis of published results indicates that annual rates of varietal improvement averaged 0.34% (range 0.19–0.52%) in the UK, Finland and Sweden (Lawes, 1977; Rekenen, 1988; MacKay, 1993) compared to 0.58% (0–0.93%) in the United States (Rodgers, Murphy and Frey, 1983; Wych and Stuthman, 1983; Lynch and Frey, 1993). Periods of stagnation and rapid progress are apparent in both continents. The evidence suggests that it is easier to breed for unfavourable rather than favourable environments.

Limited isozyme analyses of North American and European lines undertaken

at IGER (Surek, 1994) have confirmed my visual impression obtained from visiting a number of North American breeding programmes in 1987 that there appears to be greater variation in North American than European programmes. An extensive study of the genetic diversity and genetic relationship within the European oat gene pool, similar to those of Souza and Sorrells (1989) and others in the United States, would be justified. This could be extremely useful information for the selection of parental combinations that optimize genetic variances in a breeding programme, and for identifying lines with sufficient levels of polymorphisms to identify crosses for use in genome mapping and marker-assisted selection.

The potential for genetic change

Spring oats are the main form of the crop in Europe, but winter-sown oats are important in the UK (79% of total) and France, and are a small proportion of the German, Austrian and Swiss crops. Non-winterhardy forms of winter oats are grown in Italy.

An expansion of winter oats within Europe could be expected to have a major effect on the profitability of the oat crop. The source of winter-hardiness in European winter oats is almost wholly Grey Winter, apparently synonymous with Winter Turf in the US. In the US, hybridization with Red Rustproof, an *Avena byzantina* variety, and its derivatives has resulted in the release of hardier varieties. As a result, winter oats can be grown as far north as Ohio and Pennsylvania, areas with generally lower winter temperatures than, for instance, Germany (Marshall 1992). The Uniform Winter Hardiness Oat Nursery, initiated in 1926 in the US, has been a valuable tool for assessing gains. Thus, considerable scope must exist for improving winter-hardiness in Europe. Since 1980, some progress has been made at IGER in combining high yields and winter-hardiness based on Kentucky and Pennsylvania lines, using field tests, cold tolerance tests and assessment in upland sites and in Pennsylvania and Sweden.

What is urgently required is a concerted European effort to produce very hardy winter oats, involving screening genetic sources and segregating populations across the continent. This could include Eastern European countries where there have been expressions of interest in winter oats. As a difficult-to-measure characteristic, winter-hardiness is an ideal candidate for marker-assisted selection based on molecular/comparative mapping. Another approach is the use of transformation of available gene constructs associated with cold acclimation and freezing resistance from model species (monocots, dicots or bacteria). At least one cold regulated (COR) gene, BLT4, which appears to code for plant non-specific lipid transfer protein (Hughes et al., 1992) has been shown to be present in oats. This could be relevant to the changes in membrane lipid quantity and composition that occur during acclimation of rye but are less marked in oats (Uemura and Steponkus, 1992). The enzyme superoxide dimutase (SOD), which reduces oxidative stress, may be of significance in resistance to chilling and freezing.

An alternative approach is to concentrate effort on producing higher yielding, earlier maturing spring oats. We are using recurrent selection to accumulate genes from North America and Finland. By inter-crossing the products of the first cycle of selection, we have obtained considerable transgressive segregation for early maturity.

We also consider naked oats to be a major way forward for the oat crop in Europe. They currently occupy about 4% of the crop in the UK. A new winter naked oat, Krypton, has been released that is 13–19% higher yielding than the widely grown variety Kynon. This will allow further penetration of specialized markets and could be a significant step towards wider use of the crop in general livestock and human markets. Since there is no need for husk removal, naked oats have significant potential as a raw material for industrial use. Just as there are special varieties of barley for malting, so breeders are able to develop varieties that have high levels of valuable fractions.

While individual countries have their own specific problems, crown rust is a major disease throughout Europe. According to the results of the Annual Report of the European Oat Disease Nursery, no single line – not even PC68 – is resistant throughout the continent. Powdery mildew is a damaging disease in the United Kingdom and elsewhere. Other diseases include BYDV, Septoria leaf blight, Helminthosporium leaf blotch and stem rust. Cereal cyst nematode resistance is sought in Sweden. Earliness is required to escape drought in Finland and wet weather in Scotland! Aluminium toxic soils are a problem in Hungary. The list is endless!

What about climate change? It is now projected that annual-average warming in Europe will be 0.7 to 1.3°C by the 2030s and 1.9 to 2.5°C by the 2060s. The conventional wisdom, though not universally accepted, is that southern Europe will get drier and northern Europe wetter (Downing, 1995). Although no work has been done on oats, it is likely that the crop will benefit, in a way similar to other crops (Kenny and Harrison, 1993), from the interaction of CO₂ and higher temperatures in northern Europe. Doubtless, breeders will be faced with disease and other problems but will be able to handle them in the time scale involved.

New technologies

Most oat breeding is carried out using classical breeding methods, particularly the pedigree method. Recurrent selection schemes may become more widely used as a means of concentrating genes from many sources to provide parental material. It can be viewed as “population improvement,” a step sadly missing in many breeding programmes. Increasingly, one hopes that biotechnology will provide breeders with powerful additional tools.

Since oat breeding receives less investment than wheat and barley, it is inevitable that new biotechnological techniques have been hardly applied to oats in Europe. Logically, one could envisage that the science is developed in model species, transferred to major crop species and then applied to less important crops. The trouble with this scenario is that many sources of funding, particularly

at the basic end, rank innovative quality science as a prerequisite for project funding. While each crop presents its own technical challenges, the difficulty of transferring and using technology developed for other crops will, at best, result in a lag phase for the application of techniques to oats; at worst, the vicious circle will perpetuate itself.

Let us assume, however, that the crop will not be neglected in the future. Markers, maps and transformation have been considered in some depth elsewhere in the conference. *In situ* hybridization is an additional technology that will assist the achievement of a better understanding of the nature and determining mechanisms of genome structure and evolution in *Avena*. Structural rearrangements of the chromosomes, in particular translocations, duplications and deletions, add considerable complexity to the organization of the genome in cultivated hexaploid oats. This is a sharp contrast to hexaploid wheat, where differences between progenitor species tend to be genic rather than structural. Such rearrangements are a possible cause of reduced recombination between lines from different parts of the gene pool, and have hindered the isolation of a complete monosomic series and the construction of a complete molecular map in which linkage groups can be associated with individual chromosomes or genomes.

One of the differences between conventional breeding and mapping approaches is that breeders use many crosses, whereas mapping studies are usually conducted on a single, highly polymorphic cross. In this context, why do most crosses fail to produce results and will the same apply to crosses in which we have detailed information on molecular markers to assist selection? Answers to these questions might illuminate our approach to breeding in the next century.

As far as I know, IGER at Aberystwyth is the only laboratory in Europe developing a robust method of oat transformation. (Ten transformed plants of the variety Melys have been regenerated to date.) Worthwhile targets are increased freezing resistance and enhanced disease resistance above the levels present in the current gene pool; modifying carbon partitioning in the seed to produce oat varieties accumulating high levels of oil, glycolipids, beta-glucans or starch; and modifying the expression of the naked oat gene, for instance, by separating naked oat expression from the multiflorous habit (see poster by Ougham, Latipova and Valentine in this conference). Meeting some or all of these targets will require identifying and isolating the pathways and genes involved, although in some instances it will be possible to exploit syntenic relationships within the Gramineae for both mapping and transformation work.

The distant future

As recently as 1955, Sir George Thomson, Nobel Physicist, in his book *The Foreseeable Future*, predicted that a proportion of factory operations would be carried out by trained monkeys! Knowing that ionizing mutations cause genetic changes but nothing about gene structure, he predicted immense possibilities in breeding using controlled mutations. He missed molecular biology completely. My point is not to denigrate Thomson's book, which is in fact quite stimulating,

but to repeat one of his own premises. Prediction is guesswork, particularly where it involves discoveries that now appear to be major impossibilities. Therefore, when I predict that by the year 2050 keyboard-based molecular biology kits will be available from the local mall and that, by the year 2090, we will have bred the Perfect Oat, I have the nagging feeling that I will be proved *completely wrong!*

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Breeding for low-input environments

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Summary

The development of methodologies to produce suitable germplasm for difficult, high risk and low-input conditions has high priority in ICARDA's barley breeding program.

Interactions between genotype and environment (GxE) are one of the main reasons for the failure of formal breeding to serve small, resource-poor farmers. Therefore, ICARDA has decentralized its breeding activities to exploit specific adaptation and encourage national programs to use their locally adapted germplasm. To exploit further specific adaptation, farmers are involved in the early stages of selection so as to take full advantage of their knowledge of the crop and the environment.

Farmers' participation in selection will speed up the transfer and adoption of new varieties without involving the complex, bureaucratic and often inefficient mechanisms of variety release, seed certification and production, and extension activities.

Introduction

Formal, or institutional, breeding has been highly efficient in improving yield levels of several crops in favorable environments, or in environments that could be made favorable by using inputs. Resource-poor farmers, who practise approximately 60% of global agriculture and produce 15–20% of the world's food (Francis, 1986), have not known the benefits of the green revolution. Some 1.4 billion people remain dependent on agriculture practised in stressful environments (Pimbert, 1994).

In many areas of developing countries, barley is a typical crop for difficult environments either because of climate stresses, low soil fertility, or both. Where barley is the only possible crop, often there is a high risk of crop failures, and therefore there is little or no use of external inputs.

To generate useful germplasm for this type of condition, we have challenged a number of concepts that characterize conventional plant breeding programs: (1) the genetic uniformity of cultivars (pure lines, clones, hybrids), (2) the use of

optimal conditions to conduct selection, (3) the emphasis on selection for grain yield and disease resistance, (4) the importance of wide (in a geographical sense) adaptation, and (5) the disregard for the clients (the farmers), who are only involved in the final field testing of a few promising lines.

Some of the assumptions behind those concepts are that (1) selection must be conducted under favorable growing conditions where heritability is higher, and therefore response to selection is also higher, (2) yield increases can best be obtained through replacement of locally adapted landraces that are low yielding and susceptible to disease, (3) breeders know better than farmers the characteristics of a successful cultivar, and (4) when farmers do not adopt improved cultivars it is because of ineffective extension and/or inefficient seed production capabilities – the hypothesis that the breeder might have bred the wrong varieties is rarely considered.

In the last few years there has been mounting evidence that these assumptions are not universally valid, and that the special problems of lower-potential environments and their farming systems must be addressed by plant breeders in a different way.

Interactions between genotype and environment

Interactions between genotype and environment (GxE) are one of the major factors limiting response to selection and, hence, the efficiency of breeding programs. GxE interactions become important when the rank of genotypes changes in different environments (crossover GxE interaction).

Examples of GxE interactions of a crossover type have been found in a range of crops and environments: e.g., cocksfoot, maize, sugarcane, chickpea, barley (see Figure 1), wheat and pearl millet (Ceccarelli, 1994).

In Figure 1 the lines selected for high yield in favorable conditions (YP) yield more than the lines selected for high yield in less favorable conditions (YD) in all medium- to high-yielding sites. However, they yield considerably less in the lowest-yielding sites, which represent the real conditions where most barley is grown. Therefore, selection in high-yielding sites, such as the experiment stations, does not allow the identification of the best genotypes for poorer conditions and promotes genotypes that are in fact inferior under stressful conditions.

Breeding for environments below the crossover point must be based on direct selection in the target environments (Simmonds, 1991). Selection in unfavorable environments is difficult, and is further complicated by year-to-year variations in the amount and distribution of rainfall, and in temperature. To overcome such complicating factors, more precise experimental designs and field plot techniques are required.

Direct selection in unfavorable environments has revealed that locally adapted landraces can be a useful source of breeding material that would have been eliminated had the evaluation taken place only in high-yield environments, and that the exploitation of the diversity within barley landraces is a powerful means to improve barley yields in marginal environments (Ceccarelli et al., 1995). Arta,

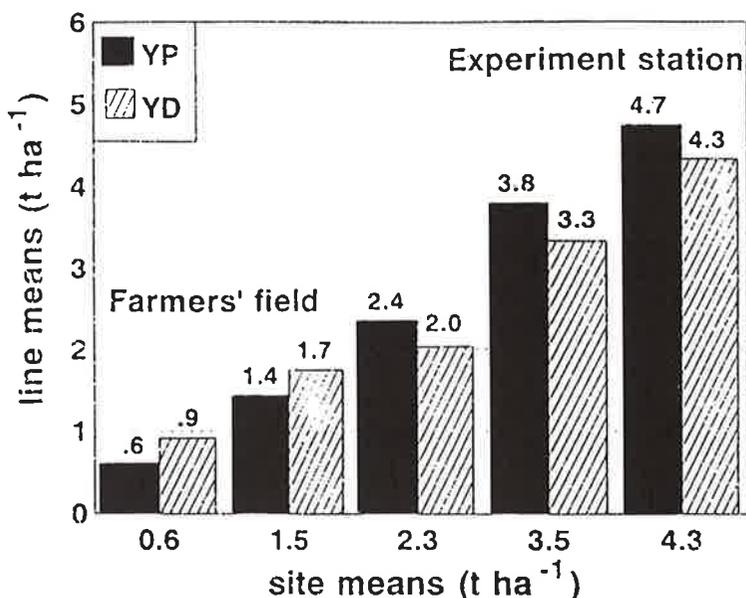


Figure 1. Cross-over type of GxE interaction: YP and YD are lines selected in high- and low-yielding locations, respectively, and then tested in a set of environments ranging from low-yielding farmers' fields to high-yielding experiment stations.

Tadmor and Zanbaka, three barley cultivars developed from Syrian landraces and adopted by Syrian farmers (Table 1), provide a strong indication that (a) it is indeed possible to make progress with selection under unfavorable conditions, and (b) that a large amount of potential improvement in unfavorable environments is missed by breeding programs using only selection in favorable conditions and neglecting the locally adapted germplasm. However, how can the mandate of an international breeding program be reconciled with the importance of specific adaptation?

Table 1. Average grain yield (kg ha⁻¹) and increase over local landraces of three barley lines obtained through pure-line selection from the two landraces commonly grown in Syria. The data are from on-farm trials.

Line	No. of environments ^a	Grain yield	% increase
Arta	51	2433	22.3
Tadmor	11	780	23.6
Zanbaka	8	945	22.4

^a location-year combinations

Decentralization: Using specific adaptation in international breeding programs

The interaction between international and national plant breeding programs has been largely a one-way process where international programs distribute their germplasm as "international nurseries," and national programs test and eventually release selections as varieties. The process has excluded the use of locally adapted germplasm, which often performs poorly in favorable conditions or experiment stations, and has in fact encouraged its displacement.

An international breeding program can exploit specific adaptation by decentralizing the selection work to national programs, i.e., by replacing the traditional international nurseries with segregating material. Early distribution of breeding material reduces the danger of useful lines being discarded because of their relatively poor performance at some test sites.

Figure 2 shows schematically the decentralized barley breeding in five countries of North Africa (Ceccarelli et al., 1994). National barley breeders are full partners in the program by providing parental material for each cycle of crosses. A similar program is being used in the case of barley breeding for the Anatolian plateau in Turkey.

However, decentralization to national programs will not respond to the needs of resource-poor farmers if it is only a decentralization from one experiment station to another. To exploit fully the potential gains from specific adaptation, selection has to be practised by farmers under their own conditions. This may be viewed as an example of extreme decentralization.

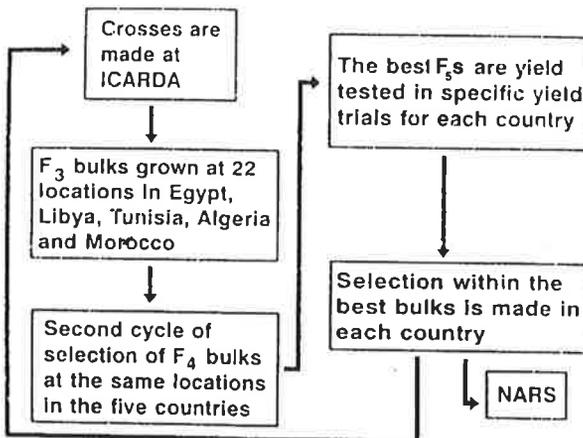


Figure 2. Decentralization of barley breeding: the example of North African countries.

Maximizing specific adaptation through farmers' participation

The idea of farmers participating in the development of new technology is not new. It was introduced in 1982 as "the farmer-back-to-farmer model" (Rhoades and Booth, 1982) and is now generally referred to as "farmer participatory research." Highly centralized breeding programs can be described as a sequential and top-down cyclical process in which (1) a large amount of genetic variability is continuously created, (2) this variability is drastically reduced through selection in experiment stations, and (3) the few lines surviving step 2 are presented to farmers who are asked to verify if the choices made for them are appropriate (Figure 3).

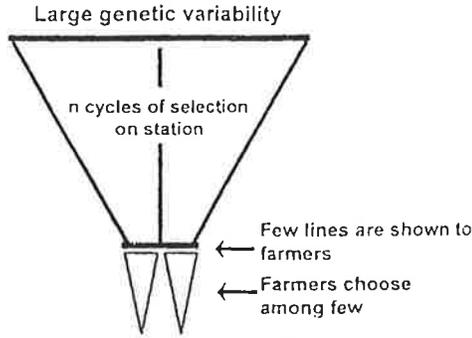
However, there is now evidence that, when breeders and farmers select in the same environment, progress with selection is enhanced (Sperling et al., 1993). This implies that farmers possess considerable knowledge that is almost totally neglected in formal "top-down" plant breeding programs. A formal plant breeding program could become more "bottom-up" by combining the concept of breeding for specific adaptation with the utilization of farmers' knowledge by evaluating a wider range of germplasm under farmers' field conditions and in conjunction with farmers.

The most significant contribution of farmers' participation to ICARDA's barley breeding program has been the incorporation of tallness under drought and softness of the straw as selection criteria in barley breeding targeted at dry and low-input areas. A crop that remains tall, even in very dry years, is important to farmers because it reduces their dependence on costly hand harvesting, while soft straw is considered important for palatability. These two characteristics represent a drastic departure from the typical selection criteria traditionally used by scientists in breeding high yielding cereal crops: short plants with stiff straw and high harvest index. Cultivars possessing these two characteristics considered important by farmers in dry areas will not be suited for cultivation in high-yielding environments because of their lodging susceptibility – a further indication of the importance of specific adaptation. One of the barley varieties shown in Table 1 (Zanbaka) was specifically selected for plant height under drought. Its adoption has been rapid, and today it is grown in some of Syria's driest areas.

Specific adaptation and agricultural biodiversity

The trend of formal, institutional plant breeding has been towards narrowing the genetic base of our crops. Broad adaptation accommodates large-scale centralized seed production, which further promotes uniformity. By contrast, resource-poor farmers in many low-input areas of the world have adopted a strategy based on both intraspecific and interspecific diversity by growing crop mixtures and genetically heterogeneous landraces. Also, in resource-poor agriculture the seed is usually produced on the farm, after some form of selection by the farmer, or is purchased from neighbors (Almekinders et al., 1994).

Formal breeding program



Farmers and formal breeding programs

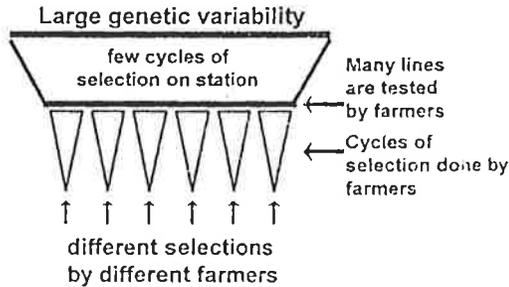


Figure 3. A centralized breeding program (top) generates a large amount of genetic variability, which is drastically reduced during a number of selection cycles on station: farmers see a small fraction of the original variability. A decentralized, participatory breeding program (bottom) also generates a large amount of genetic variability, limits selection on station, and farmers conduct further cycles of selection under their specific conditions.

Thus, breeding for specific adaptation to marginal environments under farmers' conditions is not only a way to improve agricultural production in these harsh, low-input and stressful environments, but also has positive effects on biodiversity. This occurs through the reevaluation of landraces and the dissemination of a large number of new varieties, not necessarily homogeneous, rather than few, often closely related, "widely" adapted varieties. A further advantage is that the transfer and adoption of new varieties will be accelerated without the involvement of complex, bureaucratic and often inefficient mechanisms of variety release, seed certification and production, and extension.

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Barley: Wide adaptation

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Wide adaptation was first developed in Mexican wheats by Borlaug (1968) by combining such traits as photoperiod insensitivity, resistance to diseases, and the dwarfing genes. Day-length insensitivity was incorporated by switching segregating populations among experiment stations at different latitudes twice a year, and selecting against sensitive types. During the winter cycle the germplasm was planted at CIANO Experiment Station (32 meters elevation, 27° N latitude), followed by the summer season at El Batán (2,200 meters elevation, 19° N latitude), and Toluca Experiment Stations (2,640 meters elevation, 19° N latitude) in central Mexico. The barley breeding program at ICARDA/CIMMYT has used the same locations since 1973.

The initial rating of wide adaptation in barley is based on data from international nurseries. Yield trials from the 12 International Barley Yield Nurseries (IBYT) were used to identify barley crosses with good performance. Data from 44 locations were classified into three groups: eight top-yielding lines, eight intermediate types, and the eight lowest-yielding lines. Cooperators were encouraged to sow these 24 Mexican lines with the best-adapted local variety as the check. A sister line from the cross LB Iran/UNA8271//Gloria/Come was classified in the highest yielding group at 25 locations, in the middle group at 11 locations, and in the lowest yielding group at 8 locations. The local check had similar performance. In contrast, the cultivar Tamina was classified in the highest yielding group at only 11 locations, and had the lowest yield at 22 locations. The results demonstrated the wide adaptation of the cultivar LB Iran/UNA 8271//Gloria/Come, and the narrow adaptation of Tamina. However, the ultimate test for wide adaptation is a farmer's long-term adoption of a particular variety. Two varieties were released from the cross LB Iran/UNA8271//Gloria/Come: Calicuchima in Ecuador and Kolla in Bolivia.

A widely adapted cultivar, as measured by its grain yield, might not be the most adapted under drought conditions. There are several million hectares of barley land with precipitation of 200 mm/yr or less in North Africa and the Middle East (Ceccarelli, 1989). Farmers cultivating barley under these dry conditions have survived by choosing a double-purpose barley variety. Under these circumstances, the variety can be harvested for grain if the weather is favorable, and grazed if there is little or no rain during a dry year. A barley cultivar grown in a good environment requires stiff straw and a high lignin content to support the plant and to produce high grain yield without lodging.

However, stiff straw may cause mouth injuries in grazing animals, and may therefore be rejected by farmers. In this paper wide adaptation for areas with precipitation of 250 mm or higher will be discussed.

Earliness

With the increasing world population, there is less agricultural land. China, with more than 1.2 billion people, produces food for 22% of the world population using only 7% of earth's arable land (Wittwer et al. 1987). Chinese agriculture is an example of intense land use, where farmers are interested in the total annual output of several crops from one piece of land. Combined grain yields of 24.4 t/ha were reported for a rotation of barley and two crops of rice.

The ICARDA/CIMMYT program has developed early-maturing cultivars to be harvested for grain. In addition, efforts have emphasized the development of early-maturing, hooded, day-length-insensitive barley for forage. In demonstration trials on 18 hectares in two Mexican states, Sonora and Hidalgo, a short hooded barley was sown at 60 kg per hectare, on the same field with rye grass (18 kg/ha) or medic (8 kg/ha), in two different planting operations on the same day. The barley seed was drilled and the pasture seed broadcast, to avoid competition during emergence and at later growth stages.

Typically, the flowering time for barley was 45 days. Cattle or sheep then grazed the barley for short periods (mob grazing). Grazing by many animals resulted in less competition for light and nutrients for the rye grass or medic. The results from several years of trials were an expansion of the grazing period and an increase of total biomass. More importantly, the pasture was made available at a critical time when there was a feed shortage.

Total dry matter production of tall, hooded barley cultivars was studied under rainfed and irrigated conditions. Dry matter production at dough stage was compared for three maturity groups: 58, 60, and 72 days after sowing. The highest dry matter production was 12.2 t/ha (unreplicated plots) at El Batan, and 7.2 tons/ha in a replicated trial at CIANO.

Spike morphology

According to Burger and LaBerge (1985), there are 30 physical and 40 chemical properties that qualify a cultivar as a malting type. However, a single morphological trait split Europe from North America. In Europe a malting barley variety must be a two-rowed type. In North America six-rowed and two-rowed types coexist.

The influence of spike morphology on wide adaptation is also reflected in the farmer's ideotype for yield potential. Six-rowed barleys are preferred by farmers in Morocco, with two million hectares under cultivation, while in Bolivia, with one hundred thousand hectares, two-rowed types are discriminated against. In the ICARDA/CIMMYT program, the yield potential of six-rowed and two-rowed cultivars is the same.

Kernel caryopsis

In his review on hulless barley, Bhattu (1986) indicated its potential use and limitations. Barley programs have since become interested in breeding hulless barley. The ICARDA/CIMMYT program is involved in breeding barley with multiple disease resistance for farmer's use in the Andes and Himalayas, where barley is a staple food. Hulless barley has advantages since it does not require sieving and grinding operations to separate husk from flour. In Ecuadorian markets, hulless barley fetches 10% more than hulled barley. Husk weight can reach 10 to 13% of the total grain yield of hulled barley according to Bhattu (1986). Low yields are often mentioned by farmers as the main reason for not growing hulless barley.

Yield trials conducted at CIANO during 1994 showed that two hulless barley cultivars had statistically similar yields to the hulled check. Both cultivars, Congona and Cerraja, were yield tested at the El Batan Experiment Station. The average yield was similar to the yields found the previous year, but significantly different from the hulled check in seven of eight trials. A new cultivar, Petunia, had a yield of 7.1 t/ha, compared to the best hulled check at 7.0 t/ha (Table 1). The hulled cultivar Tocte was used as a check in all experiments. The trials are being continued at CIANO.

During the last decade, six countries have released hulless varieties in South America, Australia, and Canada from germplasm developed in Mexico. With the development of hulless barley that possesses yield potential similar to that of the best covered cultivars, a major barrier has been overcome. This may result in a greater interest in hulless barley. It is too early to evaluate wide adaptation in hulless barley, but there is a range of cultivars available for improving nutritional quality for humans and monogastric animals.

Multiple disease resistance

The methodology for incorporating multiple disease resistance and for field screening to select for resistance was discussed previously by Vivar et al. (1986).

Table 1. Grain yield of hulless cultivars and the hulled check (Tocte) at the CIANO (1993-94) and El Batan Experiment Station, Mexico (1995)

Cultivar name	—Yield t/ha—		—% hulled check—	
	1994	1995	1994	1995
Cerraja	5.1	5.8	94	81
Congona	5.1	5.6	93	78
Petunia "S"		7.1		102
CMB93-855-L-4Y-6M-0Y				
Petunia "S"		6.6		94
CMB93-855-M-1Y-2M-0Y				

+ Average of eight yield trials.

In this paper several diseases, their resistance mechanisms, and the implications for wide adaptation will be discussed.

Leaf rust

There are two approaches for a breeder to develop cultivars that possess leaf rust resistance: the use of major genes (vertical resistance) and the use of minor genes (partial resistance).

Partial resistance was defined by Parlevliet (1988) as a reduced rate of epidemic build-up despite a susceptible infection type. This mechanism may enhance wide adaptation, since it provides protection against different *Puccinia hordei* virulences. In Europe, most barley varieties carry low to fair levels of partial resistance to barley leaf rust (Parlevliet, 1988).

In a recent virulence survey of *P. hordei* present in 75,000 hectares of barley in Ecuador, Brodny and Rivadeneira (1996) reported a wide spectrum of virulence capable of overcoming all the major genes present in a differential set of varieties. For Ecuadorian breeders and plant pathologists, the control of leaf rust may be obtained through the use of partial resistance, or new resistance genes.

The Ecuadorian variety Shyri carries partial resistance to leaf rust (Ochoa et al. 1996). The losses caused by leaf rust at the Santa Catalina Experiment Station (2,800 meters elevation) were estimated under a severe epidemic. Unprotected and protected plots, which received five applications of Tilt (propiconazole), were compared during the growing cycle. There were losses of 32% in grain yield for Shyri, as compared to 64% in the susceptible variety Teran.

In Ecuador the effectiveness of the single major gene for leaf rust resistance also present in Shyri was lost shortly after its release. This same gene remains effective against races 8, 19 and 30, present in Mexico. Partial resistance found in Shyri was probably inherited from the French variety Motan (Vivar, 1994). In Shyri, both resistance mechanisms were combined; vertical resistance proved to have a short life, while the remaining partial resistance mechanism can be expected to be more durable (Parlevliet, 1988).

Stripe rust

Sandoval et al. (1996) found partial resistance to stripe rust *P. striiformis f. sp. hordei*, in several studies conducted in the greenhouse at El Batán, and in the field at Toluca. Contrasting cultivars were characterized by studying parameters influencing partial resistance. Resistant cultivars had a latent period of 26 days, as compared to susceptible cultivars with 13 days LP. Large differences for the Area Under Disease Progress Curve (AUDPC) were found for resistant and susceptible cultivars.

Parlevliet and Ommeren (1975) reported a negative correlation ($r = -0.9$) between LP and AUDPC for leaf rust, while the correlation between the same parameters for stripe rust in Sandoval's study was $r = -0.7$. Selection based on LP alone could miss cultivars such as Calicuchima, with intermediate values but excellent AUDPC.

Scald

Ginkel and Vivar (1986) studied the disease development of several cultivars at Toluca. Resistant cultivars had small AUDPC values compared to susceptible types. Cultivars with this slow-scalding resistance are frequently used in our crossing program.

In summary, partial resistance to several diseases is being incorporated to ensure wide adaptation. Cultivars such as Calicuchima and Kolla were identified as having wide adaptation and partial resistance to stripe rust.

Barley Yellow Dwarf (BYD)

Webby, et al.(1993) conducted a worldwide survey on the distribution and frequency of five Barley Yellow Dwarf biotypes (MAV, PAV, RPV, SGV, and RMV). In the Andean region of South America, the biotype MAV was reported in Bolivia, Colombia, Ecuador, and Peru, while in Chile PAV was predominant.

Results from surveys were instrumental for screening large numbers of barley cultivars from the program sent from Mexico to Kenya. The biotypes reported for Kenya in the survey were PAV and RMV. The resistant lines identified in Kenya were inoculated with individual biotypes (MAV, PAV, and RPV) by Bertschinger under field conditions at Toluca. He found several cultivars resistant to both RPV and PAV .

One hundred doubled haploid lines from the cross Shyri/Galena were field inoculated with three biotypes (MAV, PAV, and RPV) at the Toluca Experiment Station to study the inheritance of the resistance to BYD. The ratio 53:47 resistant:susceptible points to a single gene present in Shyri that provides resistance to MAV. This gene was different from the Yd2 gene present in Atlas 68, which gave resistance to MAV, PAV and RPV.

Head scab

Several species of *Fusarium* cause head scab in barley and wheat. The disease is present in Argentina, Brazil, Canada, China, Europe, Japan, the United States, and Uruguay.

The economic losses caused by the disease in the upper Midwest of the United States were one billion dollars for wheat and barley in 1993 (Franckowiak, personal communication). In barley, the greatest losses were not produced by the five percent drop in grain yield, but by the rejection of infected grain with toxins such as Deoxynivalenol (DON), which are known to cause disease in humans and animals, including lung and esophageal cancer (Israel, 1989).

A survey conducted by Garns (unpublished data) on farmer's fields cultivated with barley in the Mexican states of Hidalgo, Tlaxcala, and Mexico showed 22% and 81% of fields with *Fusarium*-infected spikes for 1994 and 1995, respectively.

In the ICARDA/CIMMYT program we have screened for resistance against type I (initial) and II (hyphae spreading) infection. The cultivar Gobernadora, selected in Shanghai from Mexican germplasm, is resistant to head scab, and is grown on more than 100,000 hectares in three Chinese provinces in the lower

basin of the Yangtze river where head scab is endemic (Liu Zongzhen, personal communication).

During the summer of 1995, resistant sources were screened with a 50,000 spore concentration of *F. graminearum* at Toluca. Cultivars were previously identified and reported as head scab resistant in China, Japan, and the United States. The Ecuadorian variety Shyri was resistant to infection types I and II.

Cultivars with resistance to type II (screened only for hyphae spreading) included: Atahualpa, an Ecuadorian hullless variety; Arupo/K8755//Mora, Mexico; Gobernadora/Humai, China; Shyri/Gloria//Copal/3/Shyri/Grit, a Mexican cultivar reported to have a good malting quality profile; and three DH lines developed by Dr. Pat Hayes (OSU) from the cross Gobernadora by Shyri/Gloria//Copal/3/Shyri/Grit.

All *Fusarium*-resistant lines were two-rowed. These results agree with those reported by Takeda and Heta (1989), in which 5,000 accessions of the world collection were screened for resistance against *Fusarium*.

In close cooperation with ICARDA/CIMMYT, INIAP has developed barley varieties resistant to the main diseases present in Ecuador, including head scab. A summary of the disease reaction of three varieties is presented in Table 2.

Table 2. Multiple disease resistance of three Ecuadorian barley varieties

Disease	Shyri	Atahualpa	Calicuchima
Stripe rust	R	R	R
Leaf rust	R	R	R
Scald	R	R	R
BYD	R (MAV)	R	S
Net blotch	R	MS	MS
Head scab	R (I-II)	R (II)	?
Loose smut	S	S	?

R= resistant, MS= Moderately susceptible, S= susceptible

A more stable barley production will result for farmers growing these varieties in the near future. Yields obtained with Shyri by good farmers in Ecuador were 4.5 t/ha, a four-fold increase over the stagnant national average. Even when most Ecuadorian small farmers involved in barley production have no access to modern inputs such as fertilizer, herbicides, and certified seed, the new broadly-adapted, disease-resistant varieties offer opportunities for more stable yields. Such varieties are crucial to any barley based production system.

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The role of mobilized stem reserves in stress tolerance

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In most wheat-growing regions and especially in the Mediterranean climate grain filling is subjected to several physical and biotic stresses. Grain filling often occurs when temperatures are increasing and moisture supply is decreasing. Foliar disease of wheat also tends to spread and intensify towards and after flowering. Leaf rust, stripe rust and *Septoria* leaf blotch can result in total leaf destruction at grain filling. The common end result of all these stresses is the reduction in kernel weight, kernel shriveling, reduced test weight and loss in yield.

The current source of carbon for grain filling is assimilation by the light-intercepting viable green surfaces. This source is normally diminishing due to natural senescence and the effect of various stresses. At the same time, the demand by the growing kernel is increasing, in addition to the demand posed by maintenance respiration of the live plant biomass.

Hence, an important source of carbon for grain filling is stem reserve. Even under mild conditions, current assimilates may be limited for normal grain filling. In a three-year study in Connecticut it was estimated (Gent, 1994) that canopy respiration and grain dry matter accumulation were approximately equal sinks for photosynthate and, together, were greater than canopy photosynthesis late in grain filling. Thus, stem reserves were essential for completed grain filling.

While root storage is important in some legumes and other species, there is no evidence that roots or leaves are as important as stems for reserve storage in the small grains. In most studies of stem reserves in the small grains, stems also include the leaf sheaths, which in themselves contain reserves. In small grains, stems store carbohydrates in the form of glucose, fructose, sucrose and starch, but the main reserve is fructan (e.g., Lopatecki et al., 1962; Dubois et al., 1990; Wardlaw and Willenbrink, 1994). Storage is commonly analyzed as total non-structural carbohydrates (TNC) or water-soluble carbohydrates (WSC).

The first step, and probably the most important one in fructan synthesis, is catalyzed by the enzyme Sucrose:sucrose fructosyltransferase (SST). SST activity seems to be related to substrate (sucrose) concentration, which in itself may be affected by sucrose synthase activity in the stem (Wardlaw and Willenbrink, 1994). Fructan accumulation was greater when sucrose was high in the penultimate

internode of wheat (Dubois et al., 1990). Wardlaw and Willenbrink (1994) found that, during the accumulation of WSC in wheat stems, the mass of fructan with a DP (degree of polymerization) greater than 5 continued to increase whilst the mass of fructans of DP 3 to 5 reached a maximum and then remained constant. Fructan accumulated in internodes while they were extending, although most of the fructan in an internode accumulated after it was fully extended. When WSC was mobilized from the stem, the mass of glucose, sucrose and fructan decreased, but the mass of fructose first increased then decreased, indicating that fructan was hydrolyzed at a faster rate than its product (Bonnnett and Incoll, 1992b).

Starch is found in small amounts in wheat stems but it is not mobilized, as evidenced from shading experiments (Kiniry, 1993).

Reserve accumulation

Reserve accumulation in the stem and the size of the storage strongly depend on the growing conditions before anthesis. Total stem TNC at anthesis was shown to vary from 50 to 350 g kg⁻¹ dry mass in different experiments (see review by Kiniry, 1993).

Under optimal growing conditions with regard to temperature, water regime (Davidson and Chevalier, 1992) and mineral nutrition (Papakosta Gagianas, 1991), carbon assimilation rates are high and a proportion of the assimilates is allocated to storage. When carbon assimilation during stem elongation is reduced by stress, storage in stems is reduced. For example, remobilized WSC were 641 mg and 1047 mg in water-stressed and irrigated wheat, respectively, because the former had less storage than the latter (Davidson and Chevalier, 1992). Under dryland field conditions only half the amount of water-soluble carbohydrates was available for remobilization during grain filling, as compared with irrigated conditions. When ambient CO₂ concentration was raised to increase assimilation, more carbon was stored in the stems (Winzeler et al., 1989).

Developmentally, potential stem storage as a sink will be determined by stem length and stem weight density. Stem weight density is stem dry weight per unit stem length. Storage and its availability for remobilization may vary along the stem. In winter barley the basal internodes were found to be contributing the most to grain filling (Bonnnett and Incoll, 1992a). If this was a general phenomenon it could perhaps explain some of the interactions between sink size, monocarpic senescence and lodging, as the case is for charcoal-rot-induced lodging in droughted sorghum. However, other studies with barley showed that the peduncle and penultimate internode (and leaf sheath) had the most storage (Daniels and Alcock, 1982). Work with wheat indeed confirmed that the peduncle and the penultimate internode had the most storage (Wardlaw and Willenbrink, 1994) and that variations in storage and remobilization under different experimental conditions were larger in the penultimate than in the fourth stem internode (Bonnnett and Incoll, 1992a). Various aspects of stem anatomy with respect to storage were not thoroughly investigated, except for the finding that there seems to be no consistent difference in total stem reserves between solid and hollow-

stemmed wheats (Lopatecki et al., 1962).

Stem length, as affected by the height genes, is important in affecting stem reserve storage. The Rht_1 and Rht_2 dwarfing genes of wheat were found to reduce reserve storage by 35% and 39%, respectively, as a consequence of a 21% reduction in stem length (Borrell et al., 1993). However, under the favorable conditions the advantage of the tall (*rht*) genotype in reserve storage was not expressed in greater mobilization to the ear. Under these favorable grain filling conditions only about 20% of grain yield was contributed by stem reserves in all genotypes. The contribution of stem reserves to grain yield was greater in a tall than in a short barley cultivar, but absolute yield was the same in both, indicating that the taller cultivar was lacking in current assimilation compared with the shorter one (Daniels and Alcock, 1982). A tall (2-dwarf) isogenic line of sorghum, as compared with a shorter counterpart (3-dwarf), had more storage, and by that token had better grain filling under stress (Blum, in preparation).

Reserve utilization

Stem reserve mobilization or the percentage of stem reserves in total grain mass is affected by sink size, by the environment and by cultivar. The demand by the grain yield sink is a primary factor in determining stem reserve mobilization. When sink size was reduced by degrading, more reserves were stored in the stem, as compared with intact ears (Kuhbauch and Thome, 1989). The interaction between ear size and the demand for stem storage seems to depend on the environment (Bonnert and Incoll, 1992a), either before or during grain filling.

Environmental conditions that decrease current assimilation during grain filling pose an increased demand for stem reserves for grain filling. Thus, shading of barley plants after anthesis promoted the use of stem reserves for grain filling (Bonnert and Incoll, 1992a). When wheat plants were shaded during grain filling, up to 0.93g of grain was produced per gram of assimilates exported from the stem (Kiniry, 1993).

Stem reserve mobilization is affected by water deficit during grain filling. Even the rate of development of water deficit may affect mobilization. Hence, Palta et al. (1994) found that total grain carbon with fast development of water deficit was reduced by 24% relative to a slow rate, whereas postanthesis assimilation was reduced by 57%, while remobilization of reserves was increased by 36%. Interestingly, water deficit during grain filling also induced carbon mobilization from tillers to the main stem ear.

It is therefore to be expected that estimates of the relative contributions of stem reserves to total grain mass per ear or to grain yield would vary among the different reports, according to the experimental conditions and cultivars used. These contributions were estimated to be anywhere between 6% and 100% (Austin et al., 1980; Papakosta and Gagianas, 1991; Pheloung and Siddique, 1991; Davidson and Chevalier, 1992; Borrell et al., 1993; Blum et al., 1994; Gent, 1994; Palta et al., 1994).

It may be concluded that the reduction in current assimilation during grain

filling, under different stresses, will induce an increase in stem reserve mobilization and utilization by the grain. What is important is the reduction in assimilation and not the nature of stress causing the reduction. Thus, stem reserve mobilization is a solid source of carbon for grain filling under any stress that would inhibit current photosynthesis, including biotic stresses such as late developed leaf diseases. Tolerance to *Septoria* leaf blotch in wheat is expressed in sustained grain filling under severe epiphytotics. It has been demonstrated that mobilized stem reserve is a major component of *Septoria* tolerance in wheat (Zilberstein et al., 1985).

Drought conditions during grain filling often also involve heat stress, which reduces the duration of grain filling. There is normally an increase in the rate of grain dry matter accumulation under high temperatures, but it is not sufficient to compensate for the decrease in duration. When grain filling under such stress depends on remobilized stem reserves, the rate at which these reserves are metabolized and transported to the grain becomes critical. It seems that this rate is not sufficiently high to compensate for the reduction in grain filling duration at very high temperatures, such that a genetically longer grain filling duration seems to be an advantage in this respect (Blum et al., 1994). Hence, short grain filling duration may allow some avoidance of terminal stress, while a long duration may increase utilization of stem reserves for grain filling under stress.

Improving stem reserve utilization for grain filling

Improving the capacity for supporting grain filling by stem reserves is an important breeding target in cereals subjected to environmental and biotic stresses during grain filling. Genotypic variations exist for various aspects of grain filling from stem reserves. The effect of height has already been mentioned. With very few exceptions it seems that taller cultivars have greater capacity to support grain filling from stem reserves because of their greater storage.

The capacity for maintaining large storage in stems appears to be a constitutive trait (e.g., Blum et al., 1994; Hunt, 1979), and it is most probably linked with assimilate partitioning during stem elongation and the developmental characteristics of the stem. If greater partitioning to the stem is at the basis of high reserve storage, then it might be at some expense of grain yield potential. Indeed, Pheloung and Siddique (1991) in Australia found that the higher yielding cultivars Gutha and Kulin had less reserve storage and suffered greater reductions in grain yield under drought stress during grain filling, if compared with the potentially lower yielding cultivar Gamenya. This trend was also noted for winter wheat in Kansas, where new cultivar releases were less capable of grain filling from stem reserves than old cultivars (Hossain et al., 1990). Landraces of wheat were better at grain filling from stem reserves than a modern semi-dwarf cultivar (Blum et al., 1989), but this could be ascribed also to their taller stature.

On the other hand, some studies with wheat did not indicate a strong negative relationship between yield potential and reserve utilization for grain filling

(Blum et al., 1994; Davidson and Birch, 1992). Indeed, exceptions were noted also by Hossain et al. (1990), such as the winter wheat cultivar Bounty-310, which had a fairly high yield potential and also good grain filling from stem reserves. Still, it remains that cultivars designed for tolerance to stress during grain filling must have the capacity for high stem reserve storage, if necessary even at the expense of a certain reduction in yield potential.

Delayed monocarpic leaf senescence (i.e., "non-senescence" or "stay-green") has long been considered to be a desirable trait in cereal breeding (e.g., Thomas and Smart, 1993). It is to be expected that longer leaf area duration would contribute to grain filling and yield. It has often been noted that modern high yielding cultivars of C_3 and C_4 plants tend to express longer leaf area duration. However, in two repeated cases for wheat (Blum et al., 1994; Fokar et al., 1996), cultivars of high capacity for stem reserve utilization for grain filling had accelerated leaf senescence under both stress and non-stress conditions. Inherently accelerated leaf senescence in such cultivars would indicate that stem reserve mobilization to the grain is a constitutive trait. This seems to be linked with accelerated export of nitrogen from leaves, thus enhancing their senescence (e.g., Pell and Dann, 1991). It may therefore be suspected that non-senescence as a sustained source of current assimilation on one hand and stem reserve utilization on the other may be mutually exclusive. While inherently delayed senescence may be advantageous for yield under optimal growing conditions, it may be of no consequence under post-anthesis stress because then the stress factor will impose accelerated senescence or leaf killing. Selection for non-senescence under non-stress conditions would probably even prefer genotypes that do not use stem reserves for grain filling.

It seems that large TNC storage is the primary factor for sustaining kernel growth from stem reserves. For example, use of storage for grain filling was found to be proportional to the size of storage across 20 winter wheat cultivars (Hunt, 1979). Also, better grain filling under stress was proportional to stem sugar concentration at flowering across different wheat cultivars (Nicolas and Turner, 1993). It was, however, noted that some wheat cultivars (e.g., cv. TAM-101) had sufficient storage but were lacking in the extent of remobilization into the grain (Hossain et al., 1990). The remobilization and utilization of the stored carbon depends also on demand. There may be genetic differences among cultivars in enzymatic activity involved with remobilization, but such activity may also be a function of demand and substrate concentration (Dubois et al., 1990).

Another source of imbalance between storage size and its remobilization is the capacity to deposit starch in the kernel endosperm under heat stress. Soluble starch synthase is a key enzyme in endosperm starch biosynthesis. Compared with all the other endosperm starch synthesis pathway enzymes, it is highly thermosensitive, especially at temperatures above 34°C (Keeling et al., 1993). A more thermostable form (or a thermoprotected form) of this enzyme has been identified in a wheat cultivar (Kumar et al., 1996). With this form of heat tolerance in the endosperm, stem reserves were well utilized for kernel growth

at temperatures reaching 38° to 40°C. (Blum et al., 1994). Thus, while the size of the storage is preeminent, the size of the sink and its capacity to utilize the imported carbon is also important for allowing grain filling from stem reserves.

Methodology and selection

Clarke et al. (1984) demonstrated very well that simple relationships between stem reserve storage or remobilization and varietal drought resistance in terms of yield (such as by the "stress susceptibility index") are not to be expected. The impact of stem reserves should be evaluated only under stress conditions that equally inhibit crop assimilation during grain filling in all materials tested.

Selection for better reserve-supported grain filling under stress may be performed by subjecting the population to the actual stress condition in the field, be it drought, heat or disease epiphytotic. It has been repeatedly argued that a standard level of biotic or abiotic stress is difficult to achieve during grain filling in diverse genetic materials. The first difficulty is in the techniques for imposing stress in large breeding populations in the field. The second difficulty is in the variable phenology of breeding materials, which would not allow the researcher to affect the same timing and degree of stress after flowering in all materials (Blum et al., 1983b; Mahalakshmi et al., 1994; Clarke et al., 1984).

Blum et al. (1983a, 1983b) proposed the use of chemical desiccation of the canopy after flowering as means for inhibiting plant photosynthesis and thus revealing the capacity for grain filling by stem reserves. The treatment does not simulate drought stress. It simulates the effect of stress by inhibiting current assimilation. With this method they applied a chemical desiccant (magnesium chlorate or sodium chlorate; 0.4% w/v) as a spray to the canopy, including the ears. The treatment was applied to each genotype at 14 days after anthesis, when kernel growth entered its linear phase. At maturity, kernel weight was compared between treated and non-treated (control) plants, calculating the rate of reduction in kernel weight caused by the treatment. The rate of reduction was typically between 5% and 50% in different wheat materials. An important component of this test is that it must be free of any biotic or abiotic stress, simply because if it is stressed then grain filling would also be reduced in the controls, as noted also by others (Regan et al., 1993).

Nicolas and Turner (1993) confirmed the utility of chemical desiccation as a means for revealing genetic variation in grain filling from stem reserves, and proposed the use of a leaf spray of potassium iodide (0.4% w/v) in wheat as a milder treatment that mainly destroys chlorophyll. Potassium iodide was working well also for millet (Mahalakshmi et al., 1994) and sorghum (Blum, unpublished).

The correlation across diverse genetic materials between the rate of reduction in kernel weight by chemical desiccation and the rate of reduction by drought stress was found to be significant and reasonably high. It was $r=0.81^{***}$ and $r=0.79^{**}$ over two years in Blum (1983b); and $r=0.48^{**}$ and $r=0.81^{**}$ over two years in Nicolas and Turner (1993). The relationship also held well for several millet genotypes treated with KI (Mahalakshmi et al., 1984). Hossain et al.

(1990) noted that winter wheat cultivars of stable kernel weight over years and locations sustained relatively less reduction under sodium chlorate desiccation of the canopy. Blum et al. (1994) found a correlation of $r=0.94^{**}$ across five wheat cultivars between the reduction in kernel weight by chemical desiccation and the reduction in kernel weight by heat stress ($35^{\circ}/25^{\circ}\text{C}$ day/night temperatures) during grain filling. The rate of reduction in kernel weight under harsh heat stress conditions was well correlated across different wheat cultivars ($r=0.74^{**}$), with the reduction in kernel weight caused by post-anthesis defoliation and shading of plants under optimal temperatures (Fokar et al., 1996). Finally, the reduction in kernel weight by chemical desiccation was significantly correlated across different wheat cultivars ($r=0.48^{*}$) with reduction in kernel weight caused by late epiphytotics of *Septoria* leaf blotch disease (Zilberstein et al., 1985).

Chemical desiccation can be incorporated into breeding programs in two ways. Firstly, it can be used to assess responses of individual advanced lines or families, always compared with non-treated controls under non-stress conditions. This can be easily performed with nursery ear-rows. Secondly, it can be used in mass selection. Blum et al. (1991) performed mass selection, where six spring wheat F_2 bulks were chemically desiccated with magnesium chlorate, after which grain was divergently selected for kernel weight by mechanical sieving. After two or three cycles of selection, random lines were selected and tested for their response to chemical desiccation stress. Mass selection for large kernels under chemical desiccation significantly improved kernel weight and grain yield under chemical desiccation stress, as compared with controls, where selection for kernel weight was performed without chemical desiccation. There was no shift in phenology or plant height under chemical desiccation selection, probably because the variation in these traits within the populations used was small.

Haley and Quick (1993) performed a similar selection program under chemical desiccation with sodium chlorate in winter wheat. Two cycles of selection produced F_4 bulks that were indeed more resistant to chemical desiccation stress.

Conclusion

It is concluded that stem reserves offer a powerful resource for grain filling under any type of stress that inhibits current assimilation. In 1983 chemical desiccation was proposed as a method of selection for improved grain filling from stem reserves. Since then the method has been confirmed by several independent studies to be useful and effective.

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Developmental variation, adaptation and yield determination in spring barley

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Abstract

Date of heading is a common criterion used in selection for adaptation. Within the constraint that heading date imposes on the duration of the life-cycle, the barley plant has to accumulate dry matter and partition it among the hierarchical and co-ordinated sequence of sinks that characterize developmental progress toward maturity. Factors determining the timing of heading do so by influencing the durations of the successive phases/stages into which the life-cycle can be divided and, indirectly, the production of dry matter, the numbers of component structures contributing to the determination of yield, and the manner in which that dry matter is partitioned among them. This paper discusses the role of factors influencing developmental variation, and comments on its consequences for adaptation and yield determination.

Introduction

Most of the world's temperate cereals are grown for the grain formed during the third and terminal phase of the life-cycle. The potential yield of grain that could be harvested, however, is largely a function of developmental events occurring in the preceding phases, each of which is sub-divisible into stages (Figure 1).

Firstly, and during the vegetative phase, from planting to floral initiation, leaves and tillers are initiated to establish, very early in the life-cycle, the vegetative foundation upon which final yield will depend. In the second, or ear/stem growth phase, ears develop on the tillers present and grow in size and complexity, and in competition with the growth of leaves and the elongation of stems to determine, by the time of heading/anthesis, and subject to ear density, potential grain number per metre. Over the terminal phase, from anthesis to maturity, that potential is translated into actual grain numbers and the sizes of the grains.

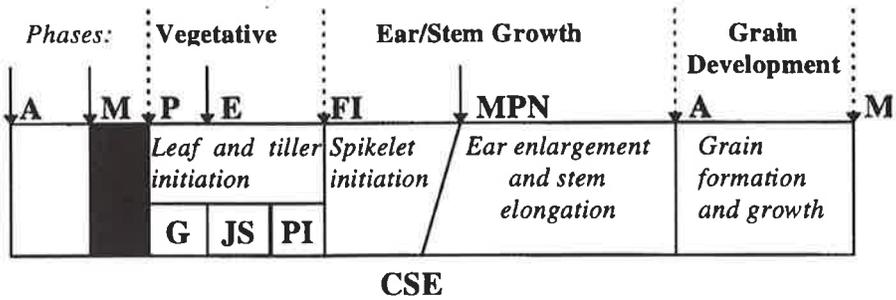


Figure 1. Phases and stages in the life-cycle of spring barley, with subdivision of the vegetative phase for: stages of germination (G), juvenile stage (JS) and photoperiod induction (PI), and for spikelet initiation and ear growth/stem internode elongation within the ear/stem growth phase. A = anthesis, M = maturity, P = planting, E = emergence, FI = floral initiation, MPN = maximum primordia number and CSE = commencement of stem elongation.

The above sequence of developmental events leading to grain production is subject to two constraints. Firstly, the life-cycle must be completed within the duration of the growing season and, secondly, within that constraint, it is limited by the amount of dry matter produced and the manner in which it is partitioned. Developmental variation influences both constraints, and the interrelationships between them.

This paper reviews the effect of factors contributing to variation in the duration of the vegetative and ear/stem growth phases, and comments on their consequences for establishing the early vegetative foundation for yield determination and its translation into yield potential. To put these relationships into a realistic perspective, let us begin with a general comparison of growing season conditions at Saskatoon (Canada) and Perth (Western Australia), and of phase durations among four varieties.

Comparison of growing season conditions and phase durations at Saskatoon and Perth

Subject to variation in temperature and rainfall, the growing season commences at both locations in May: spring in Saskatoon, but autumn in Perth. Harvest proceeds about 100 and 175 days from planting, respectively. Climatic reasons for this difference, which are summarized in Table 1, indicate growing conditions at Saskatoon are more conducive to rapid vegetative growth and development than are conditions at Perth. This is reflected in a substantially shorter life-cycle at Saskatoon (Table 2), due mainly to a major reduction in the duration of the ear/stem growth phase. By comparison, the contribution of the vegetative phase was about 20% of the length of the life-cycle for all varieties at both locations, with the duration of the grain development phase similar among varieties and locations.

Table 1. Monthly mean temperatures ($^{\circ}\text{C}$), global radiation ($\text{MJm}^{-2}\text{s}^{-1}$), and photoperiod (h) at Perth (W. Australia) and Saskatoon (Canada), with calculations of PTQ ($\text{MJ}/^{\circ}\text{C}$) and thermal time ($^{\circ}\text{Cd}$, base 0°C). Photoperiods for the 15th of each month.

	—Perth (Western Australia, 32°S)—					—Saskatoon (Canada, 52°N)—				
	Temp $^{\circ}\text{C}/\text{d}$	MJ m^2s^{-1}	Photo h.min	PTQ	$^{\circ}\text{Cd}$	Temp $^{\circ}\text{C}/\text{d}$	MJ m^2s^{-1}	Photo h.min	PTQ	$^{\circ}\text{Cd}$
May (from 15th)	15.2	11.3	10.36	0.74	243	11.8	17.7	15.46	1.50	189
June	12.3	9.2	10.14	0.74	369	16.9	21.2	16.41	1.25	507
July	10.9	10.1	10.24	0.92	338	18.2	19.8	16.21	1.09	583
August	11.3	13.4	11.02	1.18	350	17.9	18.6	14.18	1.04	555
September	13.1	17.6	11.54	1.26	393			12.49		
October	17.6	22.1	12.48	1.26	546			10.50		

Table 2. Durations ($^{\circ}\text{Cd}$, base 0°C) of the vegetative, ear/stem growth and grain development phases, for the whole life-cycle, and for main stem leaf numbers, in each of four varieties sown at Perth on 17th May and at Saskatoon on 10th May. M = Mona, B = Bonus, H = Harrington and S = Stirling.

	—Perth (Western Australia)—				—Saskatoon (Canada)—			
	<i>M</i>	<i>B</i>	<i>H</i>	<i>S</i>	<i>M</i>	<i>B</i>	<i>H</i>	<i>S</i>
Vegetative	240	430	390	370	260	340	310	240
Ear/Stem	580	1010	960	850	350	440	390	330
Grain Dev.		about 650				about 700		
Life-Cycle	1470	2090	2000	1870	1310	1480	1400	1270
Leaf number	7.7	11.9	10.9	9.6	7.4	10.8	10.1	6.5

Variation in the duration of the vegetative phase and of the stages within it

Despite the modest contribution of the vegetative phase to the duration of the life-cycle, there were major differences among varieties that, within locations, were strongly correlated with durations of the ear/stem growth phase and main stem leaf numbers ($r = > 0.9^{**}$ in all cases). Differences among varieties for duration to coleoptile emergence (about 100 °Cd) were negligible. However, varieties do differ in the duration of their juvenile stage and in their sensitivity to photoperiod. The former provides a measure of an interval, following coleoptile emergence, during which plants are insensitive to the inductive effects of photoperiod (Roberts et al., 1988). This pre-inductive period, or juvenile stage, varies with variety in a quantitative manner (Figure 2), and is correlated with a minimum number of main stem leaves. That duration may be modified within narrow limits, without change in rank order of variety, by a number of factors, including: location (Kaveeta, 1993), seeding date and depth, seed size (>2.8 mm vs. <2.2 mm), seed age (main stem vs. late tillers) and low levels of nitrogen (Men, submitted). In all F1s involving parents differing in the duration of the juvenile stage, we find a strong bias toward the parent with the longer duration. Based on the surrogate measure of time to heading under an 18-hour photoperiod, segregation among F2 and F3 progeny was distributed in a continuous manner, with transgressive segregation a common feature. Under similar conditions, large QTLs for time to heading and main stem leaf number, in the doubled haploid population of Chebec/Harrington, map closely together on chromosome 2HS (Karakousis et al., unpublished), whether the population is grown at 24/14C, or at lower temperatures in the field. A second QTL for main stem leaf number mapped to chromosome 7S. A major QTL for heading date under long days has also been reported on chromosome 2 for the Dicktoo/Morex and Steptoe/Morex mapping populations (Oberthur et al., submitted).

Floral initiation proceeded in all varieties we have tested at photoperiods down to 9 hours (Figure 3a), with variation among varieties in their

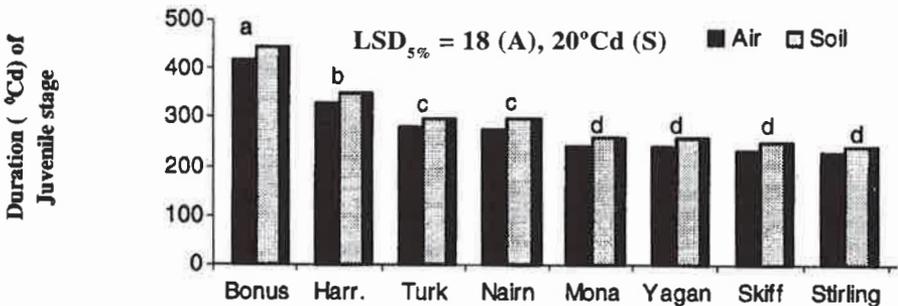


Figure 2. Variation among varieties for thermal time (°Cd, base 0°C) of the juvenile stage based on measurements of ambient and soil temperatures. Data from Men (submitted). Harr. = Harrington.

sensitivity to short photoperiods ranging from Mona (insensitive) to Turk (very sensitive). This sensitivity decreased differentially, and at different rates of response, with increase in photoperiod (Figure 3a), and increase in the number of day/night cycles from coleoptile emergence (Figure 3b). In both studies the rank order of varieties remained the same. If the juvenile stage has the effect of delaying photo-induction, then variation in its duration would be included in measures of sensitivity to photoperiod. The two traits are reported to be independent (Kaveeta, 1993), and this is supported by evidence of variation in sensitivity (Figures 3a and 3b) combined with juvenile stages of different duration (Figure 2). Among F1s we have examined, from parents differing in sensitivity, the duration to floral initiation was biased toward the most sensitive parent.

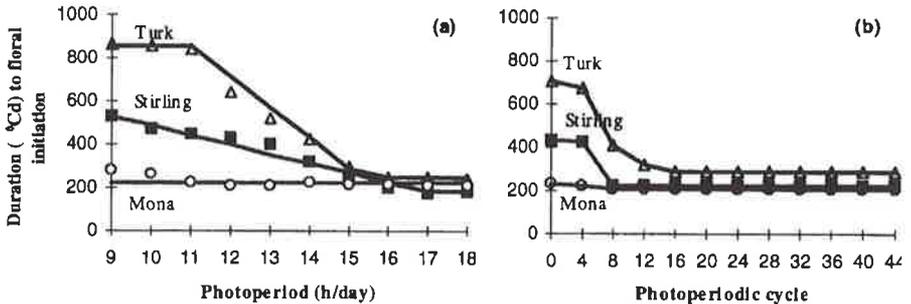


Figure 3. Thermal times ($^{\circ}\text{Cd}$, base 0°C) to floral initiation among three varieties due to increase in photoperiod from 9 to 18 hours in a growth room set at $24/14^{\circ}\text{C}$ (a), and with increase in the number of 18-hour day/6-hour night photoperiod cycles from a field planting when natural photoperiod was 10.2 hours (b). Data from Men (submitted).

Variation in the duration of the ear/stem growth phase and stages within it

Data presented in Figures 4a and 4b demonstrate correlated differences among varieties in their response to extending photoperiod from 11 to 18 hours, for the intervals both before (Figure 4a) and after (Figure 4b) floral initiation. These data apply to measures of the two phases among plants exposed to the respective photoperiod treatments from planting to awn appearance. When reciprocal transfers of plants were made between photoperiod treatments at the time of floral initiation, the durations of the ear/stem growth phase among the transferred plants were largely determined by the photoperiods to which they were exposed at that time (Figure 4c).

The correlated sensitivity/response of varieties, before and after floral initiation, suggests a common mechanism that operates independently for each phase: reducing duration and leaf number before floral initiation, and reducing duration by increasing the rates of stem elongation and leaf appearance after that event. Variation among varieties for the duration of the ear/stem growth phase extends to the durations of the two stages into which that phase can be divided (see Figure

1). Under an 11-hour photoperiod, varieties differed significantly for the durations of ear formation (FI to MPN) and ear/stem growth (MPN to AA). For Skiff (data not shown), the duration of the first of these stages was significantly shorter than for Stirling, but the latter duration was significantly longer. Under an extended (18-hour) photoperiod, both durations were reduced; more so for the interval FI to MPN in Stirling, and for the interval MPN to AA in Skiff. As a result of these differences, the rank order of varieties for the duration of the ear/stem growth phase under an 11-hour photoperiod (Skiff > Stirling) was reversed under an extended photoperiod.

Genetic control of the effect of photoperiod on correlated developmental events is complicated by variation among varieties for sensitivity/response in each of the stages contributing to the duration to heading. The duration of the vegetative phase combines variation in the juvenile stage with variation in sensitivity to photoperiod, and the duration to heading includes the above plus variation in the durations of stages comprising the ear/stem growth phase. In the absence of reported studies of variation in the timing of floral initiation among segregating populations, genetic control of variation in the duration of the vegetative phase has had to be inferred from the surrogate measure time to

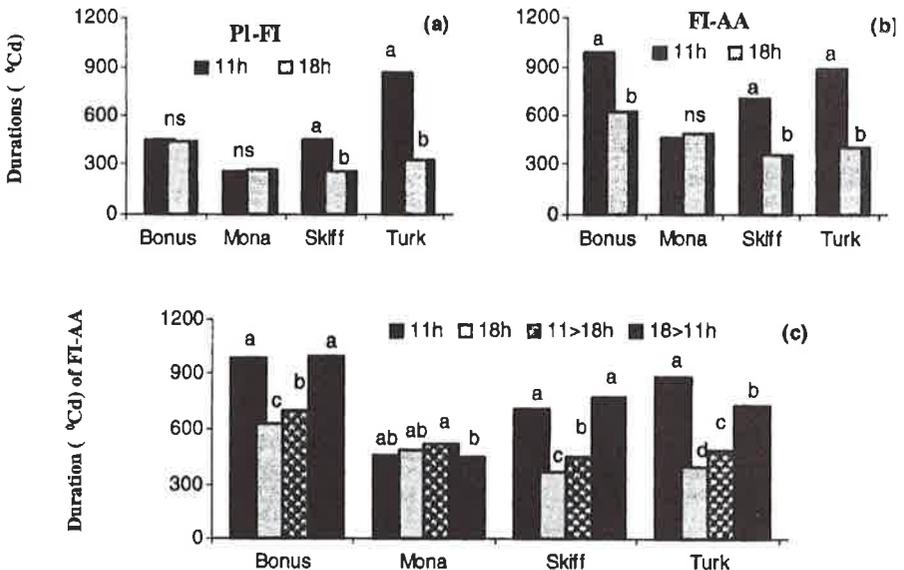


Figure 4. Thermal times (°Cd) from planting to floral initiation (a), and floral initiation to awn appearance (b), when photoperiods of 11 and 18 hours were maintained from planting to awn appearance and, in (c), when the durations from floral initiation to awn appearance shown in (b) are compared to those from plants reciprocally interchanged between photoperiod treatments at the time of floral initiation. PI = planting, FI = floral initiation, AA = awn appearance. Data from Men (submitted).

heading. Under short days, segregation among F2 progeny from parents differing in sensitivity is generally discontinuous, with the early and less sensitive sub-population being in the minority. Recessive genes conferring insensitivity, or early heading under short days, have been reported on chromosomes 1H, 3H, 4H and 6H. Major QTLs for heading under short photoperiods (<10.5 hours), from mid-May and mid-June field plantings at Perth, map, in the Chebec/Harrington population, to the centromeric region of chromosome 2, with an additional QTL on 7HS for the June planting (Karakousis et al., unpublished). There were less significant QTLs on chromosome 6 (June planting), and on 5HS and 7HS (May planting). For both the Dicktoo/Morex and Steptoe/Morex populations, QTLs for time to heading under short days were located on 1H, 2H and 5H, with another on 7H for the Dicktoo population (Oberthur et al., submitted). Sensitivity/response to photoperiod is clearly a complex trait involving multiple genes.

Adaptation and yield determination

Commercial yields of barley crops in Saskatchewan and Western Australia are similar, despite major differences in the growing season conditions (Table 1) and the phase/life-cycle durations of the varieties listed in Table 2. Stirling, the dominant Western Australian variety, flowers very early and is of low yield at Saskatoon, as is Mona at both locations. Bonus flowers too late for the growing season at either location, but grows well. Harrington, the dominant variety in Saskatchewan, flowers later than Stirling in Western Australia and has been released for the longer growing season areas in that state. These details illustrate a relationship between heading date and adaptation to very different growing season conditions.

Heading date is a readily exploitable but complex trait, which is strongly correlated with the timing of floral initiation and, that event, with the duration of the juvenile stage, sensitivity to photoperiod, and the duration of the ear/stem growth phase. It is a characteristic of most commercial varieties grown over a summer season (e.g., Bonus and Harrington) that their juvenile stage is relatively long (Figure 2). This ensures that the vegetative phase is not truncated by their sensitivity to long photoperiods at the time of emergence. In contrast, commercial releases for winter growing conditions in Australia are characterized by a relatively short juvenile stage (Figure 2), hence the rapid flowering of Stirling at Saskatoon (Table 2). Data presented in this paper demonstrate that the duration of the juvenile stage and sensitivity to photoperiods are independent quantitative traits, with the timing of floral initiation at any one location, or seeding date within location, likely to depend on the levels of each in relation to photoperiod at the time of emergence.

The timing of floral initiation has other implications. Relative to seeding date, floral initiation determines the duration of the vegetative phase and, subject to rates, the number of initiated leaves and tillers, and leaf area and dry matter potentials at the conclusion of this phase, i.e., the vegetative foundation for yield determination. Even though the duration of the vegetative phase may be modest

relative to the length of the life-cycle, the timing of floral initiation triggers the initiation and growth of ears, and elongation of their subtending stems. This combination of events leads to an exponential increase in leaf area, dry weight and assimilate supply and, at the same time, a corresponding increase in demand for those assimilates. It is this balance between supply and demand (i.e., competition) that is pivotal to the translation of the early vegetative potential for yield into the actual potential at the time of heading. That competition commences shortly after the commencement of stem elongation (Figure 1) by establishing priorities amongst available sinks, even though the effects do not become manifestly clear until shortly before heading. The effect photoperiod has on reducing the duration of the ear/stem growth phase, and variation among varieties in their sensitivity to the effects of photoperiod over this phase, are major factors contributing to the intensity of this competition and to the survival of tillers and spikelets.

Acknowledgements

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Whole plant physiology in oat

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Oat (*Avena sativa* L.) has always struggled with its past and borne the stamp of a secondary crop, even in many important production areas. Oat cultivation in patches of arable land not deemed worthy for barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) is a clear indication of this disparagement. Furthermore, the world status of oat as a cereal crop has clearly decreased by some 60% since its golden days during the early half of this century. These diminishments have also been reflected in the intensity of research on oat: e.g., interest in oat crop physiology has been relatively modest compared to barley and wheat. This article represents an attempt to describe briefly some of the major components of source-sink relationships in oat, especially in connection with formation and expression of yield potential.

Formation of yield potential

An important objective in relation to formation of yield potential is to ensure early canopy closure, which allows efficient interception of photosynthetically active radiation (PAR) early during the growth stages, and hence, possibly increases set of spikelets and fertile florets through the direct effect of the large photosynthetic area and the indirect effect of formation of a large root mass. There is a close association between grain yield and vegetative growth rate in oat (Takeda and Frey, 1977; Bregitzer et al., 1987). Increasing growth rate is likely to be especially important because harvest index (HI), which is one of the three yield-correlated factors (along with growth rate and growth duration), is already probably close to its optimum in oat. However, this association between HI – an important indicator of the success in synchronization between sources and sinks – and grain yield varies according to genotype: e.g., McMullan et al. (1988) and Salman and Brinkman (1992) did not establish a trend of decline in grain yield when the maximum HI of the material was close to 46% and 54%, whereas Takeda et al. (1987) and Peltonen-Sainio (1991) found that association between grain yield and HI was curvilinear and the maximum grain yield occurred when HI was 45% and 55%. In the case of northern European oat material, an HI as high as 60% was associated with a slow rate of accumulation for vegetative phytomass, and the consequent inability to satisfy the demands for photosynthate of spikelet set, floret set and grain-fill (Peltonen-Sainio, 1991).

Reaching the optimum leaf area index (LAI) rapidly, and maintaining it, is

essential for high yield. There are crucial points of growth and development where the assimilate-producing source area could be further modified (Figure 1). The shaded area could be reduced by developing appropriate management practices or breeding for improved LAI. Leaf area duration (LAD), i.e., LAI integrated over cumulated degree days, seems to vary greatly: differences in LAD were about five-fold in Finland, and were linked to growing conditions, crop management and choice of cultivar (Table 1). The relationship between LAD and grain yield was curvilinear, but did not in general become negative, even at the highest LAD values, indicating oat's limited capability to benefit from high LAI in northern growing conditions.

Evans (1994) postulated that the attempts of cereal breeders to produce cultivars with a low proportion of sterile tillers has resulted in assimilate savings that are likely to be invested in set of florets and spikelets in addition to grain-fill. However, results of the experiments of Lauer and Simmons (1988) indicated that barley tillers, including non-surviving ones, paid the main shoot back with interest for the assimilates used for their growth prior to autotrophic growth and maintenance. In oat cultivars adapted to northern growing conditions, tiller death coincided with floret abortion, indicating a lack of photosynthate for simultaneous maintenance of multiple sinks (Peltonen-Sainio and Peltonen, 1995).

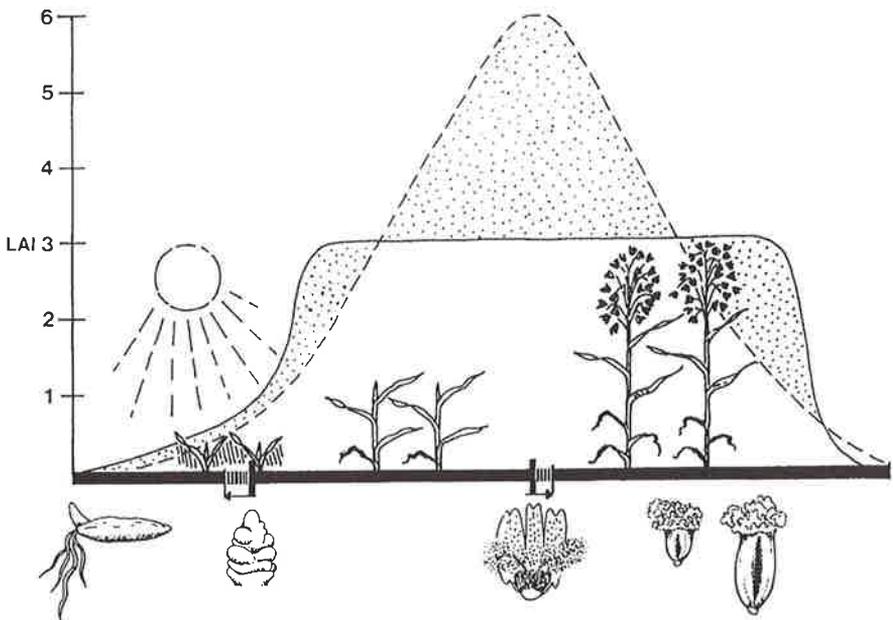


Figure 1. Hypothetical differences (shaded area) between theoretically optimal leaf area index (LAI) and actual LAI in oat. Suggested changes in length of vegetative, generative and reproductive phases of oat grown in northern growing conditions are indicated with arrows (Peltonen-Sainio, 1996).

Shortening of oat straw and the consequent increase in HI has been one of the main reasons for genetically determined yield increases in this century. Stem elongation occurring at the time of intensive floret set, in conjunction with competition caused by unproductive tillers, represent unnecessary competition for photoassimilates. Therefore, introduction of short cultivars has possibly enabled the use of photosynthetic products for set of spikelets and florets. Reduced partitioning of photoassimilates to stem elongation in short-stature cultivars, particularly at late pre-anthesis, may improve the ability to maintain a high number of florets through reduced abortion. Peltonen-Sainio and Peltonen (1995) indicated that in the oat panicle 75–90% of aborted florets were either fertile or incompletely developed, indicating an inability to realize a high yield potential. The number of initiated florets was low and the proportion of aborted florets was high at low rates of nitrogen fertilization application (Table 2). For such reasons it could be hypothesized that introduction of oat cultivars carrying the dwarfing gene would be beneficial for building and maintaining yield

Table 1. Representative data for the effects of crop management on leaf area duration (LAD) and grain yield of oat

Crop management	Total LAD	Tiller LAD	Grain yield (g m ⁻²)
<u>N fertilizer rate:</u>			
	-----Control-----		
0 kg ha ⁻¹	602	15	120
40	874	49	140
70	956	87	220
110	1354	104	290
150	1265	163	280
	-----Green manuring-----		
0	743	18	170
40	880	40	200
70	1139	110	260
110	1762	242	340
150	1639	189	330
S.E.	121	24	24
<u>Seeding rate:</u>			
	-----Naked lines-----		
400 seeds/m ²	2354	935	340
600	2700	794	360
800	3250	688	370
	-----Hulled lines-----		
400	2402	475	440
600	2701	239	480
800	2944	152	420
S.E.	84	66	10

potential. The main-shoot panicle of one dwarf line had, however, few florets and grains, and had low yielding ability in northern growing conditions. Such a disadvantageous characteristic, in a particular Minnesota-adapted dwarf line when grown in Finland, may result from the pleiotropic effects of dwarfism and an imbalance in the relative duration of growth phases.

Table 2. Response of two oat lines (Ryhti and Puhti), grown in a greenhouse, to nitrogen fertilizer application rates in terms of formation of yield potential and abortion of florets (Peltonen-Sainio and Peltonen, 1995)

Treatment	Number of florets		Number of grains		Aborted florets (%)	Panicle yield (g)
	maxi- mum	at DVS23	filled	empty		
Ryhti						
C	197	28	28	2	86	0.94
PM	197	28	27	3	86	1.01
EP	512	105	88	4	83	3.17
EM	512	105	86	3	83	3.07
S.E.			2	<1		0.07
Puhti						
C	238	35	21	4	91	0.75
PM	238	35	24	5	90	0.91
EP	400	122	98	7	76	3.31
EM	400	122	99	7	75	3.46
S.E.			2	<1		0.07

Abbreviations: C = control; PM = extra N from pollination until maturation; EP = extra N from emergence until pollination; EM = extra N from emergence until maturity; DVS23 = pollination

Table 3. Comparison of grain yield and tiller characteristics of dwarf (MN 90202), semi-dwarf (Hja76416), modern (Puhti) and landrace oat across years, nitrogen fertilizer application rates and seeding rates

Oat type	Grain yield (g m ⁻²)	Grains per		Head-bearing tillers per main shoot	Tiller contribution to grain yield per panicle (%)
		main shoot	tiller		
Dwarf	510	28	18	0.9	32
Semi-dwarf	630	46	11	0.5	14
Modern	660	47	5	0.2	7
Landrace	600	44	6	0.3	8
S.E.	84	<1	<1	<0.1	<1

Realization of yield potential

Photosynthetic activity of green tissue frequently declines before potential grain size is realized (Evans, 1994). Salman and Brinkman (1992) found that high-yielding oat cultivars had better growth rates and dry-matter accumulation during post-anthesis. Such superiority requires increased post-anthesis photosynthate production or an increased ability to build up (stem) reserves, the importance of which has not been investigated in depth for oat. The importance of the flag leaf as an assimilate source for grain-filling is evident, not least due to the free capture of PAR (least shading effects) and the short distance between the source and sink tissues. In the experiments of Bregitzer et al. (1987), grain yield of oat lines was not related to the photosynthetic area above the uppermost leaf sheath, whereas in northern European growing conditions, plant breeding has increased flag-leaf area by up to 40% during the last 60 years, and concomitantly grain yield has increased by 20% to 30% (Peltonen-Sainio, 1990). Crop management has had a great impact on realization of yield potential. For example, use of plant growth regulators decreased flag leaf area, which may indicate reduced ability of this leaf, closest to the panicle, to support grain growth, whereas nitrogen is likely to have the opposite effect on green leaf area (Table 1). This is, in addition to its effect on maintaining photosynthetic activity at post-anthesis, most likely to take place through increased production and translocation of cytokinins synthesized in root caps.

The requirement for highly synchronized source-sink interaction throughout the growth period can be somewhat exceeded at grain-filling if stem and additional reserves compensate for the reduced production of assimilates *per se*. Such reserves comprise excess products of photosynthesis at pre-anthesis, in particular. In addition to the indubitable role of stem reserves under stress conditions, they might also have some potential in non-stress environments. For example, "late abortion" of oat (husks developed without groat) could be avoided with an adequate continuous flow of photoassimilates for grain-filling. Stem reserves built up with excess carbohydrate could also decrease spikelet and floret death that occur particularly prior to heading (Peltonen-Sainio and Peltonen, 1995), because intensity of abortion is likely to be connected to severity of competition between several active, demanding sinks.

Even though, in general, high panicle weight is an indication of high yielding ability in oat, the internal structure of the panicle is crucial to the capacity of the vascular system to translocate photosynthetic products and to the rate of grain- and panicle-filling. Housley and Peterson (1982) found that a large number of spikelets per panicle – and not a high number of grains per spikelet – correlated positively with vascular tissue traits, indicating the translocation capacity for photoassimilates. Peltonen-Sainio (1991) indicated that improved productivity in oat has been achieved in Finland, not through prolonged duration of green area, but rather through development of cultivars with a shorter grain-filling period and an increased panicle-filling rate (by 30–35%). This was possible through increasing the number of grains per panicle (Peltonen-Sainio, 1990), but

not per spikelet. Furthermore, one of the main reasons for lower groat yield in naked oat cultivars, compared with husked ones after dehulling, is that naked cultivars produce a small number of spikelets per panicle, associated with a higher number of grains per spikelet (Peltonen-Sainio, 1994). Therefore, invariably, small tertiary grain was more often present in the spikelets of naked cultivars than those of conventional cultivars. In addition to limited capacity for translocation and filling, earlier differentiation of lower order grains in spikelets may also contribute to the differences in grain sizes within a spikelet.

Summary

Some of the important traits associated with formation and realization of yield potential are shown in Figure 2. Traits characterized are: short, lodging-resistant straw with high translocation and reserve building capacity; unculm growth habit with few vegetative tillers that contribute to rapid canopy closure and pay the main shoot back with interest before wilting (for northern growing conditions, in particular); high panicle weight associated with a high number of spikelets that include only primary and secondary grains; reduced abortion of florets; seasonal changes in flag-leaf angle to secure capture of PAR throughout the growing season; and phenotypic stability including resistance against barley yellow dwarf virus (BYDV).

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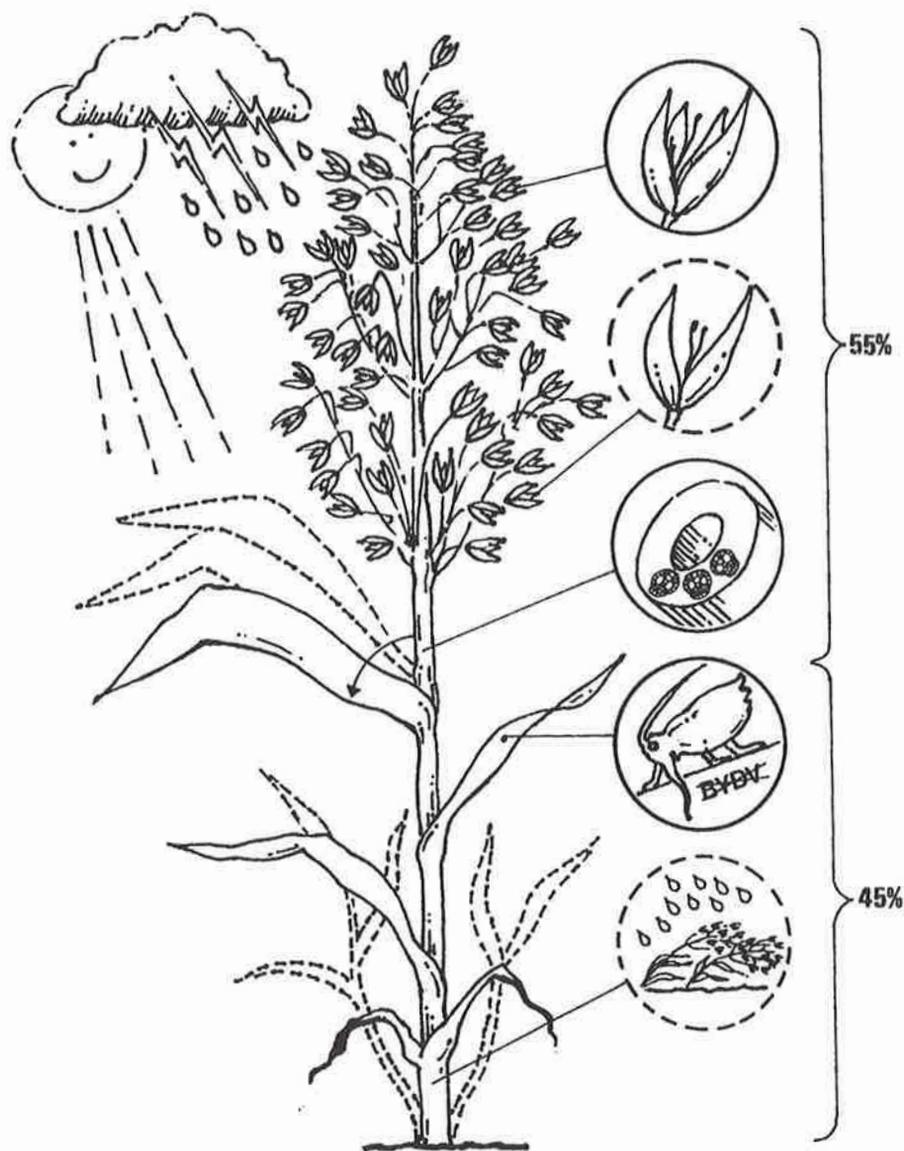


Figure 2. Hypothetical representation of oat characterized by traits that are important for formation and realization of high yield potential

Involvement of environmentally regulated genes in the adaptation of barley to cold and drought

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Introduction

Barley is grown from sub-arctic to semi-desert regions, showing a wide adaptability to a range of different ecological situations, but extreme stress, of course, can overcome its resistance, causing severe yield loss. Genetic studies have focused on the selection of those plant genotypes that are less subject to environmental stress factors so as to increase yields and yield stability. Thus, genetic variability plays a primary role in determining positive adaptation to environmental stresses and, hence, in supporting the spread of various barley genotypes to extreme climatic conditions (Cattivelli et al., 1994). Frost resistance in barley is an important agronomic characteristic because growing barley has been shifting over a number of years from spring to autumn sowing, since winter varieties are higher-yielding than spring cultivars.

The tolerance of winter cereals to low temperatures depends on the physiological process known as hardening or acclimation that occurs when plants are exposed to temperatures ranging from 0 to +5°C. Cold acclimation is defined as a physiological process involving a number of biochemical and molecular changes that enable certain plant species to withstand periods of very intense cold (Cattivelli and Bartels, 1992). In many barley growing areas where cold is not a problem, drought is probably the most important factor limiting the barley yield. Yet, compared to other cereals, barley is relatively well adapted to environments with a reduced water availability due to its better water-use efficiency (WUE). The adaptation of the plants to drought environments has been studied for a long time; nevertheless, very few traits are considered to be important in breeding today, and we still need to understand the drought-resistance mechanism to develop new genotypes for drought areas.

Genes regulated by low temperatures

The ability of the plants to survive under freezing temperatures involves many modifications at physiological and molecular levels (Cattivelli and Bartels, 1992). Frost-resistant plant cells show variations in some fundamental biochemical pathways (i. e. sugar and amino acid metabolisms – Murelli et al., 1995) as well as in the properties of the membranes (Wolter et al., 1992) and of the photosynthetic apparatus (Huner et al., 1993). Studies undertaken to understand the molecular processes controlling such variations have led to the isolation of many cold-regulated (COR) genes. In barley, probably more than 20 cDNA clones in which expression is affected by low temperatures have been isolated (Cattivelli and Bartels, 1990; Dunn et al., 1990; Dunn et al., 1991; Goddard et al., 1993; Hughes et al., 1992). Most of these genes are expressed only at low temperatures, while only a few are involved in plant response to other environmental stress situations (see below for the relation between cold and drought). The expression of the COR genes is controlled by different regulation mechanisms: some COR sequences have been shown to be transcriptionally regulated, while others are controlled by post-transcription mechanisms (Dunn et al., 1994). The analysis of the expression pattern reveals that the COR mRNAs reach their steady-state within 2–3 days of exposure to cold, while, when the plants are moved from low temperatures to 20°C, the level of COR mRNAs drops in a few hours (Cattivelli and Bartels, 1990). Even though many barley COR genes have been sequenced, important homologies have been found in only a few. The clone *blt4* shows an interesting homology with genes coding for lipid transfer proteins (Hughes et al., 1992); a second barley COR gene (*blt63*) codes for a translation elongation factor 1 α (Dunn et al., 1993); while the clone *cdr29* (regulated by cold and drought) is homologous to the genes coding for acyl-coenzyme-A oxidase (Grossi et al., 1995).

In order to understand more about the possible function of COR genes, specific antibodies can be used to study the corresponding COR proteins. A detailed description of the accumulation of a small barley COR protein of 14 kDa (COR14), encoded by the COR gene *pt59*, has been reported by Crosatti et al. (1995). The accumulation of COR14 was monitored throughout hardening and subsequent de-hardening in leaves of barley plants. COR14 was detected after about 7 days' exposure to low temperatures and gradually accumulated over a period of up to 4 weeks. When the plants were subsequently transferred to 20°C, the COR14 persisted for at least 6 days, a finding in contrast with a previous study showing that the messengers corresponding to *pt59* disappeared a few hours after the temperature was raised (Cattivelli and Bartels, 1990).

Analysis of the proteins in the subcellular fractions indicates that the COR14 is accumulated in the stroma fraction of the chloroplasts. The chloroplast localization suggested a possible involvement of light in regulating COR14 accumulation. The role of 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, light markedly stimulates gene expression and is needed for the protein's accumulation. The regulation of gene *pt59* does not require the concomitant presence of both factors: indeed, a plant only needs to be exposed to light for a short time (5 minutes) 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-minute light exposure is enough to induce this factor enough 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 light, or of chloroplast activity, influences COR14 accumulation (Crosatti et al., 1995).

The accumulation of COR proteins is associated with the development of cold hardening

Since the function of many COR proteins is still unknown, one possible approach to demonstrating the importance of the molecular response to low temperatures is to look for a correlation between the amount of mRNAs or proteins and the degree of frost resistance.

For instance, the analysis of the COR genes' expression patterns has sometimes shown a positive correlation between the accumulation of COR transcripts and the degree of cold resistance in some barley cultivars (Dunn et al., 1990), though in other cases the same approach did not allow cold-susceptible cultivars to be distinguished from cold-resistant cultivars of barley via COR-transcript expression (Dunn et al., 1991) or COR protein accumulation (Crosatti et al. 1994).

A clear correlation between the degree of freezing tolerance and the accumulation of a specific COR protein has been found for the wheat protein WCS120. The corresponding antibody not only discriminates between frost-resistant and frost-susceptible wheat cultivars, but, because it also recognizes the WCS120 homologous protein of related cereals, it has been proposed as a marker to select for freezing tolerance in all Gramineae species (Houde et al., 1992).

The physiological evidence that frost-resistant cultivars have faster hardening and slower de-hardening compared to frost-susceptible genotypes (Rizza et al., 1994) suggested that the cold acclimation process may have different threshold induction temperatures for spring and winter barleys. We have tested this hypothesis by using the antibody raised against the COR14 protein and 30 barley cultivars previously evaluated for their level of frost resistance (Crosatti et al., 1996). All these cultivars were cold-acclimated for 7 days at constant temperature (6°, 8° or 10°C) and subjected to Western analysis. When the winter frost-resistant cultivars were compared with the spring frost-sensitive ones, a clear difference was detected: at a temperature of 8°C, COR14 was accumulated in the former but not in the latter cultivars, indicating that, in barley, a higher degree of frost resistance is associated with a higher threshold induction temperature for the accumulation of COR14 protein. These results provide a good example of how molecular markers, such as the antibodies for WCS120 or COR14, can be either

a breeding tool for selecting superior genotypes with increased frost resistance or a useful marker for studying plant adaptation to different thermal environments.

The adaptation of the plant to cold and drought involves a common set of genes

The ability of the plants to survive freezing temperatures also involves the capacity to withstand drought. Therefore, it is not surprising that several genes involved in cold response are also expressed during dehydration and vice versa. Of course, a number of modifications of gene expression specific for cold or for drought response have also been observed (Grossi et al., 1992). When barley plants are subjected to a reduction in water availability, the typical molecular response leads to the accumulation of a class of dehydration-related proteins called dehydrins. These polypeptides are characterized by a consensus 15 amino acid domain rich in lysine (EKKGIMDKIKEKLPG) that may be present several times in the sequence. In many dehydrins an additional characteristic domain rich in serine is present adjacent to the lysine rich domain (Close et al., 1993). In barley about 10 dehydrin genes have been cloned; studies of their chromosomal localization have shown that dehydrin sequences are spread over all the barley genome. Genes *dhn1* and *dhn2* are localized on chromosome 7 (van Zee et al., 1995), while *dhn3*, *dhn4* (Heun et al., 1991) and *dhn5* map to chromosome 6, and *dhn6* to chromosome 4 (van Zee et al., 1995).

While probably all dehydrin genes are expressed under dehydration or by an exogenous abscisic acid (ABA) application, only a few are induced during cold acclimation in barley. In Crosatti et al. (1994) we described a COR protein of 75 kDa (COR 75), representing the most abundant COR protein in barley. COR 75 was shown to be induced by an exogenous ABA application as well as by drought stress. By using a polyclonal antibody, COR 75 was found to be immunologically related to the high-molecular-weight COR proteins of wheat (Crosatti et al., 1994). After purification and N-terminal microsequence, this protein was identified as DHN5 (F. Rizza, unpublished results). Recently, van Zee et al. (1995) have shown that among the dehydrin gene family, from *dhn1* to *dhn6*, *dhn5* is the only one that, besides the induction by ABA or drought, is also expressed under low temperatures.

A feature common to all dehydrins is their induction by ABA, and indeed promoter sequences that mediate the ABA responsive transcription have been described for several dehydrin genes (Robertson et al., 1995); nevertheless, we have found that in barley there are also genes coding for proteins very similar to the dehydrins whose expression is independent from ABA. An example is the cDNA clone *paf93*: this clone was originally identified as induced by low temperatures (Cattivelli and Bartels, 1990), though recently its involvement in the dehydration response has also been described (Grossi et al., 1995). The protein sequence deduced from the cDNA clone *paf93* shows a serine cluster and four lysine-rich domains, demonstrating its homology with the dehydrin proteins.

The role of ABA

The exposure of plants to water deficit during growth results in an increase in ABA, and this hormonal variation has been often associated with the expression of ABA-responsive genes (typically the dehydrins). Many experiments with different plant species have also found a correlation between the accumulation of ABA and the development of cold hardening (Ryu and Li, 1994; Lång et al., 1994). So far, it seems that ABA plays a key role in the adaptation of the plants to limited environments, though the mechanisms through which the hormone acts are still not completely understood. In barley, the accumulation of ABA in response to low temperatures is clearly different from response to drought. While during dehydration a fast and linear increasing in ABA content occurs (Grossi et al., 1995), during hardening no variations in ABA were detected, with the exception of a 24-hour peak during the first few days. This peak was shown to be higher in frost-resistant cultivars (Murelli et al., 1995). Several other works have recently reported that plants respond to low temperatures with a transient ABA accumulation at the beginning of acclimation that correlates with frost resistance (Ryu and Li, 1994; Lång et al., 1994). Because it is well known that ABA can modify gene expression, even a transient accumulation of ABA might affect the molecular response to low temperatures by inducing ABA-regulated genes.

In barley a large number of ABA-regulated and drought-induced genes are known; however, several non-ABA-regulated genes have also been identified in response to desiccation or drought stress (Grossi et al., 1995). It is arguable whether all these genes are really expressed by cereal plants during their life cycle when a drought stress event occurs. Our findings indicate that, despite the real water deficit induced in non-irrigated plants grown in a typical Mediterranean environment (south Italy) that led to a significant reduction of grain yield and rise in ABA content, only drought-induced but ABA-independent genes (i.e., *paf93*), and not the dehydrin genes, were expressed in the still-green plant tissues. It would therefore seem that the expression of dehydrin genes under field conditions requires a more severe water deficit and/or a greater rise in the ABA content than that produced by a drought condition able to reduce plant yield of about 25% (Lacerenza et al., 1995).

Conclusions

The cloned genes provide tools for the understanding of the molecular mechanisms involved in the adaptation of the plants to limited environments. It is generally accepted that the expression of stress-related genes is an essential part of the plant adaptation processes, though for many genes direct evidence of their function is still lacking. The use of transgenic plants expressing sense and antisense constructs is expected to be the most powerful tool in understanding the role of cloned genes. Unfortunately, the transformation of barley is still not routine; nevertheless, in model plant species such as tobacco and *Arabidopsis*,

new genotypes with an improved adaptation to cold (Murata et al., 1992) or drought (Holmström et al., 1996) conditions have already been produced.

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Use of rhizobacteria as biofertilizers for enhancing growth and yield of crops

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Introduction

How often we look out over a field of growing wheat or other crops, and think to ourselves: "How great and healthy this crop looks; it's going to be above average yields this year." As we marvel at the visible plant biomass we see, it is easy to forget that below the soil surface exists a hidden world of microorganisms that are essential for healthy and productive soils. These microorganisms do many things that affect crop production. They cycle nutrients between available and unavailable forms, produce chemicals that stimulate or inhibit root growth and elongation, and may cause and prevent plant diseases.

It has long been recognized that many different kinds of soil bacteria and fungi can be used as seed or soil inoculants to promote the growth of various vegetables and field crops [Brown, 1974; Gaskins et al., 1985; Okon and Harder, 1987]. However, it is impossible in a short review to examine the past 80 years of research on using microbial inoculants to enhance crop production. Likewise, it is impossible to cover all of the different types of organisms that have been tested and heralded as effective inoculants. Consequently, my objective is to focus on the use of rhizobacteria as *biofertilizers*. My definition of a biofertilizer is *any microorganism added to seed or the soil to increase or sustain plant growth*. I hope to convince readers that biofertilizers can be successfully used in agriculture by providing information on the nature of rhizobacteria, where they live, what they do to affect plant growth and examples of field trials using biofertilizers. At the very least, perhaps my review will stimulate some readers to seek more information on this topic.

The rhizosphere

The term *rhizosphere* was coined by Hiltner in 1904, and was used to describe the zone of intense bacterial activity around roots of Leguminosae [Curl and Truelove, 1986]. Since then, the term has been defined and redefined many times as researchers debate the definition of a special habitat near the root surface that

consists of a zone of soil as small as one or two millimeters. In fact, a number of other terms are sometimes found in the literature in reference to the rhizosphere, including: the *endorrhizosphere* (defined as the various layers of the root itself); the *ectorrhizosphere* (the area immediately surrounding the root, but which can sometimes extend substantial distances into the soil); and the *mycorrhizosphere* (which refers to the area of soil under the influence of the hyphae of mycorrhizal fungi). Although these terms may be used as synonyms for the rhizosphere, they are not as universally accepted.

The rhizosphere is recognized as the region of soil immediately surrounding the root that is directly under the influence of root exudates, and which is characterized by an increased level of microbial activity. The *rhizoplane* refers to the actual root surface. Hiltner recognized that plant root exudates attracted both beneficial and detrimental microorganisms that could (and often did) affect plant nutrition and growth. It is the composition of these exudates that ultimately influences the types of organisms one finds in the rhizosphere, and the interactions occurring between microorganisms and plants. Therefore, different crops such as corn, canola, lentil and wheat will support distinct populations of microorganisms. During the past 80 years extensive research on various crops has expanded our understanding and appreciation of the rhizosphere and rhizoplane as unique environments supporting the growth and activity of a multitude of microorganisms, including many groups of bacteria and fungi. As we isolate new bacteria and study their association with roots, it becomes clear that these organisms may be classified based on their functional diversity. For example, deleterious rhizobacteria (DRB) live on plant roots and can cause root rot when environmental and biological factors are favorable. Endophytic bacteria live inside roots, in the xylem or phloem, and may be beneficial or detrimental to plant health. Plant growth-promoting rhizobacteria (PGPR) and yield-increasing bacteria (YIB) are rhizosphere bacteria that have the ability to sustain or increase plant growth, and have the most potential for use as biofertilizers.

Plant growth promoting rhizobacteria as biofertilizers

The term *plant growth promoting rhizobacteria* (PGPR) refers to those bacteria that enhance plant growth either directly, by affecting plant physiology or metabolism, or indirectly, through mechanisms such as solubilizing nutrients and making them more available for plant uptake [Kloepper et al., 1989]. Many different types of bacteria, including species of *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Clavibacter*, *Micrococcus*, *Pseudomonas*, *Serratia* and *Xanthomonas*, are recognized as PGPR and have been studied as biofertilizers. These bacteria may be endophytes or colonize the rhizoplane or rhizosphere of host plants. Many have unique metabolic properties such as the ability to fix atmospheric nitrogen in a free-living state (e.g., *Azotobacter* and *Azospirillum* spp.), others exhibit rapid growth rates or produce extracellular polysaccharides that facilitate adhesion to roots (e.g., *Pseudomonas* spp.), and some form resting cysts or spores that allow them to survive stress

conditions (e.g., *Azotobacter* and *Bacillus* spp.). Usually these bacteria are identified based on their ability to promote plant growth in lab or growth chamber studies, but extensive field testing is also important to verify their potential as biofertilizers.

Biofertilizers (i.e., PGPR) affect plant growth in a number of ways, which vary depending on bacterium and plant. Because many PGPR are used as seed inoculants, the most common effect on plant growth is enhanced or altered root development (Table 1). These effects are sometimes reflected in early seed germination, plant emergence and increased seedling vigor; plant yield may or may not be affected. The success or failure of a biofertilizer often depends on the initial cell density applied to a seed. Too few PGPR cells and the inoculant is unable to out compete indigenous bacteria and fails to colonize roots, whereas too many PGPR cells is detrimental to seed germination and initial root development. Field variability can also mask the effects of biofertilizers, and usually this relates to the mode of action by which a PGPR affects plant growth.

Table 1. Some effects of biofertilizers on roots

- Stimulates the density and length of root hairs
 - Stimulates the rate of appearance of lateral roots and root surface area
 - Stimulates leakage of root exudates
 - Stimulates membrane activity and levels of free auxins such as indole-3-acetic acid
 - Stimulates root nodule formation of legumes
 - Stimulates arbuscular mycorrhizal colonization of roots
 - Stimulates uptake of minerals and water by roots
-

Modes of action of PGPR biofertilizers

Many mechanisms are proposed to explain how PGPR affect plant growth, but five modes of action have received the most attention. One popular theory is that PGPR produce auxins or metabolites that act as phytohormones and stimulate root hair development and/or root length and biomass. There is an increasing body of evidence that many effective PGPR biofertilizers do produce auxin-like compounds, and this would explain the typical response of roots to PGPR inoculants [Glick, 1995]. A second mechanism is that PGPR produce enzymes or metabolites that increase root permeability, and thus alter nutrient and water uptake. The direct solubilization or mineralization of nutrients, making them more available to the plant, is another way PGPR biofertilizers can affect plant growth. This mechanism has been demonstrated for a number of biofertilizers, some of which are being commercially marketed. A fourth mechanism is the ability to act as biological control agents (i.e., BCAs). In this case, PGPR act through the competitive displacement of pathogens, or by producing metabolites that inhibit pathogen activity. There is a great deal of evidence that many effective PGPR produce siderophores or antibiotics that inhibit growth and

activity of root rot fungi. A number of BCA products are commercially available. Recently, some PGPR have been shown to stimulate natural plant defense mechanisms, such as the induction of systemic resistance [Tuzun and Kloepper, 1994]. In these cases, the PGPR either interact with plant roots directly, which results in the plant turning on defense mechanisms, or, alternatively, the PGPR affect other rhizosphere microorganism(s), which then interact with plant roots. In either case, the plant is protected from diseases or insect pests.

Field assessment of PGPR biofertilizers

Once potential PGPR biofertilizers are identified in growth chamber and greenhouse studies, it is necessary to validate their ability to affect plant growth and yield under field conditions. Unfortunately, many promising PGPR are not effective under field conditions. Field variability is a common problem encountered when testing PGPR, and might reflect the mode of action through which a PGPR acts [Okon and Labandera-Gonzalez, 1994]. For example, PGPR selected because of their ability to serve as biocontrol agents against root rot fungi will appear ineffective if there is no disease pressure in the field. Similarly, PGPR that act as biofertilizers by solubilizing nutrients such as phosphorus will not be effective if soil-P levels are adequate for plant growth. Two additional key reasons for

Table 2. Examples of reported crop yield increases in field studies with PGPR biofertilizers from 1974 through 1988

<u>Inoculant/Crop</u>	<u>% yield difference from control</u>	
	<u>lowest</u>	<u>highest</u>
<i>Azospirillum</i> spp.		
Maize	6.7	75.1
Millet	-12.1	31.7
Mustard	16	128
Rice	4.9	15.5
Sorghum	12	30.5
Wheat	-9.6	31
<i>Bacillus</i> spp.		
Peanut	-6.1	37.4
Potato	-16	12
Sorghum	15.3	33
Wheat	0	114
<i>Pseudomonas</i> spp.		
Canola	0	57
Potato	-14	37
Rice	3	160
Sugar beat	-11	32
Wheat	1.9	26.3

Source: adapted from Kloepper et al. (1989).

PGPR failures are: (1) the inability of the inoculant to effectively colonize the host plant root system, and (2) the variability in soil chemical and physical factors that limit inoculant activity [Kloepper and Beauchamp, 1992].

Despite the problems encountered in field testing biofertilizers, there is good evidence that plant growth promotion does occur, and benefits to the producer are significant. A large number of field trials have been conducted by workers around the world with many different PGPR biofertilizers [Kloepper et al., 1989; Okon and Labandera-Gonzalez, 1994; Tang, 1994]. Yield increases ranging from 6% to 160% above control have been reported (Table 2), but on average the difference from control ranges from -2% to 42%. A number of studies in Canada since 1990 have shown that some PGPR biofertilizers isolated from one crop (e.g., wheat) are more effective on that crop than other crops, whereas other studies have shown that biofertilizers are effective on a range of crops [Chanway and Nelson, 1990; De Freitas and Germida, 1990; 1992; Germida and De Freitas, 1994]. Repeated field trials with these PGPR biofertilizers on wheat and canola show yield increase of about 2 to 2.5 bu ac⁻¹. The reasons for the variations in response of some plants to specific inoculants probably is related to the way the field trials were conducted. In fact, a major problem in the assessment of PGPR biofertilizers is the lack of a standard inoculant formulation and delivery system. These issues are being addressed by the seed coating and microbial inoculant producers, and a number of new, effective delivery systems are in development.

Conclusions

The more we learn about the diversity and activity of microorganisms living in soil, the better our chances to exploit these organisms for our own use. For example, farmers have long used rhizobium inoculants to reduce nitrogen fertilizer inputs. They accomplish this by exploiting the nitrogen fixation potential of the legume-rhizobium symbiosis. Rhizobia inoculants are produced commercially and successfully marketed because we understand how this plant-microbe system works, and we can identify and explain the factors responsible when it does not work. However, we do not fully understand how all PGPR biofertilizers work and why they are not always effective. Additional research will resolve these issues. Furthermore, because producers and the industry seek alternatives to chemical inputs, commercial production and marketing of various microbial inoculants as “*biofertilizers*” is now recognized as a growing agbiotech industry.

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Cereal pathology in the twenty-first century

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It does not do to leave a live dragon out of your calculations if you live near to him (Tolkien, 1937). Cereal diseases are just such a dragon, and all of us who are working with cereals are living near to him. The intensity of the dragon's fire and smoke may vary from season to season, but he is alive and well and capable of causing damage in most cereal-growing areas of the world.

Cereals are affected by a range of economically important diseases that may reduce both yield and quality. Before we look forward to the 21st century, it is important to pause and look back at the incidence of diseases and the origins and aims of breeding for resistance to set the scene.

Pests and diseases of cereals are as old as agriculture itself. The effects of mildew diseases and of insect pests, including locusts, are recorded in the Old Testament. Cereal rusts are known to have been important during the period of the Roman Empire, and it has been postulated that exhaustion of the soil at this time was probably caused by parasitic nematodes. The Romans invoked the aid of gods to keep their crops free from disease, and epidemics were attributed to the wrath of the gods (Russell, 1978).

Historically, the Chinese had a good knowledge of the causes of plant disease and for more than 2000 years have used practices to control crop diseases. These measures include crop rotation, fallow, using quality seed, seed treatment, sanitation, soil management, timely sowing, harvesting and suitable crop density (Bin-Cheng Zhang, 1989). Plant resistance to disease and insect attack is the rule rather than the exception.

Humans, in the course of the development of agriculture, have removed the onerous task of producing their own food from the vast majority of the community while maintaining or increasing the quantity and quality of food products. This

capacity of the few to feed the many is the key to the standard of living enjoyed by many of us. The consequence of this agricultural efficiency has greatly increased the potential for disease and pest attacks.

To some degree this has been brought about by:

1. moving crops into new areas. Diseases and insects that were of little consequence on the natural vegetation may become problems on introduced crops.
2. spread of diseases into new areas. This spread is the result of increased global travel, which began in the mid-1800s, and increased the possibility of transport of pathogens by non-natural means around the world.
3. crop production practices, including some tillage practices, irrigation, fertilization and herbicide usage, causing lush plant growth that is often more susceptible to disease.
4. plant breeders who have been selecting for yield, plus uniformity in crop maturity. The range of germplasm used for crossing has often been limited, so it is not surprising that problems of susceptibility to diseases have arisen. This highlights the need for preserving diverse plant material, so that there is a wide genetic basis available for breeding.

The interest in breeding for disease and insect resistant plants is by no means recent: it has been mentioned in the literature for over 200 years. This interest is due in some part to the following factors:

1. concern, whether well founded or not, over the contamination of the environment by chemicals used for disease or insect control.
2. the fact that insecticides and fungicides are not specific and may eliminate beneficial species
3. plant resistance is the only alternative for crops that have low profit margins per hectare.
4. plant resistance is the only alternative for the control of virus diseases that are spread from alternative host crops by insect vectors such as aphids.
5. the cost of breeding disease or insect resistant plants is low relative to the recurring costs of chemical control.
6. plant resistance to disease or insect attack often extends over much or all of the growing season, while chemical controls may be short-lived.

From the preceding points it might appear that we are suggesting that chemicals are obsolete, but this is not the case. Chemicals are often a very important first step in any disease or insect control program; however, we believe genetic resistance is the ultimate aim.

The ability to identify, modify, transfer and clone genes will transform breeding for disease resistance. Currently, DNA-based markers for genes imparting resistance to stem, leaf and stripe rusts; powdery mildew, scald, net blotch, BYDV and BYMV have been identified. Marker-assisted selection will not necessarily decrease the time for cultivar development but will allow for more directed improvements, especially for disease resistance. Perhaps the greatest impact of marker-assisted selection will be for the effective utilization of resistant genes from wild relatives. There is a danger that the application of these markers could

lead to an overuse of race-specific genes in many breeding programs throughout the world, so these resistances would be at risk of breaking down. Pyramiding genes aims at increasing the durability of resistance but may enhance the selection for more complex forms of pathogen virulence. Perhaps we need to move from strategies involving gene deployment based on geography and initiate strategies based on rotation of genes, especially for pathogens that overwinter on stubble. Disease resistance genes have been identified in plants, and subsequently cloned and characterized.

In the future, resistance genes may be cloned instead of attempting to identify markers for resistance through mapping. A great deal of progress has been made for genetically engineered disease resistance through plant transformation. However, only time will prove if these resistances will persist. It is probable that plants do not use many different ways to effect disease resistance, and that virulent pathogens do not overcome resistant mechanisms but avoid detection. If we focus on understanding recognition we may be able to extend the durability of recognition genes and reduce the ability of the pathogen to avoid detection. Durability of pathogen recognition is the real challenge facing plant breeders.

With recent advances in molecular biology it is often easy to overlook the potential influence of other factors on plant diseases. The most important change is a shift from conventional to conservation tillage practices, where crop residues are left on the soil surface, which may favour the build-up of some plant pathogens (Watkins and Boosalis. 1994).

The combination of increased adoption of conservation tillage and the continued popularity of rotations where cereal follows cereal increases the potential impact of residue-borne diseases like net blotch and scald of barley, and tan spot and septoria leaf and glume blotch of wheat under moisture conditions that promote disease. Recent survey results from Alberta suggest that the increase in net blotch severity observed under zero or minimum tillage may be reduced by planting a non-host crop between barley crops rather than using a rotation with back-to-back barley. The use of disease-resistant varieties may also help to counteract any potential increase in disease severity under conservation tillage. Plant diseases can be managed quite effectively under conservation tillage by the use of sound rotations and agronomic practices, and careful choice of variety. Advances in the development of disease resistance could counteract any increase in disease risk as crop production systems move from conventional to conservation tillage. It may also be necessary to monitor soil biology in conservation tillage systems to determine mechanisms of disease suppression that have been observed in experiments and farm fields.

In the 21st century, access to research information will become more private. Recent reports have identified potential biological control agents for a range of plant diseases. Additionally, compounds isolated from soil microflora, plants and pathogens will likely be identified that will contribute to the control of diseases. However, much of this information is proprietary and will probably be commercialized through industry rather than the public domain.

Disease outbreaks will still occur in the 21st century, so researchers must make disease resistance, cultural and biological control of diseases and sustainable agriculture the frontiers to be conquered.

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Breeding for partial resistance in oat to rusts

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Experience with the cereal rusts has shown that hypersensitive resistance conferred by single genes gives protection for only a short time before races of the pathogen appear that render it ineffective. Several strategies are being employed or being considered to provide more durable protection. Combining two or more genes for hypersensitive resistance in a single cultivar aims to create the same complete control of rust that was the original goal of using single resistance genes. This strategy places intense selection pressure on the pathogen, but the presence of more than one resistance gene in the host is thought to reduce the likelihood that the pathogen will accumulate all the required matching virulence genes in an otherwise fit genotype. This strategy seems to control wheat stem and leaf rusts in the spring wheat regions of North America.

Other strategies are based on a philosophy of disease management rather than disease control. The goal is not to suppress the pathogen population entirely, but to prevent it from reaching destructive levels. Among these strategies is the use of partial resistance. In this discussion, I will distinguish between moderate resistance, whose phenotype is an intermediate infection type, and partial resistance, whose phenotype is a susceptible infection type coupled with a quantitative resistance that interferes with pathogen reproduction (Ohm and Shaner, 1992). Moderate resistance may be conditioned by single genes, such as *Pc-35* or *Pc-47* (Harder and Haber, 1992). Partial resistance, as used here, and as defined by Parlevliet (1979), is equivalent to slow rusting (Ohm and Shaner, 1976).

Oat crown rust disease, like other cereal rusts, can be thought of as a process of growth of a pathogen population in a host substrate. The size and physiological status of the host population set a limit on growth of the pathogen. The closer the pathogen population approaches its limit, the greater the damage to the host. Partial resistance retards growth of the pathogen population. Partially resistant cultivars are a less congenial substrate for the pathogen, but whether this is due to active defense responses or by virtue of being a poorer source of nutrients is not known. A completely resistant cultivar is an entirely unsuitable substrate, and the pathogen fails to reproduce. A partially resistant cultivar is not completely inimical to growth of the pathogen population, but retards it to some degree. For purposes of disease management this retardation should be sufficient to prevent

unacceptable damage to the host.

Partial resistance is a quantitative trait. It lies anywhere between complete resistance and full susceptibility. Breeding for partial resistance poses the same challenges as breeding for any quantitative trait. One must be able to distinguish environmental variance from genetic variance. Partial resistance may be under the control of several to many genes, and expression may vary with age of the plant. In contrast to quantitative traits generally, the pathogen, as well as the plant (host) and the environment, influence variability in partial resistance. The pathogen's fitness depends in turn on its own genetic makeup and the environment. The amount of primary inoculum may have a large effect.

Partial resistance is a complex trait, the consequence of the interaction of several components: reduced probability that a spore will infect, longer latent period, smaller pustules that produce fewer spores, and possibly a shorter infectious period (Parlevliet, 1979). These components of resistance delay or reduce the magnitude of various stages in the pathogen's reproductive cycle (Shaner and Hess, 1978). Their combined effect, magnified with each cycle of infection, slows the progress of rust development in the field. The limited work on components of partial resistance in oat suggests that reduced infection frequency, slower growth of the fungus in host tissue, and smaller pustules contribute to this resistance (Heagle and Moore, 1970; Kochman and Brown, 1975; Luke et al., 1984).

Disease progresses slower in the field on partially resistant cultivars than on susceptible cultivars because the pathogen population grows slower. Breeders who work with hypersensitive resistance to rust typically make a single assessment in the field to detect lines that have little or no disease and show a characteristic infection type. If partial resistance retards growth of the pathogen population, then a single quantitative assessment should identify lines with the desired degree of resistance. Traditionally, rust workers have used the percentage of leaf or stem area occupied by pustules as a measure of disease severity. This concept was introduced by Nathan Cobb in 1892. Cobb drew leaf diagrams with different densities of rust pustules to assist in visual estimation of percent severity. Later, Peterson et al. (1948) elaborated these diagrams by depicting pustules of various sizes. They set an actual pustule coverage of 37% equal to 100% severity, because this is about the maximum density of pustules that can be supported on a leaf. For most cereal rusts, a distinct pustule of determinate size is produced as a consequence of each infection, so disease severity is a direct measure of pathogen population size.

Although visual estimates of severity are sometimes referred to as subjective, with the implication that they are therefore not reliable, they are not subjective in the same sense that appreciation of a painting or a musical composition depends on the eyes, ears, and personality of the observer. When two or more observers examine the same rusted leaf, if they understand the principles behind severity assessment and something of the biology of rust, they should arrive at similar estimates of severity, within the limits of "instrument" error. In fact, experienced observers give remarkably similar assessments. To improve the reliability of visual

assessments, it is important that comparable leaf layers are observed on each line. For cereal leaf rusts, often the flag leaf or the leaf below is assessed, rather than the whole plant. It is informative to note the host growth stage when disease is assessed.

A more objective method of assessment would reduce observer error, but no truly objective method has been devised that is rapid enough for most field applications. Counting and measuring pustules is not practical unless many observers can be enlisted. Photographic or video imaging techniques have potential, but these are expensive and slow. It does not take long for an experienced observer to examine a plot and record severity, perhaps only a few seconds, and no device I am aware of can match this speed. If hundreds of lines must be examined in a day, as is typically the case in a breeding nursery, I see no practical substitute for visual assessment at the present time. Perhaps, in the future, some kind of scanner that reliably distinguishes rust from other spots and blemishes, and that instantly yields a quantitative severity value, will be devised.

In practice, a single assessment may not suffice to detect partial resistance in the field. Rust is a polycyclic disease that typically begins with very low levels of infection, even if plots or disease spreader rows are inoculated heavily. If severity is assessed too early, there will not be sufficient distinction among genotypes to recognize partial resistance reliably. Likewise, if assessment is overly delayed, senescence and the surge in inoculum pressure late in the epidemic may obscure effective levels of partial resistance in all but the most resistant lines. In general, it seems advisable to delay assessment until disease is well developed on susceptible check cultivars, but not to wait until the upper leaves are beginning to senesce. Different dates of heading among cultivars and lines will confound assessments. The upper leaves of earlier-maturing lines will have been exposed longer to inoculum than comparable leaves of later-maturing lines. At any given time, for lines of equal partial resistance, the earlier-maturing line is likely to show a greater severity of rust than the later-maturing line. If weather conditions are not uniformly conducive to rust during the season, interactions between weather and rates of plant development may confound interpretation of disease assessment data.

In research on partial resistance in the field, workers often make multiple assessments of disease to deal with the problems outlined above. Various summary statistics are then used to compare lines for partial resistance. Least squares regression is applied to a linearizing transformation of severity data to estimate the rate parameter for disease progress (Campbell and Madden, 1990). For cereal rusts, in which severity is based on the density of pustules on leaves or stems, this rate parameter should estimate rate of rust fungus population increase. Partial resistance is expected to retard the rate of rust increase, but rust often increases on partially resistant cultivars as rapidly as on susceptible cultivars. The disease progress curves differ in position, but not in slope. In practice, resistance that reduces the rate of disease increase also reduces initial severity, and this will displace the disease progress curve to the right (Gilligan, 1990). The displacement that is commonly observed, without an associated reduction in infection rate,

may arise from interplot interference. For example, Luke et al. (1972) compared disease progress on several cultivars of *Avena byzantina*. There were substantial differences in final severity, but the logistic rate parameter was not correlated to final severity ($R=-0.059$). We have observed a similar phenomenon for partial resistance in wheat to leaf rust (Shaner and Buechley, unpublished).

A useful summary statistic for quantifying partial resistance is the area under the disease progress curve (Shaner and Finney, 1977). This statistic requires no assumptions about the shape of the disease progress curve, and it reveals small differences in degree of partial resistance (e.g., Singleton et al., 1982). To be reliable, area under the disease progress curve for cereal rusts requires that sequential assessments be made no more than 1 week apart, and that a minimum of three assessments be made. This is not practical for a large breeding nursery containing early-generation head rows, but is feasible for testing potential parents in a breeding program. For large breeding nurseries, two assessments are probably all that time permits, the first when rust severity on susceptible checks is 40–60% and the second about one week later. Because the reliability of assessment depends on uniformity of rust development throughout the nursery, it is important to have a susceptible check cultivar planted frequently in the nursery. Parents with partial resistance should be planted frequently to measure $G \times E$ effects.

Many genes for race-specific, hypersensitive rust resistance have been identified in cereal crops. Most modern cultivars and well adapted breeding lines carry some of these genes, even though they may not be resistant to all races of the pathogen where they are cultivated. Because breeders use this elite germ plasm in their crosses, genes for hypersensitivity are likely to be segregating in breeding populations for which it is desired to improve partial resistance. When a plant is inoculated with races that lack matching genes for virulence, such genes are epistatic to genes for partial resistance. It is impractical to eliminate these genes from breeding populations. The only practical way to eliminate their epistatic effect is to use races of the pathogen that carry corresponding genes for virulence. This poses the dilemma of whether to use a diverse mixture of pathogen races, so that collectively all genes for hypersensitivity are matched by virulence, or to use only a single race. The dilemma arises because, if a mixture of races is used and a line carries a gene for hypersensitivity that is effective against some of the races in the mixture, this line may appear to have partial resistance because only a portion of the inoculum that lands on it will infect successfully. This situation is somewhat like the retardation of rust development that is achieved with multiline cultivars or cultivar mixtures. A line thus falsely identified to have partial resistance may prove to be very susceptible to inoculum that consists mainly of virulent races. The false identification of partially resistant lines can be avoided by use of a single race to screen material in the breeding nursery. In this case, lines that show a hypersensitive resistance cannot be assessed for partial resistance, and would either be discarded or would need to be tested later to another race.

Interplot interference is a concern when partial resistance to an airborne pathogen is studied. It is presumed to operate in both directions. Positive

interference occurs when a partially resistant line receives more inoculum from susceptible neighboring plots than it would receive if the neighboring plots had the same degree of partial resistance (as would occur in a solid stand of the partially resistant line). Thus, partial resistance may be somewhat overcome by the influx of inoculum. Conversely, a highly susceptible line will export a certain amount of inoculum it produces, but will not receive a like amount back if it is surrounded by lines with partial resistance, and it may not develop as much disease as it would in a pure stand. Interplot interference diminishes the differences between degrees of partial resistance. Interplot interference may also contribute to the lack of difference in infection rates among genotypes. The greater the degree of partial resistance in a line, the more its resistance will be underestimated when grown in small plots adjacent to more susceptible lines. Despite this, small plots (1-meter rows or hill plots) were used successfully to distinguish degrees of partial resistance to rust (Das et al., 1992). Ranking of durum wheat lines for partial resistance to stem rust in small plots was the same as in larger plots, even though absolute differences in degree of resistance were smaller (Broers and Lopez-Atilano, 1995). Susceptible or moderately susceptible cultivars usually had about the same severity in small, closely spaced plots as in large isolated plots, suggesting that negative interference was not very important. We found that partial resistance to wheat leaf rust was expressed about equally in isolated 10 m x 10 m plots as in adjacent 1-m rows (Lehman and Shaner, unpublished).

Compared to rusts on wheat and barley, little work has been done on partial resistance to oat crown rust. Luke et al. (1972) compared various cultivars of *A. byzantina* that differed in maturity and rust resistance. The later the maturity, the greater the degree of resistance, even when rust severity was recorded on the most severely affected leaf. Simons worked on field resistance to crown rust in oat (e.g., Simons, 1975 and 1985). He assessed partial resistance by yield and seed weight relative to the same plots kept free of rust with fungicides. Unfortunately, he published little information on qualitative or quantitative disease development traits associated with high relative yield and seed weight. While many of the sources of resistance he worked with evidently contributed resistance to progeny, readers of his work cannot determine what characters might be selected for in a breeding program. Simons's replicated hill plot designs, with duplicate and replicated disease-free plantings, are not feasible for handling early generation material in a typical breeding program. Nonetheless, the germ plasm Simons identified as having adult plant resistance certainly warrants further work and utilization.

No oat line has been identified as far as I know that has a degree of partial resistance that would provide adequate and durable protection against crown rust in all environments where oats are grown. A number of lines with partial resistance have been identified, and it may be that different sources of partial resistance can be combined from which transgressive segregants can be selected. To use this approach, initial selection pressure should not be too stringent. Lines with only modest degrees of partial resistance should be saved and crossed to other lines with partial resistance.

The USDA oat collection has not been adequately screened for partial resistance, and this work should be undertaken in a systematic fashion. To begin with, evaluation should concentrate on accessions reported in the literature to be tolerant or partially resistant, and on modern cultivars. Most modern cultivars were originally bred for hypersensitive resistance, but this resistance has generally been overcome by new races of the pathogen. However, these cultivars are the product of long breeding efforts in rust-prone environments, and they may contain a residual degree of partial resistance that can be effectively exploited, along with other useful characteristics. As cultivars and lines of *A. sativa* are identified that have partial resistance, these should be tested in several environments, perhaps in a reinstated international rust nursery, to determine the stability of expression of this resistance.

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Breeding for resistance to the major fungal leaf pathogens of barley

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Leaf fungal diseases are considered to be a limiting factor in barley production. This is more important in high-input agriculture, especially in favourable environments. Increased demand has resulted in the use of intensive cultural methods that are sometimes incompatible with sound plant protection methods. The lack of resistant cultivars, especially in the developing countries, has resulted in the use of fungicides by farmers who in most cases are insensitive to environmental issues and do not have adequate education on the safe use of pesticides. There is now more demand for disease-resistant cultivars.

Efforts to improve disease resistance in breeding programs has often been a second priority compared to yield. However, with the apparent yield plateaus that have been reached in most crop plant varieties more attention is being given to the improvement and stability of yield through the incorporation of disease resistance genes.

Barley is the host of many pathogens, but only a few of them are recognized to be of economic importance. In this paper, I will attempt to present what has been achieved so far in terms of breeding for disease resistance to the major barley pathogens other than the rusts, which will be covered in another paper.

Net blotch

Net blotch, caused by *Pyrenophora teres*, is a major fungal disease of barley, and is commonly found wherever barley is grown. The disease is a major constraint to increased barley production in some parts of the world. Net blotch is a disease that typically occurs in cool, humid areas. Recently it became a serious disease in the dry areas of North Africa, the Middle East, New Zealand and Australia. Smedegaard-Petersen (1971) reported two forms of the net blotch fungus, *P. teres* f. *maculata* (spot form) and *P. teres* f. *teres* (net form). The former causes dark brown spots and the latter produces the typical net-type lesions. The net form is more common in North Africa and the Middle East. Yield is significantly reduced by the disease but protein yield and percentage of protein seem not to be affected (Arabi et al., 1992).

Efforts to control the disease through breeding have been conducted since

the early 1950s. Mode and Schaller (1958) reported three genes for resistance, which they designated *Pt*, *Pt2* and *Pt3*; these were mapped by Bockelman et al. (1977) to chromosomes 3, 5 and 2, respectively. Khan and Boyd (1969) designated *Pta* in the extensively used genetic stock (CI 2330) in California. These genes, however, were not effective in many parts of the world, which led to increased efforts to identify new sources of resistance (Bockelman et al., 1977; Caddel and Wilcoxson, 1975; Buchannon and McDonald, 1965; Wilcoxson et al., 1992). Table 1 is a partial list of some barley genotypes with known resistance. Most of the resistance genes described so far are race-specific. *P. t. f. teres* consists of different pathogenic races in many parts of the world suggesting that the pathogen may rapidly adapt to resistant cultivars after they are released. Harrabi and Kamel (1990) tested 30 isolates from the Middle East and North Africa, and found four major virulence groups. It appeared that the North African isolates were more complex than those from Egypt and Cyprus. This may be due to the fact that barley in North Africa is more widely grown. None of the cultivars tested were resistant to all races. In a later study, we tested 47 isolates from Morocco, Algeria, Tunisia and Lybia, and found that practically all (45) were moderately to highly virulent on the cultivars grown in the region (unpublished data). Other studies have confirmed the highly variable nature of the pathogen. Steffenson and Webster (1992b) isolated 13 pathotypes from a collection of 91 isolates from California alone. They found that the population of *P. teres f. teres* in California is genetically diverse (Shannon index 1.30 to 1.96) and it differs from other pathotypes collected from Minnesota, Mexico and England. Douissi (personal communication) has reported high variation among 15 isolates collected from Morocco. The diversity of the fungus has been

Table 1. Barley genotypes with their corresponding resistance genes to *P. t. teres*

Genotype	Number genes	Gene designation	Chromosome
Tifang (CI* 4407-1)	1-2	<i>Pt</i>	3
Ming (CI 4797)	1-2	<i>Pt2</i>	5
Manchuria (CI 739)	1	<i>Pt2</i>	
Manchuria (CI 2330)	1	<i>Pta</i>	
Harbin (CI 4929)	1	<i>Pt2</i>	
Canadian Lake Shore (CI 2750)	1-2	<i>Pt2-Pt3</i>	
CI 7584	1	<i>Pt3</i>	2
CI 4922	2	<i>Pt2 + Pt3</i>	
Robust	1	?	
M 76-160	1	?	
M 81-111	1	?	
Steptoe	2	?	
Park	1	?	

* CI = Cereal Investigation Number, US Department of Agriculture.

recently confirmed with random amplified polymorphic DNA markers (Peever and Milgrom, 1994).

The race-specific resistance and the high genetic variation of the *P. teres* population has led barley researchers to investigate other genetic means to control the disease. Quantitative resistance was reported by Steffenson and Webster (1992a) using four criteria for evaluation of resistance. Cultivars Cape and UC 603 appeared to possess quantitative genes for net blotch resistance as measured by AUDPC. However, their resistance was highly dependent on the environment. In crosses between susceptible but otherwise adapted barley cultivars, we have observed transgressive segregation for resistance when the segregating generations were tested against 9 isolates of *P. teres* f. *teres* (Cherif and Harrabi, 1993). The relatively higher level of resistance was confirmed by the lower number of spores/mm² in the F3 and F4 generations as compared to the parents (Table 2). In some crosses, there was a significant increase in resistance in the F2 and F3 generations as measured by infection type, average lesion size and number of lesions per unit leaf area (Figure 1). The resulting resistant progenies were tested against pathotypes from Morocco, Algeria and Tunisia with limited evidence for race-non-specific resistance (Harrabi et al., 1993).

Transgressive segregation was first reported in the barley-net blotch system by Bordelon (1981) and recently confirmed by Steffenson et al. (unpublished data). They have also reported, using quantitative trait loci (QTL) analysis, two chromosome regions contributing to net blotch resistance at the seedling stage: one near the centromeric region of chromosome 4 and the second on the long arm of chromosome 6. They have further identified seven QTL conditioning resistance at the adult stage. These mapped to chromosomes 1P, 3P, 2P, 3M, 7P, 6P and 4. The seven QTLs accounted for 67.6 % of the phenotypic variance. Narrow sense heritability was 0.92. This result further confirmed what we have

Table 2. Average number of spores/mm² on leaves of two crosses between two susceptible barley cultivars and derived populations tested with 3 isolates of *P. t. teres*.

Cross	Generation	Number of spores/mm ²		
		Isolate 1	Isolate 2	Isolate 3
P1 x P2	P1	15	45	48
	P2	55	35	50
	F3	12*	18**	24*
	F4	10**	15**	15**
P3 x P4	P3	42	26	36
	P4	33	28	33
	F3	20**	20**	20**
	F4	15*	18*	12*

* Significantly lower than the mid-parent value at (P= 0.01)

** Significantly lower than the mid-parent value at (P= 0.05)

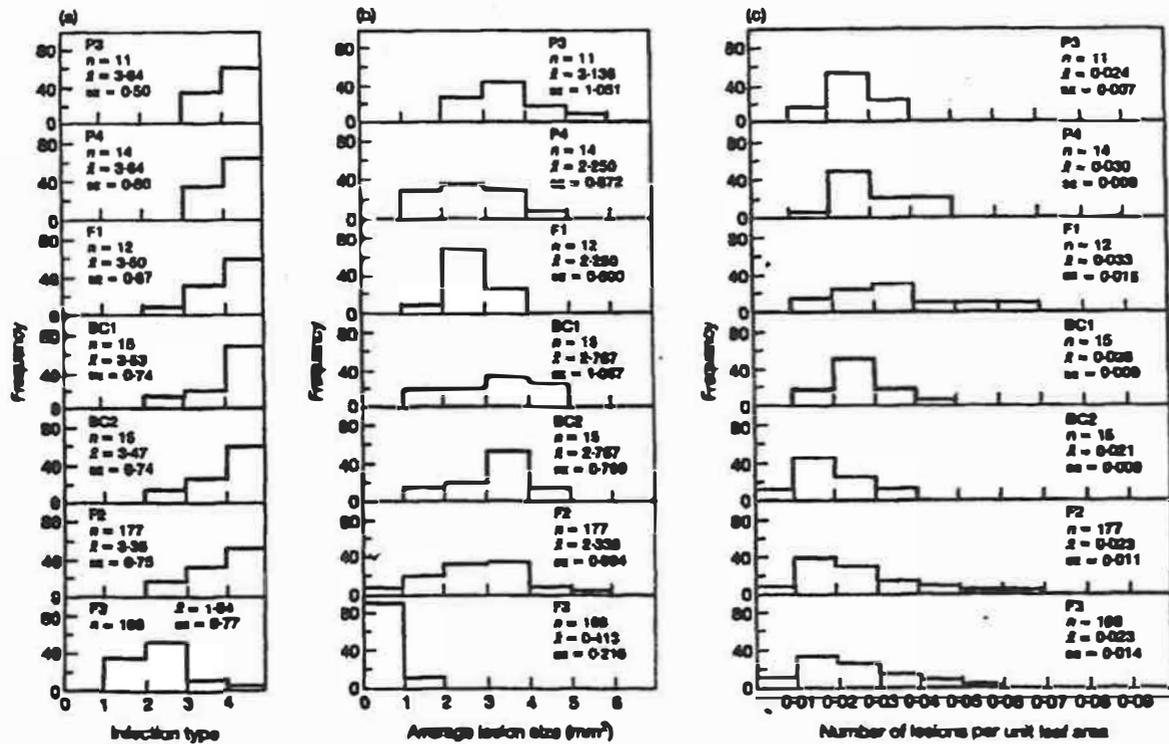


Figure 1. Frequency distributions of infection type (a), average lesion size (b) and number of lesions per unit leaf area (c), on barley cultivars P3, P4, and the F_1 , $F_1 \times P3$ (BC1), $F_1 \times P4$ (BC2), F_2 , and F_3 populations inoculated with a mixture of five isolates of *Pyrenophora teres* (n = number of plants, \bar{x} = population mean and SE = population error).

reported earlier, i.e., that resistance to *P. teres* f. *teres* may be explained by additive gene action and in some cases by epistatic genes (additive x additive) (Cherif and Harrabi, 1990). It appears, therefore, that selection for resistance to *P. t. teres* in barley can be achieved if there is a thorough knowledge of: a) the structure of the pathogen population; b) the genetic mechanism governing resistance; and c) the use of proper selection techniques. We strongly believe that pyramiding various QTLs may be a good approach in selecting for durable resistance in this pathosystem.

Spot Blotch

Spot blotch of barley, caused by *Bipolaris sorokiniana*, is a widely distributed disease of barley in North Africa, the Middle East, the Upper Midwest of the United States (where it is considered the major pathogen of barley), Europe and Australia. In wet and warm conditions, the fungus attacks the leaves and the spike, whereas in drier conditions the crown and roots are more prone to the disease. On leaves this disease can be confused with net blotch, especially when the symptoms are not typical. Only examination of conidia morphology can distinguish between the two pathogens. In North Africa and the Middle East, the disease is not as important as net blotch. Yield reductions of 20% have been reported (Wilcoxson et al., 1990). The search for sources of resistance goes back to the early 1920s when Stakman first reported that the disease can be serious in Minnesota. Hayes et al. (1923) were the first to report a single factor for resistance in a cross between Lion and Manchuria. Based on correlation studies, Griffee (1925) inferred that resistance to spot blotch at the adult stage is conditioned by three unlinked genes on chromosomes 2, 5 and 7. A fourth gene was designated using the data of Arny (1951). Two genes for resistance were reported in the cultivar Bowman (Gonzales Ceniceros, 1990). Recently, Steffenson et al. (unpublished data) identified a fifth gene on chromosome 1P for resistance to spot blotch at the seedling stage in a Morex/Steptoe population. This gene was designated as *Rcs5*. They also identified two QTLs for resistance at the adult stage; the largest QTL effect mapped to chromosome 5P and the second mapped to chromosome 1P. Narrow sense heritability was 0.91 indicating a large additive genetic effect for resistance to spot blotch.

Scald

Scald or leaf blotch of barley, caused by *Rhynchosporium secalis*, is known to occur wherever barley is grown, particularly in cool and humid areas. There is disagreement among researchers as to the existence of races. Jackson and Webster (1976) reported 75 pathogenic races, identified from a collection of 175 single-spore isolates. Reed (1957) showed the presence of races in Canada and the United States. Skoropad (1960) found no clear evidence of pathogenic races in Canada. However, Tekauz (1991) reported a high degree of pathogenic variability in Canada, and identified 45 pathotypes on a set of 10 barley

differentials with known genes for resistance. The occurrence of "races" was reported in Italy, the United Kingdom, Australia, Norway, Argentina and, recently, in Denmark, where 28 races were identified (Jorgensen and Smedegaard-Petersen, 1995). They also reported that known sources of race-specific resistance may not be effective in Denmark. Goodwin et al. (1990) proposed a standard nomenclature based on octal numbers for each possible combination of pathogenicities in the fungus. Twenty-four barley differential cultivars were included in this nomenclature. McDermott et al. (1989), using four putative isozyme systems, a colony colour dimorphism and 20 ribosomal DNA restricted fragment length variants, identified 49 different haplotypes from a total of 163 isolates.

The most extensive work on the genetics of scald resistance was reported by Dyck and Schaller (1961), who reported five genes for resistance (Rh2, Rh3, Rh4, Rh4² and Rh5). They also identified a single dominant gene (Rh2) in Atlas. Using an isolate from Montana, Harrabi (1982) identified another dominant gene in Atlas. The first gene is incompletely dominant and conditions resistance at the seedling stage; the second confers resistance at the adult stage. Atlas 46 has a second gene (Rh5) in addition to Rh2 and may contain an additional recessive gene, rh6 (Harrabi, 1982). Atlas 46 is a derivative of a cross between Atlas and Turk. This latter cultivar may have three genes for resistance, two dominant and one recessive – i.e., Rh3, Rh2 and rh6. It is thus logical to assume that the recessive gene in Turk was carried into Atlas 46. The Rh3 gene in Turk is closely linked to Rh4, found in La Mesita. Habgood and Hayes (1971) found that La Mesita contains two genes (Rh4 and Rh10). However, when an isolate from Morocco was used, La Mesita was found to have two recessive genes (Harrabi, 1982). Baker and Larter (1963) found two temperature-sensitive complementary recessive genes in Stuedelli and Jet. These genes were designated rh6 and rh7 on chromosomes 4 and 3, respectively (Bockelman et al., 1977). The use of temperature-sensitive genes in a breeding programme is therefore limited. Abyssinian (CI 668) and Kitchin each have one single incomplete dominant gene, designated Rh9, on chromosome 4. This gene confers full resistance only in the homozygous condition. Another line, CI 3940, seemed to have a high level of resistance to three diverse isolates, from Montana, Tunisia and Morocco, and was found to have one recessive gene when a Moroccan isolate was used. A second dominant gene was identified when tested with an isolate from Tunisia (Harrabi, 1982).

It appears that most resistance alleles belong to the Rh-Rh3-Rh4 locus complex. There are at least five alleles or pseudo alleles at this locus on chromosome 3H, with the Rh-Rh3-Rh4 being clustered on the short arm. Barua et al. (1993) identified a DNA marker, SC10-65-H400, linked to the resistance locus (*Rh*) on chromosome 3L. The use of DNA markers can be very useful to barley breeders since most of the genes conditioning resistance to *R. secalis* were reported to interact with the environment. DNA markers can therefore circumvent this problem and are also useful when one is pursuing pyramiding genes in a single genotype. Recently Backes et al. (1995) identified 5 QTLs

associated with resistance on chromosomes 2H, 3H, 6H and 7H. Four positive QTLs explained 52% of the genetic variance. In total, there are probably more than 11 loci conditioning resistance to scald in barley. A summary of resistance genes and the probable cultivars in which they are found is presented in Table 3. A comprehensive but non-definitive list of referenced resistant gene sources is given by Goodwin et al. (1990).

Barley stripe

There has not been much research related to resistance on this disease compared to other foliar diseases of barley. This is probably due to the effectiveness of seed treatment as a control measure. Barley stripe caused by *Pyrenophora graminea* is a seed-born disease and the only "*Helminthosporium*" species known to be systemic in barley. The disease has gained importance worldwide since the exclusion of organic mercury compounds. Unlike other foliar diseases of barley, the symptoms usually are not visible until the end of the tillering stage. The disease is becoming important in Europe, North Africa and the Middle East, where imported seed is common and very little seed treatment is being utilized by farmers because of the high cost of fungicides compared to the price of barley. Yield losses approach an amount equal to the percentage of infected plants. The slight difference between percent leaf stripe and percent loss was attributed to compensatory effects, resulting in increased yield in healthy plants (Suneson, 1946). Races of the fungus were reported by Army (1945), Smedegaard-Petersen and Jorgensen (1982), Tekauz and Chiko

Table 3. Reported genes conditioning scald resistance in barley

Cultivar	Gene	Chromosome
Hudson (CI 8067)	Rh	
Bey (CI 5581); CI 8162	Rh3	3
Forrajera, Osiris	Rh4	3
Atlas (CI 4118)	Rh2 rh or Rh2	
CI 3940	Rhrh6	
LaMesita	Rh10rh6rh7 or Rh4Rh10	3
Atlas 46 (CI 7323)	Rh2Rh3Rh5 or Rh2Rh3	
Turk (CI 14400)	Rh3Rh5rh6 or Rh3Rh5	
Jet (CI967)	rh6rh7	3, 4
Nigrinudum	rh8	
Kitchin, Abyssinian (CI 668)	Rh9	4
Osiris (CI 1622)	Rh10 or Rh4	
CI 4364	rh11	
Trebi	Rh4, Rhrh6	
CI 2376	Rh4Rh9	
Modoc (CI 7566)	Rh2rh6 or Rh4rh?	
E224/3	Rh4Rh10	3

(1980) and Knudsen (1981). However, Kline (1971) did not find any physiologic specialization in *P. graminea*.

The concept of resistance is not well defined in this pathosystem. Classification of reaction is generally based on the percentage of infected plants, and may therefore be arbitrary. The lack of reliability of artificial inoculation techniques makes it difficult to draw firm conclusions about the genetic control of resistance to *P. graminea*. Tekauz (1983) proposed disease reaction be based on percent infection as well as a yield loss of 0.6% for each percent of leaf stripe. The two-rowed barleys had less leaf stripe infection than did the six-rowed spring types. All the winter cultivars tested were susceptible. Tekauz (1983) also identified five cultivars (Betzes, an apparent universal resistant, Mingo, Klages, Olli and Summit, a highly susceptible cultivar) that can be used to differentiate several biotypes of *P. graminea*. Isenbeck (1930) was probably the first to study genetic resistance and concluded that resistance was conditioned by several dominant genes. Arny (1945) reported three genes in some crosses and many genes in other crosses. Later, Suneson (1950) found six genes for resistance. Nilan (1964) reported three resistance genes (Rhg1, Rhg2 and Rhg3), although neither the chromosome location nor the number of alleles at each locus are known yet. Two dominant genes, one in Betzes and the other in Tokak, were reported by Konak (1983). Boulif (1988) found two recessive genes with cumulative effects in Minn.23, but had difficulty classifying resistance and based most of his conclusions on genetic variances. In Denmark, it appears that commercially grown barley cultivars have a high level of resistance, as demonstrated by the immune reaction of Zita (Smedegaard-Petersen and Jorgensen, 1982). Recently, Vale (1994) showed the accumulation of three mRNA families coding for peroxidase, thaumatin-like and thionin, in response to infection of barley by *P. graminea*, although these three genes alone did not explain the process of resistance. Other as yet unknown genes must also be involved in the plant defense mechanism.

Powdery Mildew

Powdery mildew, caused by *Erysiphe graminis* (DC) Merat f. sp. *hordei* Marchal, is the principal foliar disease of barley in Europe resulting in a substantial loss in quantity and quality. The disease is widely distributed but its economic importance in other parts of the world is not well documented. In North Africa and Tunisia in particular, barley powdery mildew attained epidemic levels in the 1995/96 crop season, to the point where farmers had to spray fungicides beginning at the three-leaf stage, an uncommon practice in that part of the world. The disease is caused by an unusual fungus in the sense that it does not require free moisture for completion of the infection process, as do most of the other foliar pathogens of barley. The barley-mildew interaction is probably the most extensively investigated pathosystem. Conidia of the fungus are spread over continents (Europe-Africa), since they are easily detached from leaves by small movements. In many parts of the world, ascospores are the most important primary inoculum.

The fungus is extremely variable and known to occur as many races. In Europe there is a tendency to standardize virulence nomenclature. Designation of virulence is usually made using the symbol V (virulence), followed by the code for the corresponding resistance, e.g., Var for virulence on Arabische; however, if virulence refers to individual resistance genes, the symbol V is followed by the gene symbol, e.g., *Va12* for the virulence gene corresponding to *Mla12*. There have been significant changes in the powdery mildew population in Europe caused by selection pressures from use of resistance genes and the extensive use of fungicides. When the area using specific genes increases, the frequency of the corresponding virulences usually increases (Munk et al., 1991). It appears that virulences (*Vla*)+*Vg*+*Va6*, *Va6*, *Va12*, *Va9*, *Va7*, *Va13* and *Va1* are the most frequent in Europe. A detailed virulence survey in Europe is presented by Limpert et al. (1991).

An integrated approach has been proposed to control the disease. Varietal mixture, fungicides and genetic resistance are commonly used. Wolfe (1991) showed that a varietal barley mixture showed considerable restriction of the disease, and therefore can slow the rate of epidemic development. A large number of mildew resistance genes have been identified, most of them found in three loci, *Mla*, *Mlp* and *mlo*. *Mla* is a complex locus with a high degree of polymorphism. Twenty-one alleles or closely linked genes have been confirmed at this locus (Moseman and Jorgensen, 1971). The *mlo* apparent durable resistance, based on the spontaneously arisen gene *mlo11* from Ethiopia, has become widely used in the European barley breeding programmes. The percentage of the barley areas planted with cultivars with *mlo* had reached 25% in 1989 in five European countries (Andersen, 1991). Most cultivated barley varieties in Europe have more than one gene for resistance to *P. mildew*, and, according to Jensen and Jorgensen (1991), the most widely used sources of resistance are We Mlg and La (*Mla7*, *Mla12* and *Mla6*). The lack of linkage between these and other resistance genes has allowed these alleles to be used in combination with other genes. A complete list of barley cultivars, with their corresponding genes for resistance, is given by Brown and Jorgensen (1991). In North Africa and the Middle East, the most effective resistance alleles are *Mla7*+*Mla4*, *Mla9*+*Mla4*, *Mla10* and *mlo5* (Yahyaoui, personal communication).

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The rusts of barley and oat: A prospectus

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Both barley and oat may be affected by one or more of a number of rust diseases. The diseases are generally well known, and it is not the intent in this paper to review these in detail. Rather, a comparative overview will be presented, outlining occurrence, distribution, new developments or outbreaks, their importance, and approaches to control.

Barley

Leaf rust

Two leaf rusts, caused by *Puccinia hordei* Othh or one of several *Uromyces* sp., are known in barley. *Uromyces* is found on wild barley (*Hordeum spontaneum* C. Koch. and *H. bulbosum* L.) in Israel, and one species, *U. viennot-bourginii*, is found on cultivated barley (Anikster and Wahl 1966). Cummins (1971) described *U. turcomanicum* Katejev as the species infecting cultivated barley, listing *U. v-bourgonii* as a synonym. This species is distributed from southern Russia to the Middle East, but has not been reported to cause significant damage.

Puccinia hordei is the most important rust pathogen of barley. Although widespread severe epidemics historically have been rare, more recently leaf rust has increased, and substantial localized losses may occur. Much of this has been attributed to increased barley cultivation in cool temperate regions, changes in rotational and fall-sowing practices, increased fertilizer use and widespread use of susceptible cultivars (Clifford 1985). The disease has the potential to cause serious losses in Europe, depending on cultivation practices and how extensively resistant cultivars are grown (Clifford 1985). In New Zealand, leaf rust is sporadic, but localized losses of about 20% may occur (Arnst et al. 1979; Teng et al. 1979), and in some circumstances yield losses of up to 45% have occurred (Cromeey and Viljanen-Rollinson 1995). In Queensland, Australia, occasional epidemics may result in losses up to 26% (Cotterill and Rees 1983; Luig 1985). In the US, leaf rust is generally a minor disease, but there may be sporadic outbreaks in the south-east and mid-west (Steffenson et al. 1993), and in California (B.J. Steffenson, personal communication). Considerable damage may be caused by lowering yield, grade and malting quality (Levine and Cherewick 1956). The disease is observed at low levels most years in the Red

River Valley of Manitoba, Canada, but is not serious. In Argentina, leaf rust first became serious in 1951, and has since become one of the most important barley diseases (Rodriguez-Amieva and Frecha 1971). In Uruguay, however, leaf rust is not very important (S. German, personal communication).

Virulence in North American populations historically has been lower, based on the differentials used, than in European populations (Clifford 1985). Races of *P. hordei* in Europe, North Africa and the Middle East have virulences to genes that have not been widely deployed in these regions (Parlevliet 1976, 1983; Reinhold and Sharp 1982). Use of specific resistance genes in barley quickly results in selection of virulent races of *P. hordei*. Gene *Rph7* from Cebada Capa, which has been one of the most widely effective leaf rust resistance genes in barley, was widely deployed in the southeastern US beginning in the late 1960s. This gene remained effective until the early 1990s, when virulence was detected in collections from the southeast and California (Steffenson et al. 1993). Although virulence to *Rph7* occurred previously in Israel and North Africa, the origin of this virulence in the US was considered most likely to be due to mutation and selection (Steffenson et al. 1993). Similarly, in New Zealand, Cromey and Viljanen-Rollinson (1995) noted that races of *P. hordei* differed from those in Australia, indicating that the occurrence of new pathotypes in New Zealand occurred through evolution locally rather than by migration.

Until about 1970, most leaf rust resistance breeding programs utilized specific *Rph* (*Pa*) genes as sources of resistance, but few of the genes have been widely used commercially (Clifford 1985). The resistance genes that were used usually occurred singly in released cultivars; thus, not surprisingly, the resistance has not been durable. Parlevliet (1983) concluded that, except for *Rph7*, individual *Rph* genes were not worth deploying commercially. Interest in more durable resistance to leaf rust began in Europe about 1970 by the observation of "non-hypersensitive" resistance, and its further evaluation as "slow rusting" (Clifford 1972). This resistance was typified in the cv. Vada. Parlevliet (1978) demonstrated the polygenic additive nature of an apparently non-specific resistance in several cultivars, including Vada. Parlevliet (1983) noted that most European barley cultivars carried no *Rph* genes, but "partial" resistance was widespread, readily available and relatively easy to transfer. The "partial" resistance to leaf rust in barley is effective, and has shown little evidence of serious erosion (Parlevliet 1993), despite an observed increase in fitness of leaf rust populations to Vada (Clifford and Clothier 1974). This type of resistance is now widely used in European cultivars, and may be useful in Australia (Cotterill and Rees 1993). Resistance of a more durable nature has not been utilized in the US, though some genes contributing to durability may have been transferred from Cebada Capa along with *Rph7* (Steffenson et al. 1993).

Stem rust

The main stem rust affecting barley worldwide is *P. graminis* f. sp. *tritici*; in North America and Australia, *P. graminis* f. sp. *secalis* is also a problem. A hybrid of *P. g. tritici* and *P. g. secalis*, designated as *P. g. hordei*, was detected

in Australia in 1963. This hybrid, avirulent to wheat, rye and oat, but virulent and well adapted to commercial barley, may cause significant losses in barley (Luig 1985). *Puccinia graminis* f. sp. *avenae* is normally not a pathogen of barley, but may occur on wild barley (*H. leporinum*) in Australia (Luig 1985), and has been found to be moderately virulent to some barley cultivars in New Zealand (Martens et al. 1977) and North America (Martens et al. 1983).

In North America, epidemics on barley have mainly occurred when wheat was severely infected (Roelfs 1978, 1985). In Australia, increased severities of stem rust occurred in the early 1980s, and were associated with increased plantings of susceptible cultivars (Dill-Mackey et al. 1990). Since the release of cultivars with gene *Rpg1* in the US in 1942, there have been few significant losses in barley, even during the severe wheat stem rust epidemic of 1954 (Steffenson 1992).

Although phenotypes of *P. g. tritici* with increased virulence to the *Rpg1* resistance were identified periodically in North America, the barley crop did not appear to be threatened. In 1988, however, a race designated as QCC appeared (Martens et al. 1989), which has posed a greater threat. Although no widespread losses have occurred, localized infections of up to 60% were observed in the northern Great Plains (Roelfs et al. 1990) and over 65% in Manitoba (Harder et al. 1996). Race QCC has increased in prevalence, and is now the predominant race in north-central North America. Why does this race appear to pose a greater threat? Normally, *P. g. tritici* has been considered a less specialized and less aggressive pathogen on barley (Green 1971). Race QCC combines virulence to gene *Rpg1*, is highly aggressive relative to other races, and has increased fitness on barley and susceptible wheats (Liu et al. 1996). Therefore, this race poses a threat should favorable conditions for a stem rust epidemic occur.

Presently, genes *Rpg1*, *Rpg2*, *Rpg3* (Jin et al. 1994a), *Rpg4*, and a probable second recessive gene (Jin et al. 1994b), and *RpgU* (Fox and Harder 1995) for stem rust resistance are known in barley. Most observations indicate, however, that there are complex interactions involving these genes and other probable minor-effect genes. Highly variable host line reactions are normally encountered in resistance screening nurseries. Through utilizing the known sources of resistance and yearly intense screening in nurseries, the levels of stem rust resistance have been improving in western Canadian barley breeding programs. It usually is not certain, however, what the resistance genotype combinations are. Because of complex interactions, higher levels of resistance in parental lines may not be retained in progeny from crosses. Molecular markers have been developed for genes *Rpg1* (Kilian et al. 1994, Penner et al. 1995) and *Rpg4* (Borovkova et al. 1995), which will enable easier tracking of these resistances in breeding.

Stripe rust

Severe epidemics of stripe rust, caused by *Puccinia striiformis* West. f. sp. *hordei* Eriks., have occurred in Europe, the Indian subcontinent, China (mainly Tibet), Japan, parts of the Middle East, North Africa and Ethiopia (Chen et al.

1995, Stubbs 1985). In 1975 the disease, probably introduced from Europe, first appeared in Colombia, and within seven years through most of the barley growing areas of South America (Dubin and Stubbs 1986). By 1987 the disease had spread to Mexico, and by 1991 into Texas (Marshall and Sutton 1995). Presently, barley stripe rust in the United States occurs throughout the western states, but not in the Great Plains or eastern regions (Chen et al. 1995). It has not been observed in Canada. Observations of stripe rust on barley in South America, Central America, the US and Canada were made as early as the late 1800s, but in all cases the causal pathogen appears to have been *P. s. tritici* (Stubbs 1985).

In South America, particularly in the Andean region, stripe rust has become one of the most serious diseases of barley. In this region, barley as well as several collateral wild barley host species occur throughout the year. Yield losses up to 70% occurred in Colombia, 61% in Bolivia and 33% in Ecuador (Dubin and Stubbs 1986). In Europe, barley has traditionally been planted as a spring crop in areas that are at risk for stripe rust, so it frequently escapes serious infections. Severe outbreaks, however, may occur (Stubbs 1985). Yield losses of about 40% in winter barley have occurred in Germany, and up to 80% in north-eastern Holland (Hassebrauk 1962). The disease likely would increase with increased plantings of winter barley.

Although Eriksson originally designated a specialized f. sp. *hordei* form, later European and other workers did not distinguish between isolates collected from wheat or barley in the differentiation and nomenclature of races (Stubbs 1985). This was because of overlapping host ranges where isolates from barley may be virulent to some wheats, or isolates from wheat may be virulent to some barleys. The barley and wheat forms, however, have been shown to be distinct based on pathogenicity data (Stubbs 1985), isozyme data (Newton et al. 1985) and pathogenicity and RAPD analysis (Chen et al. 1995). Isolates of this pathogen collected from various parts of the US are highly variable (Chen et al. 1995).

The resistant cultivar Dorada was released in Ecuador in 1971, and by 1981 occupied most of the area sown to barley in Ecuador, effectively limiting losses (Dubin and Stubbs 1986). Chen et al. (1995) have indicated a useful diversity of resistance sources. Brown et al. (1995) screened about 30,408 barley lines for resistance in Bolivia, from which numerous resistance sources were identified. The diversity of the *P. striiformis* f. sp. *hordei* population offers the opportunity to select and pyramid effective seedling resistance genes, and to possibly identify other more durable rate-limiting forms of resistance (Chen et al. 1995).

Crown rust

In 1991 and 1992, cultivated barley, wild barley (*H. jubatum* L.) and quackgrass (*Elytrigia repens* L.) in Nebraska and the Red River Valley were found to be heavily infected with crown rust (Jin and Steffenson 1992). Collections of this rust infected a wide range of grasses (Jin and Steffenson 1993). Of 30 barley lines tested, all were susceptible (Jin and Steffenson 1992). The causal pathogen is a variant of *Puccinia coronata* Cda. (Y. Jin, personal communication). Because of the susceptibility of numerous grasses and the widespread occurrence of the

alternate host *Rhamnus cathartica* L., this pathogen has the potential to become an economic problem.

Oat

Stem rust

Stem rust, caused by *Puccinia graminis* Pers. f. sp. *avenae* Eriks. & Henn, has been a problem mainly in the great plains of North America and in Australia, though severe outbreaks may occur wherever oat is cultivated. In North America the stem rust races have been relatively stable for many years (Harder 1994), and the sources of resistance used in breeding programs in the Red River Valley of North Dakota and Manitoba have effectively controlled the disease. In this region, stem rust is receiving lower priority in terms of incorporating new sources of resistance. In Australia, however, the virulence of the pathogen to currently available sources of resistance is causing concern, and improved sources are being sought (R.A. McIntosh, personal communication).

Crown rust

Crown rust, caused by *P. coronata* f. sp. *avenae* Eriks., is more widespread than stem rust, and is one of the most important diseases of oat worldwide. The pathogen is notable for its very highly variable virulence (Chong and Kolmer 1993). The high variability in North America was not so evident in the past, but appears to have increased in recent years, where as many as 107 virulence phenotypes were identified from only 167 isolates collected in Manitoba in one year (Chong and Seaman 1994). This variability has led to rapid erosion of resistance in North America. The eastern and prairie populations of *P. coronata* were previously generally dominated by avirulent phenotypes (Chong and Kolmer 1993). The release of cultivars with gene *Pc39* in Ontario and with genes *Pc38* and *Pc39* in Manitoba in the early to mid-1980s led to strong directional selection of races with virulence to these genes. By 1990 these virulences had become distributed through a large number of phenotypes. The alternate host, European buckthorn (*Rhamnus cathartica*), occurs widely in Ontario and in parts of the prairie regions of the US and Canada, and may in part be responsible for the reassortment of virulences in this pathogen. Buckthorn also plays an important epidemiological role by providing an indigenous inoculum source capable of infecting the oat crop early in the season. Under suitable conditions, severe crown rust may now occur in the prairie region (Chong and Seaman 1994).

Earlier optimism of an abundant supply of specific genes for resistance from wild oat accessions (Harder et al. 1992) now must be tempered due to the high variability of the pathogen. None of the genes isolated from hexaploid sources have offered durable resistance. For example, virulence to a highly effective gene, *Pc68*, was detected in 1993 (Chong and Seaman 1994), before there was any exposure of this gene to the pathogen population. A large reservoir of resistance may exist in diploid and tetraploid wild oat accessions (Harder et al.

1992), but the resistance is difficult to transfer and may be of limited durability. To maximize the effectiveness of specific resistance genes, a range of carefully chosen genes must be pyramided in different combinations in different cultivars. Because of the limited durability of these genes, there now is increasing interest in exploiting potentially more durable quantitative and less race-selective resistance in breeding programs for the Great Plains of the US and Canada. Since breeding programs have traditionally relied on specific gene resistance, however, the effective utilization of potentially more durable quantitative resistance will require a number of years to achieve.

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Genetically engineering plants for enhanced disease resistance*

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Introduction

With the ability to produce transgenic oats and barley will come the potential of inserting into their genomes new genetic information for modifying agronomically important traits. Compared to traditional breeding, the new methods of gene transfer offer the advantage of utilizing an essentially unlimited source of genetic information that can be transferred as very specific segments of DNA, thus preventing the potential co-transfer of closely linked deleterious genes. If experience with other crops is any indication, a great deal of effort will be concentrated on trying to generate transgenic oat and barley plants that are resistant to viral, bacterial and fungal pathogens. It is anticipated that transgenic technology will provide a means of enhancing protection in cases where natural resistance is not available, and improving the efficacy and long-term durability of currently existing resistance.

This chapter aims to illustrate some of the strategies that have been exploited to improve disease resistance in crops where genetic transformation is feasible. Because of their ease of transformation, many studies have been performed in Solanaceous dicots such as tobacco and potato. In addition, some new developments in molecular pathology will be highlighted, because these may influence transformation-based disease resistance strategies in the future.

Engineering resistance to viral pathogens

Transgenic plants with variable levels of protection against viral pathogens have been produced by introducing into their genomes DNA sequences originating from the pathogens themselves (reviewed by Lomonossoff, 1995). This strategy, known as pathogen-derived resistance (PDR), has now been demonstrated to be effective against almost all classes of viruses in a number of different plant species (Fitchen and Beachy, 1993). In many cases, levels of resistance are sufficiently high to be of commercial use. PDR appears to hold up well under field conditions, and has led to the recent release of a transgenic virus-resistant squash variety (Shah et al., 1995; Tricoli et al., 1995).

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A number of different segments of the viral genome have been used to confer PDR, including those encoding coat proteins (CPs), viral replicases, and movement proteins. In fact, it has been speculated that any part of a viral genome can potentially give rise to PDR (Lomonossoff, 1995). PDR appears to be mediated at the protein or RNA level; in general, protein-mediated resistance (i.e., resistance is dependent on translation of the viral-derived transgene) results in low-level, but broad-spectrum resistance, whereas RNA-mediated resistance (i.e., resistance is dependent only on transcription of the viral-derived transgene – a protein need not be synthesized) can yield high levels of protection and is very narrow. There is at least one example of both types of resistance being conferred by the same transgene (reviewed by Lomonossoff, 1995). It is noteworthy that the specificity of resistance may also be explained on the basis of which viral segment was inserted into the plant. For example, CP-mediated resistance is generally high and narrow, whereas resistance conferred by full-length, unmodified viral replicase genes is relatively broad (Fitchen and Beachy, 1993; Lomonossoff, 1995). Viral movement protein-based resistance also appears to be effective against a broad spectrum of viruses (Lomonossoff, 1995). In cases where resistance is restricted to specific viruses or strains of viruses, protection against different viruses may be achieved by introducing genes from multiple viruses into the plants (Tricoli et al., 1995).

Two outstanding questions relating to viral pathogen-derived resistance are how durable it will be under field conditions and what its potential environmental risks are. With respect to the latter question, there is concern that the approach may lead to the evolution of new viral strains that are more virulent and more difficult to control than the currently existing ones. These concerns have stemmed from reports of heteroencapsidation (i.e., the genome of one virus being “packaged” into a viral particle by the CP of a different virus) in transgenic plants expressing a CP, as well as those of *in planta* recombination between incoming (infecting) viral RNA and RNA transcripts derived from viral genes inserted into the plant genome (Falk and Bruening, 1994; Tepfer, 1993). Although the risks of producing new viral strains are thought to be low (Falk and Bruening, 1994), they should not be discounted.

Engineering resistance to bacterial and fungal pathogens

The production of transgenic plants having commercially useful levels of resistance to bacterial or fungal diseases has proven more difficult than the genetic engineering of viral resistance. Due to different modes of pathogenicity, pathogen-derived resistance has received only limited attention as a means of protection against these microbes (see below). Instead, many transgenic strategies for bacterial and fungal resistance have relied on bolstering the plant's natural ability to limit microbial invasion.

Plants respond to pathogen attack by inducing an array of defense mechanisms designed to limit microbial invasion (reviewed by Bowles, 1990; Kuc, 1995; Lamb et al., 1992). These include the elicitation of local cell death at the site of

infection (hypersensitive response; HR), reinforcement of the plant cell wall, the production of antimicrobial proteins, peptides, and secondary metabolites, as well as the production of other pathogen-related (PR) proteins of unknown function. The speed at which the defense responses are initiated has been proposed to be a critical factor in determining whether a plant will be susceptible or resistant to any particular micro-organism (Bowles, 1990; Lamb et al., 1992; Kuc, 1995). A susceptible plant will not elicit a HR, and accumulation of defense molecules is often absent, delayed and/or diminished (relative to a resistant plant). Presumably, virulent pathogens have evolved mechanisms to suppress plant defense responses or escape its detection.

Expression of antimicrobial proteins

One strategy of enhancing resistance to pathogens is to produce plants that constitutively produce high levels of proteins or peptides capable of limiting microbial growth. Conceptually, there are at least three reasons why this approach might be effective. First and foremost, it would ensure the presence of high levels of antimicrobial proteins from the earliest stages of attack, a time during which susceptible plants fail to produce substantial amounts of defense molecules. Second, it allows for the expression of antimicrobial proteins from other plant species and those usually not associated with pathogen attack (for example, those from germinating seeds or isolated from animals or fungi). Assuming that pathogens may have evolved a certain degree of tolerance to those pathogen-induced antimicrobial proteins normally produced by the host plant, heterologous compounds could represent more potent inhibitors of microbial growth. Finally, there has been speculation that expression of antimicrobial proteins capable of degrading fungal cell wall carbohydrates can enhance the production of elicitors and thereby stimulate plant defense responses (Yoshikawa et al., 1993). Regardless of the mechanism involved, the fact that many of the antimicrobial proteins characterized to date appear to be effective against many different microbes suggests that their expression in transgenic plants could lead to protection against a broad range of pathogens.

A number of studies have investigated the effects of constitutively expressing *single* antimicrobial proteins on disease progression (reviewed by Brears and Ryals, 1994; Lamb et al., 1992; Shah et al., 1995). Included among the proteins tested are:

- hydrolytic enzymes such as chitinases and beta-1,3-glucanases that can degrade components of fungal cell walls. In one of the few studies using transgenic cereal plants, Lin et al. (1995) expressed a chitinase gene in rice and observed enhanced resistance to sheath blight.
- the barley seed ribosome-inactivating protein (RIP), which cleaves an N-glycosidic bond in rRNAs from heterologous sources, thus inhibiting protein synthesis.
- lytic peptides such as plant defensins and alpha-thionin, cecropins from *Cecropia* moth, and the scorpion insectotoxin.
- hen egg-white and T4 bacteriophage lysozymes that lyse bacterial cell walls.

- PR proteins of unknown function, including the tobacco PR1 and PR5.

It is noteworthy that almost all measurements of protection reported in the above studies were performed under controlled conditions using a very limited number of test pathogens. Therefore, the extent of protection conferred under field conditions remains to be determined. Specific pathogens tested and methods used for monitoring disease symptoms also differed between studies, making it difficult to compare the relative effectiveness of different antimicrobial proteins. However, it is apparent that variable degrees of protection were observed in different studies, and that the levels of resistance achieved in some cases are unlikely to be sufficiently high to be of commercial value. In no case was protection complete, and higher levels of resistance would be desirable. Possible means of achieving this include engineering plants to express more than one class of antimicrobial proteins, increasing the amounts of transgenic antimicrobial protein produced, and the use of proteins with more effective antimicrobial properties. The first of these possibilities is considered in more detail below.

Two recent reports have demonstrated that expressing *combinations* of different antimicrobial proteins in individual transgenic plants provides much more effective protection than either protein alone, or than would be expected by simple additive effects (synergism). Zhu et al. (1994), reported that constitutive expression of a chitinase and a beta-1,3-glucanase in tobacco delayed the appearance of first visible lesions and reduced both the lesion number and lesion size following infection with the causative agent of frogeye disease (*Cercospora nicotianae*). In plants homozygous for both the chitinase and the beta-1,3-glucanase genes, the average lesion size was reduced to approximately 10% of untransformed controls, while plants homozygous for either beta-1,3-glucanase or chitinase alone developed lesions sizes of approximately 70% and 55% of untransformed controls, respectively. In greenhouse trials designed to mimic field infections, the mean number of lesions per leaf was reduced by at least 75% compared to untransformed controls. Unfortunately, the extent of reduction observed for either antimicrobial protein alone was not reported. Synergistic interactions between chitinase and beta-1,3-glucanase were also reported by Jach et al. (1995), who monitored the development of symptoms caused by *Rhizoctonia solani* on tobacco. By calculating the expected additive effects of combining the two antimicrobial proteins with the observed results, a 1.5- to 2-fold higher level of protection was apparent. Jach et al. (1995) also demonstrated that chitinase interacts synergistically with the barley RIP. Presumably, hydrolysis of the fungal cell wall by chitinase increases uptake of RIP into fungal cells, thus enhancing its potential to interact and inactivate fungal ribosomes. Degradation of fungal cell walls by hydrolytic enzymes may also result in the increased exposure of fungal membranes and thus enhance the effectiveness of lytic peptides, such as defensins, which act at the membrane level (Brears and Ryals, 1994). Similar beneficial interactions may also occur between other classes of antimicrobial proteins. In addition to beneficial synergistic interactions, combining different antifungal proteins may present a greater obstacle for pathogens to overcome, and thus result in more durable resistance than would be conferred by single genes.

Modifying phytoalexins

Plants produce structurally diverse, low-molecular-weight antimicrobial compounds called phytoalexins in response to microbial infection. Given the diversity in phytoalexin structure, and the observation that at least some pathogens appear to be less tolerant of phytoalexins from non-host plants (Kuc, 1995), one strategy for enhancing disease resistance may be to genetically engineer plants to synthesize compounds structurally unrelated to the ones they naturally produce. In the only example of this type published to date, Hain et al. (1993) engineered tobacco plants (which normally produce sesquiterpenoid phytoalexins) to synthesize the stilbene-type phytoalexin resveratrol. This was achieved by introducing a gene-encoding stilbene synthase from grapevine into the tobacco genome. Transgenic plants capable of rapidly synthesizing high levels of resveratrol were reported to be more resistant to *Botrytis cinerea*, a fungus previously known to be sensitive to resveratrol. An alternative approach to engineering phytoalexins is to modify naturally produced compounds to be more effective. It has been suggested that this may be achieved by enhancing lipophilicity or changing stereochemistry (Lamb et al., 1992).

Manipulating the production of active oxygen species and hypersensitive response

One of the earliest events following pathogen recognition is the transient production of active oxygen species such as hydrogen peroxide (H_2O_2). Wu et al. (1995) produced transgenic potato plants that constitutively produced H_2O_2 by inserting into their genomes a gene encoding glucose oxidase, which converts glucose to gluconic acid and H_2O_2 . Such plants appeared to be phenotypically normal but were significantly more resistant to bacterial (*Erwinia carotovora*, the cause of bacterial soft rot) and fungal (*Phytophthora infestans*, the cause of potato late blight) pathogens.

Another defense-related phenomenon that may be experimentally manipulated to enhance disease resistance is the HR. Localized cell death associated with HR is thought to contain invading pathogens to the site of infection and may also trigger systemic resistance throughout the plant. In order to induce cell death at the site of pathogen attack, Strittmatter et al. (1995) fused the gene for barnase, a bacterial RNase capable of causing plant cell death, to a pathogen-inducible promoter. Potato plants transformed with this chimeric gene did, in fact, undergo cell death at the site of pathogen attack and displayed an enhanced resistance to *P. infestans*. Protection conferred by barnase expression was presumably achieved by limiting the invading pathogen through localized cell death, although a possible role for systemic resistance was not investigated. In a separate study, systemic resistance associated with HR-like lesions was concluded to be the most probable determinant for the observed enhancement in resistance to viral and bacterial pathogens (Mittler et al., 1995). In this case, the necrosis was induced by constitutive expression of a bacterial proton pump, and although distinct lesions were formed, they appeared spontaneously and not in response to pathogen attack.

Pathogen-derived resistance: Toxin "neutralization"

The ability of pathogens to produce toxic diffusible compounds has been demonstrated to be an important factor determining virulence. Such toxins may be effective only on the host plants (host-specific) or on a range of organisms, including non-host plants, fungi and bacteria (non-host-specific). In the latter case, it may be assumed that the pathogens themselves must have evolved mechanisms to escape the effects of the toxins, and therefore it should be possible to identify the genes involved and transfer them to plants as a means of enhancing resistance. Two examples of this type of work have been reported. In the first, de la Fuente-Martínez et al. (1992) protected tobacco plants from the effects of the *Pseudomonas syringae* pv. *phaseolicola* toxin (phaseolotoxin) by transferring into their genome a pathogen-derived gene encoding a resistant variant of the target enzyme, ornithyl transcarbamylase. In the second example, the pathogen-derived resistance gene was an acetyltransferase that detoxified the toxin produced by *P. syringae* pv. *tabaci*, the cause of wildfire in tobacco (Anzai et al., 1989). In both cases, the resulting transgenic plants were less sensitive to the toxins and displayed enhanced resistance to infection by the respective pathogens. Given that an appropriate source of a resistance gene can be identified, this general strategy should be applicable to cases where host-specific toxins are involved. It is also noteworthy that these strategies are likely to be effective only against those pathogens that produce, and rely on, the specific toxins for pathogenicity. As such, the spectrum of resistance conferred will be narrow.

Recent developments in molecular plant pathology

Research into optimizing and extending the strategies described above is already underway. For example, several groups are either searching for more potent antimicrobial proteins, testing combinations of proteins for synergistic interactions, elucidating the structure, biosynthesis and metabolism of phytoalexins and phytotoxins, or isolating new promoter elements that will allow more flexible and efficient targeting of transgenes. In addition, research into fundamental aspects of plant-pathogen interactions will reveal new factors important for virulence and resistance, and should place biotechnologists in a better position to devise more effective strategies for enhancing plant resistance to pathogens. For example, it has recently been demonstrated that the ability to degrade saponins (pre-formed glycosylated triterpenoids or steroids having antifungal activity) is an important factor determining pathogenicity of the fungus *Gaeumannomyces graminis* var. *avenae* on oats (Bowyer et al., 1994). The gene encoding the enzyme responsible for detoxifying the oat saponin avenacin has been isolated and found to cross-hybridize to DNA from other phytopathogenic fungi. This has led to speculation that saponin detoxification may be widespread among phytopathogenic fungi and that specific combinations of saponin/saponin-detoxifying-enzyme may be more important in determining

host-range than previously recognized. The challenge now is to exploit this information to engineer transgenic plants having enhanced resistance to saponin degrading pathogens.

One of the key experiments in proving the importance of saponin detoxification in pathogenicity of *Gaeumannomyces graminis* var. *avenae* on oats was to "knock out" the gene function involved through mutagenesis. Similar mutagenesis strategies should be helpful in confirming the role of other genes suspected of being involved in pathogenesis, as well as in identifying and isolating new genes based solely on a mutant phenotype. Mutagenic screens in plants are also uncovering a number of genes involved in various aspects of protection against pathogens (see, for example, Ausubel et al., 1995; Chasan, 1994a, 1994b; Freialdenhoven et al., 1996; Kunkel, 1996). One important class of genes that has been recently isolated (in part through mutagenesis) is the *R* genes (reviewed in Staskawicz et al., 1995). These correspond to the major disease resistance loci that plant breeders have been manipulating for crop improvement since the beginning of the century. So far, the molecular structure of *R* genes suggests that many are likely to be involved in signal transduction events. Interestingly, *R* genes conferring resistance to very different pathogens, including virus, bacteria, and fungi, share a considerable amount of structural similarity, suggesting that common mechanisms may be used to recognize and defend against a wide range of pathogens. Already, a long list of possible biotechnological applications for *R* genes is being proposed. Possibilities include simplified schemes for introducing, detecting, and combining multiple resistance genes, interspecies transfer of *R* genes, and engineering radically new *R* gene specificities (Michelmore, 1995; Staskawicz et al., 1995). Based on the observation that massive cell death resembling a "systemic" HR can be induced by expressing certain *R* genes in heterologous plants (see Rommens et al., 1995) or by generating plants that express both an *R* gene and the corresponding pathogen avirulence (*avr*) gene (Hammond-Kosack et al., 1994), it may also be possible to exploit *R* genes to induce rapid HR at the sites of pathogen infection. As with other strategies based on expressing potentially toxic genes, it will require the availability of promoters that are activated strictly in response to pathogen attack.

Finally, the phenomenon of systemic acquired resistance (SAR) has generated much interest in recent years. SAR refers to the long-lasting, broad-range systemic resistance that results from localized infection with a necrosis-inducing pathogen (for specific references to SAR, see Delaney et al., 1994; Gorkach et al., 1996). Chemical induction of SAR is also possible and has recently been shown to provide wheat with very good protection to powdery mildew infection under field conditions (Gorkach et al., 1996). Plant mutants compromised in SAR have been isolated (Bowling et al., 1994; Cao et al., 1994; Delaney et al., 1995) and their characterization should provide a better understanding of the process, and possibly genes to allow modification of SAR through biotechnology.

Concluding remarks

Disease in crop plants creates two major problems. The first is a substantial loss of income, easily reaching several billions of dollars on a worldwide basis. The second is the environmental damage resulting from the application of chemicals used in disease control. It is generally accepted that genetic resistance is the "most economical, efficient, environmentally acceptable and long-term sustainable" means of disease control (Martens et al., 1988). It is hoped that the examples included in this chapter were helpful in illustrating some of the possible ways by which biotechnology, through the production of transgenic plants, can be applied to achieve these goals.

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