

18th North American Barley Researchers Workshop and 4th Canadian Barley Symposium

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Breeding for malt and feed quality barley in northern Australia

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Most countries that produce barley classify their varieties as either malt or feed with the feed class consisting of varieties that are not biochemically suited for malting. However, these varieties have probably not been tested for any animal feed value. In a number of countries, including Australia, more barley is used annually for feeding animals than used in beer production. Under Australian feedlot conditions, anecdotal data had suggested that malt varieties were best for feeding cattle but little data was available to support this generalisation. We have undertaken a study comparing over 30 Australian varieties and breeding lines to ascertain some scientific basis to this theory. Genotypes from two sites and two years replicated trials were evaluated for malt and feed analysis. Results indicated that the levels of resting grain components were similar for each end-use. There was no apparent difference in total starch content between malt and feed. However, there were differences for the *in sacco* Dry Matter Digestibility with the good feed and malt genotypes having low levels. While there was no strong relationship for particle size (hardness) between malt and feed quality there was a relationship within a genotype with feed type being slightly harder. This relationship was independent of protein content. The most significant area of difference is the need for malt varieties to produce moderate to high levels of enzymes to breakdown endosperm components during malting and mashing. Varieties that performed especially well in both end-uses, ie good malt quality and improved animal performance, were current malting varieties. The biochemical results to date demonstrate that breeding programs could effectively select for improved malt and feed quality in breeding lines by focusing on malt quality and selecting lines with high level of enzymes.

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Milling energy and grain hardness in barley

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Grain hardness is a product of the complex interaction between compositional and structural endosperm components, including starch, protein and beta-glucan. Hardness may contribute significantly to barley quality. Grain hardness can be evaluated by measuring the energy required to mill (milling energy) or crush (hardness) the grain, with harder grain requiring more force. Our research examines the relationship between milling energy and hardness of several feed and malting barley genotypes grown at multiple locations and the influence of protein and moisture on grain hardness.

Seven feed, one malting variety and one malting barley breeding line were grown in field trials at six Western Canadian sites during 2003 and 2004 and evaluated for milling energy, hardness, moisture and protein content. Milling energy was determined using the 'Comparamill' at the Scottish Crop Research Institute (Scotland). Hardness and moisture were determined using the Perten Single Kernel Characterization System (SKCS). Grain protein was estimated using Near Infrared Transmittance (NIT).

Analysis of variance showed significant differences between genotypes and sites for all measured traits ($P = <0.001$) with no variety by site interaction ($P = >0.99$). Milling energy of genotypes ranged from 617 to 736 joules ($SE = 5.9$). McLeod and CDC Dolly required significantly more energy to mill, followed by Valier, Newdale, Xena and CDC Helgason. CDC Bold, TR253 and CDC Trey required the least energy to mill, indicating a softer endosperm. Milling energy ranged from 625 to 709 joules across sites. SKCS hardness of genotypes ranged from 38.5 to 56.6 ($SE = 0.77$). McLeod was hardest, followed by Valier, Xena and CDC Dolly. CDC Trey, Newdale, TR253, CDC Helgason, and CDC Bold followed with CDC Bold being softest. SKCS hardness ranged from 40.4 to 55.8 across sites. Protein concentration of genotypes ranged from 10.8 to 12.0% ($SE = 0.16$). McLeod, CDC Dolly and Newdale were highest followed by Valier, CDC Helgason, TR253, CDC Bold, Xena and CDC Trey. Protein concentration ranged from 8.8% to 13.3% across sites. Moisture of genotypes ranged from 10.1% to 10.5% ($SE = 0.06$), with larger differences between sites (7.4% to 13.1%). Milling energy was correlated ($n = 9$) with SKCS hardness ($r = 0.81$, $P = <0.008$) and protein concentration ($r = 0.79$, $P = <0.01$). No significant correlation was detected between milling energy and moisture ($P = 0.90$), SKCS hardness and moisture ($P = 0.89$) or SKCS hardness and protein concentration ($P = 0.20$).

Low phytate barley (*Hordeum vulgare L.*) development at the Crop Development Centre, University of Saskatchewan

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Phytate, a complex of phytic acid (myo-inositol 1,2,3,4,5,6-hexakisphosphate) and other minerals, is the primary form of phosphorous in barley grain. Monogastric animals do not effectively digest phytate because they do not produce the phytase enzyme. Diets must be supplemented with inorganic phosphorous (P) or a microbial phytase to meet minimum nutritional requirements. Consequently, excreted phytate generates high levels of P in effluent resulting in possible environmental pollution or eutrophication of waterways. Low phytic acid mutants (with corresponding increases in free and available phosphorous) have been developed in Harrington barley by Dr. V. Raboy, U.S.D.A. Hvlpa1-1 has 50% less phytate and M635 and M955 have 75% and 95% less, respectively. Initial hybridizations of the low phytate genotypes were made in 1998 at the Crop Development Centre (CDC) to adapted hullless parents in the combinations: Hvlpa1-1/CDC McGwire and M635/CDC Freedom. Based on the uniformity of the original mutants from an observation trial in 1999 the initial crosses were subjected to a rapid backcross breeding strategy with CDC McGwire and CDC Freedom as recurrent parents. Four backcrosses were made for each hybrid combination between 1999 and 2000 with each F₁ being screened for phytate to retroactively identify the low phytate F₁ plants for the correct backcross in the greenhouse at the University of Saskatchewan. BC₄F₁ generations were grown as bulk populations in 2000/01 in New Zealand winter nurseries and the subsequent F₂ populations were grown as space planted bulks at Saskatoon, SK in 2001. The BC₄F₃ and BC₄F₄ generations were advanced using a modified single seed descent procedure in the greenhouses at the University of Saskatchewan during the 2001/02 winter. BC₄F₅ lines were grown in the field at Saskatoon as F₅ hill plots in 2002. Each hill plot was derived from an individual F₄ head. Selected hills were tested for phytate. Seed from selected low phytate F₅ hill plots was bulked and increased in 2002/03 winter nurseries in New Zealand. Selections were tested in CDC yield trials in 2003, two of which, SR03013 (50% phytate reduction) and SR03044 (75% phytate reduction), were advanced to the Western Canadian Hullless Barley Cooperative (WCHBCoop) yield trial during 2004 as HB378 and HB379, respectively. HB379 has been advanced for final year testing in the 2005 WCHBCoop and will be put forward for support for variety registration in 2006. Growing 2nd generation Breeder Hills in our 2004/05 New Zealand contra-season nursery has allowed for rapid production of Breeder Seed of HB379 in 2005 in anticipation of variety registration. Using this rapid breeding technique means we have moved from 1st cross in 1998 to a released variety in 2006, a period of less than eight years.

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Post-anthesis biomass yield and quality of barley cultivars developed by Field Crop Development Centre

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Over 2.5 million tonnes of barley silage is produced each year in Alberta to support the livestock industry. Barley is a vigorous, early maturing crop that makes high quality silage and is also a preferred feed grain for Alberta producers. The objective of this study was to determine post-anthesis (PA) biomass yield and quality of barley varieties and advanced breeding lines developed at the Field Crop Development Centre (FCDC), Lacombe. Tests were grown at Lacombe from 1998 to 2004, excluding data for the drought year 2002. The varieties were grown in replicated field trials. At about soft-dough growth stage (post-anthesis) the plots were harvested and wet weights determined. Samples were analyzed to determine quality and percent moisture so dry weight could be calculated. Sub-samples of biomass were analyzed for percent protein, acid detergent fibre (ADF%), neutral detergent fibre (NDF%) and relative feed value (RFV) was calculated. Overall, there were significant variations of 5 to 20 tonnes/ha of PA biomass yield among barley varieties. On average there were no significant differences between the 2-rowed, 6-rowed or hulless barley classes. Biomass protein for all varieties ranged between 8 and 15%. The 6-rowed and hulless barley classes tended to have slightly wider range of protein values compared with the 2-rowed. The ADF ranged between 20 and 40%. The overall NDF ranged between 30 and 65%, although the 2-rowed barleys showed a relatively narrower NDF range of between 39 and 59%. The overall RFV varied between 85 and 200. The 2-rowed barleys showed narrower RFV values varying between 100 and 160 compared with either 6-rowed or hulless barleys. The ADF was positively correlated ($r = 0.85$) with NDF, and grain yield was positively correlated ($r = 0.75$) with biomass yield. The biomass yields, protein % and grain yield showed no correlation to ADF, NDF or RFV. These results suggest that it is possible to breed barley for high post-anthesis biomass yields and quality.

Process development for quick cooking barley products

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Barley is among the most ancient of the cereal crops. Canada is the world's third largest barley producer with an average annual production of 12 million tonnes. Alberta produces approximately one half of the total Canadian production. A large percentage of the production is used as feed for cattle, swine and poultry, while the second largest usage is in the malting industry. A limited amount of barley is used for human food. The objective of this study was to develop processes for quick-cooking barley products to increase food barley consumption.

Alberta grown Falcon and AC Metcalfe cultivars were used for the study. The effects of variety, pearling rate and pre-treatment on moisture uptake were studied. Moisture uptake was used to evaluate the effect of each pre-treatment. Two processes were developed to produce quick-cooking barley. The quick-cooking barley products reduced cooking time from 45 minutes to 15 minutes for 35% pearled barley and from 60 minutes to 18 minutes for 5% pearled barley. Quick-cooking barley can be cooked by boiling in at least twice the volume of water for 15 to 18 minutes followed by a 5-minute stand. Quick-cooking barley products have a cooked texture, as measured by an Ottawa extrusion method, with an average force of 450 N, an average bulk density of 530 kg/m³ and an appearance similar to long-cooking barley (cooking 35% pearled barley for 45 minutes and 5% pearled barley for 1 hour).

There was a slight decrease in the beta-glucan content with the treatments except with the 35% pearled AC Metcalfe, where the beta-glucan level increased. The 5% pearled barley had much higher insoluble dietary fiber content than that of the 35% pearled barley samples. There was a slight increase in the soluble fibre content with the pressure treatment compared with the untreated barley samples.

There were no significant differences between the samples for overall acceptability or flavour, but appearance and texture were significantly different. The steamed barley samples scored significantly higher for appearance and colour than the pressure-cooked samples. The pressure-cooked barley scored significantly higher than the steam-cooked samples for texture, bite and stickiness/looseness.

The successful development of a quick-cooking barley process provides an excellent commercialization opportunity for processors to produce a human consumption barley product, which could be conveniently incorporated into our daily diet.

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PCR detection and quantification of *Fusarium* species

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Fusarium head blight (FHB) is presently the most significant disease of barley in Canada and many areas of the world. FHB is caused by many *Fusarium* species and it is very difficult to correlate visual symptoms with deoxynivalenol (DON) concentration as symptomless kernels may carry the pathogen. Conversely many blighted kernels may not be contaminated with DON. Symptoms may be caused by non-tricothecene producing *Fusarium* species. Identification of *Fusarium* species based on morphological characteristics by growing on selective media is cumbersome and requires considerable expertise and experience. In addition DON estimation is slow, labour intensive and expensive and demand for this service outstrips supply. PCR-based assays to quantify fungal DNA in infected plant tissue could indirectly estimate DON levels. PCR assays have been standardized to detect *Fusarium* species with species-specific primers (reported by Parry and Nicholson 1996, Schilling et al. 1996, Nicholson et al. 1998, Yoder and Christianson 1998, Aoki and O'Donnell 1999, Williams et al. 2002) using 22 isolates of seven different *Fusarium* species. The protocol to detect *F. graminearum* associated with FHB symptoms produced under artificial and natural infections has been standardized. Primer-pair Fg16N amplified the desired band (280 bp) in all infected barley samples from a greenhouse experiment and no band was amplified from uninoculated and uninfected samples. The vast majority of samples from artificially and naturally infected samples from barley fields showed the desired band. Protocol to quantify *F. graminearum* DNA using a competitor DNA template (obtained from Dr. P. Nicholson, John Innes Centre, Norwich, UK) has been standardized. Fungal concentration ranging from 1 pg to 100 ng with a constant competitor DNA template concentration (1fg or 3fg/ μ l) was evaluated and fungal DNA concentration as low as 100 pg (0.1ng) was detected. Work is in progress to quantify fungal DNA from FHB infected barley.

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Can we use Australian identified molecular markers for barley net blotch resistance in western Canadian barley breeding programs?

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Net blotch of barley caused by *Pyrenophora teres* Drechs. is an important disease in western Canada (Tekauz 1990) and elsewhere (Steffenson 1997). Two types of leaf symptoms are associated with the net blotch disease: the net form (NFNB), caused by *P. teres* f. *teres*, which causes a dark brown crisscross venation pattern that sometimes turn chlorotic; and the spot form (SFNB), caused by *P. teres* f. *maculata*, which causes dark brown circular or elliptical spots accompanied by chlorosis of the surrounding leaf tissue (Khan and Tekauz 1982). Yield losses of 20 to 30 % in susceptible cultivars have been reported in western Canada (van den Berg 1988) and up to 40 % in other parts of the world (Khan 1987). More important than yield losses, the pathogen reduces thousand kernel weight, plumpness and test weight, negatively affecting malting and feed quality. The most effective and economical method to control this disease is the use of resistant cultivars, however most commonly grown barley cultivars are susceptible to most isolates of *P. teres* (Tekauz 1990, 2000). The variability observed in *P. teres* and failure to find lines resistant to all isolates suggests breeding for resistance should emphasize pyramiding resistance genes to develop broad-based durable resistance. Molecular markers allow breeders to rapidly introgress resistant genes into elite lines and to pyramid more than one resistant gene into a cultivar. Molecular markers linked to net blotch resistance in barley have been recently reported from Australia (Cakir et al. 2003, Raman et al. 2003, Williams et al. 2003). There is need to determine whether we can use Australian developed molecular markers in western Canadian barley breeding programs. Thirty-nine barley lines were screened with 6 NFNB (WRS102, WRS858, WRS1607, WRS1906, LO256, LO246) and 4 SFNB (WRS857, WRS1881, LO233, LO231) isolates at the seedling stage in the U of S, College of Agriculture Phytotron. Parents of Australian barley mapping populations used to identify/validate net blotch markers in Australia, western Canadian lines, US lines and one Ethiopian accession and lines from International collections were included. The majority of the Australian parent lines were susceptible to western Canadian isolates. Australian 'R' lines Franklin, Alexis, Kaputar, Baudin, Hamelin were susceptible to the majority of the isolates tested. However, Pompadour, Halcyon, Tilga and Chebec were resistant to some isolates. Accession CI9214 showed the best overall resistance to all isolates and several western Canadian lines/cultivars viz. TR253, TR251, TR244, CDC Helgason and CDC McGwire were resistant to the majority of the isolates. We will evaluate Pompadour/Stirling, WPG8412/Stirling and Sloop/Halcyon populations to validate Australian NFNB markers using western Canadian NFNB isolates. For SFNB marker validation, potential populations are CI9214/Stirling, Keel/Gairdner, Chebec/Harrington and Tilga/Tantangara.

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Selection for improved scald resistance in the Crop Development Centre barley improvement program

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Scald, caused by *Rhynchosporium secalis*, is a significant barley disease in western Canada in most years, especially in the moist regions of Alberta and north west Saskatchewan. Control depends primarily on genetic resistance in varieties planted and cultural practices including crop rotation and tillage to bury crop residue. Sources of resistance have been identified; however, many resistance sources break down rather quickly. Success in using the strategy of genetic resistance to control scald has been hampered by evolution and pathogenic variability within scald populations. Improved scald resistance is a part of the Crop Development Centre (CDC) barley improvement program and involves repeated testing of selected lines in a search for putative sources of resistance as well as to monitor scald resistance inherent in the program. Annual testing is conducted in collaboration with T.K. Turkington et al, AAFC, Lacombe at screening nurseries at Lacombe and Edmonton, Alberta. Scald reaction data has been collected at both locations for more than 10 years. Data from 2000 through 2004 is presented for a number of resistant CDC selections and six check varieties. Intermediate resistance derived from CDC Dolly is holding but appears to be less effective than it was initially. Resistance derived from PC11 continues to be effective. Resistance tracing to Arizona hulless waxy (AzHull) remains effective with a large number of selections from crosses with that resistance currently being tested at advanced levels in the CDC program. Lines derived from Senor demonstrate moderately resistant reactions. Resistance from *Hordeum spontaneum* or *H. bulbosum*, introduced to the CDC program via New Zealand accessions, has endured well. Several CIMMYT lines, notably Calicuchima, 18 IBON-128 and 18 IBON-75 exhibit highly resistant reactions. Progeny of BT474, a six row CDC breeding line with no apparent resistance source(s) in its pedigree and now serving as a resistant nursery check, will be screened for scald reaction in 2005 Alberta nurseries. Several selections from crosses with BT474 have now reached advanced yield test stages of the CDC program.

Selection for improved FHB tolerance in the Crop Development Centre barley improvement program

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Continued financial support by the WGRF, CWB and AAFC MII fund to operate a collaborative Fusarium Head Blight (FHB) screening project, initiated in 2000, at AAFC Brandon, attests to the serious impact of FHB on both barley growers and users. As complete resistance has not been observed in barley, genetic resistance from additive action of multiple minor genes offers the greatest potential against FHB. The Crop Development Centre (CDC) barley improvement program's participation in this collaborative project was initially limited to searching for putative sources of resistance for the breeding program and screening existing breeding lines to select for improved reaction to FHB. Repeated testing, 2001 to 2004, identified 9 hulled genotypes that consistently demonstrate DON concentrations comparable to the resistant check CI4196. Two of these lines, 2ND16092 (from NDSU) and HDE84194-622-1 (a Chinese accession from Shanghai Academy) have been used as parents in the CDC program on the basis of their FHB reaction. 114 selections from 7 populations derived from 2ND16092 and 65 selections from 2 populations derived from HDE84194-622-1 will be tested in 2005 for FHB response as Preliminary yield test entries. The remaining two-rowed hulled CDC lines all share TR251 as a common parent. SB00106, the most promising in an agronomic sense, also showed promise as an entry in the 2003 and 2004 North American Barley Scab Evaluation Nursery, where its DON level across all sites was among the lowest. SB00106 was entered in the Western Two Row Coop Test as TR04378 in 2004 and chosen to replace CI4196 as the resistant check in the 2005 Brandon FHB nursery. The larger proportion of hulless, 30 versus 9 hulled lines, repeatedly selected and tested for low DON concentration, emphasize the relationship between hull removal and DON accumulation. DON concentrations for all selected hulless lines were lower than the checks. The predominance of several parents (i.e. CI4196, CDC Freedom and TR251) in the pedigrees of these selections indicate the heritable nature of the low DON accumulation trait. SH00749, selected from an early cross between CDC Freedom and CI4196 (population 99T511-03) was in turn used as a parent to improve FHB tolerance. 115 selections from 4 populations derived from SH00749 will be tested in the 2005 FHB nursery as pre-yield test entries. The FHB response of several of the best CDC selections for lower DON accumulation, with few exceptions, seem to hold up well in other FHB screening nurseries.

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An AFLP derived tightly linked marker for true loose smut resistance (*Un8*)

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True loose smut resistance in barley is conferred by the single dominant gene *Un8*, and is the major source of resistance in cultivars bred for western Canada. Since smut is seed borne, conventional disease screening involves the manual (hand held syringe) inoculation of 10 to 12 florets per spike (line). Mature seed from inoculated plants is harvested and grown to anthesis before plants can be evaluated for the presence of smut, a process which needs to be repeated for putative resistant lines because of potential escapes. Cost estimates for conventional screening are near \$5 per line. This expense and the simple genetics of the resistance make smut resistance an excellent candidate for marker-assisted selection (MAS). PCR based markers for *Un8* (Eckstein *et al.*, 2002) have been in routine use in the Crop Development Centre barley breeding program for more than five years. While the markers are robust and simple to use, the 6cM of genetic distance (recombination) renders approximately 50% of crosses as monomorphic (unscreenable). AFLP on bulked-segregant DNA samples has generated a polymorphism that is more tightly linked with the resistance in 149 DH lines from the cross Harrington x TR306. AFLP primers E32-M58 amplify a short fragment of DNA from the resistant DNA bulk and resistant parent only. This fragment is consistently amplified from constituent lines of the resistant DNA bulk, is absent from the susceptible lines, and co-segregates appropriately with the disease reaction in all lines that showed recombination with our previous marker. Efforts to convert the AFLP polymorphism to a simple PCR-based marker are in progress to simplify linkage analysis on an additional 367 lines from the same population. The polymorphic fragment has been isolated, cloned and sequenced, and consists of 40 bases of genomic sequence from which allele specific PCR-based markers cannot be designed. A 1052bp fragment containing the original 40 bases has been identified through anchored PCR. This locus has been sequenced from resistant and susceptible genotypes in order to design allele specific primers. Analysis of sequence to date indicates that the locus likely has numerous locations in the barley genome and efforts to identify nucleotide variation between resistant and susceptible genotypes at the *Un8* linked locus continue. This tightly linked marker will be useful in reducing the error percentage in MAS, and will increase the amount of breeding material that can be evaluated through MAS. Linkage estimates obtained from the larger population will form the basis for closer examination of the locus and perhaps the isolation of the gene itself. The eventual isolation of the *Un8* gene will allow for the characterization of previously un-phenotyped materials such as can be done with the *Rpg1* resistance gene (Brueggeman *et al.*, 2002) and markers (Eckstein *et al.*, 2003).

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Cytological karyotyping of *Pyrenophora teres*

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Net blotch, caused by the fungal pathogen *Pyrenophora teres*, is a common disease of barley that adversely affects seed quality characteristics like plumpness and test weight. Despite its importance, there is limited information available about the *P. teres* genome. Karyotype analysis of this pathogen was initiated to address this and to provide a foundation for further genetic work including the creation of a molecular linkage map.

One technique available for karyotyping fungal pathogens is the germ tube burst method (GTBM) which allows cytogenetic analysis by releasing mitotic metaphase chromosomes from the actively growing tip of conidial germ tubes. Previous cytological work with fungi focused on meiotic stage chromosomes present during sexual reproduction, however, the sexual stage of many important phytopathogenic fungi is either unknown or difficult to induce in the lab, restricting the use of this method. Pulsed field gel electrophoresis (PFGE) is another popular method of karyotyping fungi which is useful for estimating the molecular weights of chromosomes, but tends to underestimate chromosome numbers due to the inability to resolve chromosomes of similar size. The combination of the GTBM and PFGE make a powerful tool for karyotype analysis.

The GTBM was applied to four isolates of *P. teres*. A time course analysis was conducted to determine the conidial germination rate and the point at which the maximum number of germ tube nuclei were in metaphase. Germination began after 30 min and was near 100% by 120 min. The proportion of metaphase stage nuclei in germlings reached a maximum of 15% at regular intervals of 60-70 min. Culture media was amended with hydroxyurea to attempt to synchronize mitosis and increase the proportion of metaphase nuclei, but only a marginal increase was observed. Cells within the germling contained an average of seven nuclei and the nuclei within each cell appeared to be at the same mitotic stage. These observations were not significantly different between the four isolates studied. Nine chromosomes were observed for each isolate after making a minimum of 20 chromosome counts. Observations on chromosome size were recorded. This karyotype analysis solidifies the only previous estimation of chromosome number in *P. teres*, made using PFGE. In that study, only six bands could be resolved, but densitometric analysis of the larger, unresolved bands led to a chromosome number estimate of nine. This study shows the power of the GTBM to accurately determine karyotypes in fungi.

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A molecular linkage map of *Pyrenophora teres*

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Genetic linkage maps provide basic information about a species' genome organisation and are important tools required for positional cloning of genes. The rice blast pathogen, *Magnaportha grisea*, provides a good example of how a linkage map of this fungus has allowed map-based cloning of several avirulence genes. The goal of this project was to construct a linkage map of the barley net blotch pathogen, *Pyrenophora teres*, that will support future mapping studies.

A mapping population of 80 single ascospore progeny were isolated from a cross between parental isolates WRS 1906 x WRS 1607. The parents were screened with 144 AFLP primer combinations from which 23 primer pairs were selected to screen across the entire population. Because WRS 1906 is avirulent on the barley variety 'Heartland' while WRS 1607 is highly virulent, the population was also evaluated for virulence on Heartland and segregated 38 avirulent: 42 virulent ($\chi^2 = 0.2$, $P = 0.70$). This suggested a single gene controlled the avirulent phenotype and this locus was placed on the linkage map. Finally, the mating type (MAT1/MAT2) locus was mapped on the population using a set of PCR primers specific to this locus. The map consists of 110 unique loci distributed over 18 linkage groups. Only eight (7.3%) markers displayed a Mendelian segregation ratio different from 1:1. The total map length is approximately 650 cM.

The present chromosome number estimate for *P. teres* is nine based on pulsed field gel electrophoresis (PFGE) and cytological observations. This clearly indicates that many of the linkage groups represent common chromosomes. Currently markers from each linkage group are being hybridized to PFGE-separated chromosomes in order to assign linkage groups to specific chromosomes. Mapping of the chromosome telomeres has also been initiated to determine how fully the current map represents the genome and to better define the region around the avr locus near the terminus of linkage group 6.

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The barley stem rust resistance gene *Rpg5* encodes NBS-LRR and protein kinase domains in a single gene

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The biotrophic fungus *Puccinia graminis* causes stem rust of barley. Several major genes for resistance (i.e. *Rpg1-Rpg5*) have been described in barley. To understand the molecular basis of stem rust resistance, we have focused on the isolation and characterization of the genes involved in the incompatible interactions between barley and *Puccinia graminis*.

The *Rpg1* gene was recently cloned and predicted to code for a receptor-like kinase with dual kinase domains (Brueggeman et. al., PNAS 99:9328, '02), representing a novel class of plant disease resistance genes. A genetic locus believed to contain the barley stem rust resistance genes *Rpg5* and *rpg4* was delimited genetically to two BAC clones and completely sequenced. The *Rpg5* locus confers resistance to the rye stem rust pathogen *Puccinia graminis* f. sp. *secalis*, isolate 92-MN-90 and the *rpg4* locus confers resistance to *P. graminis* f. sp. *tritici* pathotype, Pgt-QCC. Annotation of the BAC sequences revealed several candidate resistance genes. Allele sequencing from resistant and susceptible cultivars and recombinant lines resulted in a single candidate *Rpg5* gene. The *Rpg5* gene was confirmed by allele sequencing and it also appears to be required for *rpg4*-mediated resistance. This was indicated by the presence of recombinants resistant to isolate 92-MN-90 (*Rpg5*), but not to QCC (*rpg4*). Recombinants resistant to QCC, but susceptible to 92-MN-90 were never isolated among over 5,000 gametes examined. *In-silico* translation of the *Rpg5* sequence from the resistant line Q21861 revealed a protein containing NBS-LRR and protein kinase domains, all in one gene. Several cases are known in the literature where an NBS-LRR gene and a protein kinase gene are required for resistance to a pathogen, but this is the first case where all three domains are encoded by a single gene. Further validation of the gene is underway using a viral induced gene silencing approach as well as complementation by *Agrobacterium* mediated transformation. Characterization and validation of this gene will be presented and possible mechanism of resistance discussed.

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***In vitro* selection for pre-screening barley for resistance to *Fusarium* head blight**

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Cultivation of susceptible wheat and barley cultivars has resulted in FHB epidemics in the Midwest USA and Manitoba. The use of barley cultivars with genetic resistance to FHB is the most cost effective and environmentally sound means to manage this disease. However, the limited occurrence of FHB caused by *Fusarium graminearum* Schwabe in Alberta, especially in the central and northern regions, requires developing methodology to screen resistance under controlled conditions. Barley genotypes (*Hordeum vulgare* L.) with known field reactions to fusarium head blight (FHB) were used for *in vitro* ground grain and leaf detached assays. In the ground grain assay, the FHB reaction of the genotypes was measured based on the extent of mycelial growth of *F. graminearum* (PW027) on a mixture of agar and ground grain at room temperature ($23\pm 1^\circ\text{C}$). In addition, the deoxynivalenol (DON) level in a mixture of *F. graminearum* biomass and ground grain of each line was determined using an ELISA-based assay. *Fusarium graminearum* showed larger colony diameters (mm) for susceptible 'AC Lacombe' and 'Stander' compared with resistant genotypes, 'Chevron' H94051001, I92130, and H93120, except for resistant CI4196. *Fusarium graminearum* produced more DON on susceptible 'AC Lacombe' compared to resistant 'Chevron', I92130, and H93120, except for resistant CI4196 and H94051001. Larger colony diameters and higher DON content were also observed for susceptible genotypes compared with resistant ones in a repeated test with another 16 barley genotypes. Detached leaves of six barley genotypes were inoculated with single isolates of *F. graminearum* (PW027) and *F. culmorum* (PW0T6) for evaluation of partial disease resistant (PDR) components at 10°C and $23\pm 1^\circ\text{C}$, and DON content at $23\pm 1^\circ\text{C}$. Both isolates were pathogenic at $23\pm 1^\circ\text{C}$ with shorter incubation period and greater lesion sizes on detached leaves compared to 10°C . PW027 was more virulent than PW0T6 at both temperatures resulting in shorter incubation and larger lesions for all barley genotypes but not for spore production. *Fusarium graminearum* appeared to better differentiate between resistant and susceptible barley genotypes at $23\pm 1^\circ\text{C}$ compared with *F. culmorum*. However, there was no clear pattern in DON content between resistant/tolerant and susceptible genotype. The results of *in vitro* evaluations tended to agree with previous field reactions in terms of FHB reaction and DON level of the genotypes. Thus, both *in vitro* assays may be alternate selection methodologies for FHB resistance. The detached leaf assay has the advantage of measuring specific disease components, allowing elucidation of the potential nature and genetic components of resistance to FHB operating for this assay. The potential of screening barley embryos of different genotypes against DON as a method of identifying resistant genotypes was also evaluated. In a preliminary test, 20 days old embryos of susceptible 'AC Lacombe' and resistant H94051001 were evaluated in Murashige Skoog media amended with different concentrations of DON. DON was found to considerably reduce the embryo weight of 'AC Lacombe' compared with that of H94051001. Further embryo experiments are being carried out, with screening of more barley genotypes against different DON concentrations.

Diversification strategies for barley disease management in Alberta

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Barley is an important feed grain crop in Alberta that accounts for 40-50% of Canadian production. To ensure a constant supply of feed many farmers often grow barley continuously for several years; however, continuous barley production leads to a build-up of plant diseases and a general reduction in yield potential over the long-term. A three-year experiment was established at Lacombe in 1998 to determine if barley cultivar rotation could be used to reduce the impact of leaf diseases, while maintaining crop productivity. Treatments consisted of various sequences of two cultivars with varying degrees of scald and net blotch resistance, 'Kasota', and 'AC Lacombe'; a previously scald-resistant cultivar 'CDC Earl'; a susceptible check, 'Harrington'; and a non-host, triticale cultivar 'Wapiti'. Rotations were established in 1998 with comparisons being made in 1999 and 2000. In 1999, significant rotation differences occurred for scald and net blotch severity, with disease severity usually highest and yield lowest when a barley cultivar was grown on its own residue, especially for cultivars other than 'Kasota'. Statistical analysis using contrasts indicated that yield and kernel weight were lower, while scald and net blotch levels were higher for barley cultivar monoculture compared with barley cultivar rotation. In 2000, when a barley cultivar was grown on its own residue, scald severity was usually highest compared to barley cultivar rotation. A similar trend was also observed for net blotch, especially for 'Harrington' and 'AC Lacombe'. Poor stand establishment in some plots precluded the detection of yield differences among some treatments in 2000. Contrasts for 2000 indicated that higher levels of scald and net blotch, and decreased kernel and test weights occurred for barley cultivar monoculture compared to barley cultivar rotation. In both 1999 and 2000, barley planted on triticale residue generally had the highest yield, kernel weight and test weight, while having the lowest disease levels compared to planting barley on barley residue. Barley cultivar rotation can be a potential short-term strategy to help reduce leaf disease levels and maintain crop productivity for Alberta barley producers where crop rotation options are limited due to feed requirements or market factors. However, continuous production of barley, even utilizing cultivar rotation, will not provide long-term sustainable leaf disease management, especially for scald. The summer of 2004 was the third year of a separate trial that was established to look the interactive effects of agronomic factors such as rotational diversity, seeding rate, and time of silage removal on crop health, competitiveness, disease levels, productivity and quality in a cereal silage production system. In 2004, all plots except for the continuous triticale were seeded to the barley cultivar 'Seebe'. The rotation treatments of 'CDC Helgason' barley/'Pronghorn' triticale/'Seebe' and 'Pronghorn'/'AC Mustang' oats/'Seebe' had significantly greater emergence compared to the continuous 'Seebe' treatment, which had the lowest emergence. The remaining rotation treatments had intermediate emergence and were not significantly different from the continuous 'Seebe' rotation. Overall, silage yield on a dry weight basis was highest for the 'Pronghorn'/'AC Mustang'/'Seebe' rotation (8060 kg/ha), intermediate for the 'CDC Helgason'/'AC Mustang'/'Seebe' rotation (7822 kg/ha) and lowest for the remaining rotations (7005-7279 kg/ha, LSD = 400 kg/ha). The spot-form of net blotch was the main leaf disease present and it was highest for the continuous 'Seebe' rotation (13.8% leaf area diseased on the flag -2 leaf) and lowest for the 'Pronghorn'/'AC Mustang'/'Seebe' rotation (5.9%) with the other rotations having intermediate disease levels (8.2-11.7%, LSD = 2.1). Rotation had a significant influence on root mass assessed in the fall. Root mass was highest for the 'Pronghorn'/'AC Mustang'/'Seebe' rotation (42.4 g/2 m length of row), lowest for the continuous 'Seebe' rotation (29.1 g), and intermediate for the remaining rotation treatments (34.4-37.0 g, LSD = 8.2 g). Rotation treatments had an impact on silage yield and this appeared to be related to crop health as indicated by leaf disease levels and root mass measurements. A second 3-year cycle of this trial is being repeated starting in 2005.

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Resistance of western Canadian barley genotypes to scald in Alberta

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Barley (*Hordeum vulgare* L.) production in Alberta has averaged around 6 million metric tonnes from 2 million hectares annually from 1995 to 1999, and accounted for close to 50% of the total barley production in western Canada. Scald caused by *Rhynchosporium secalis* J. J. Davis is one of the major foliar diseases causing significant yield loss in Alberta as a result of intensive barley production, and cool and wet environmental conditions that favor disease development. In the current study, nine barley differentials were grown up in hill plots at multiple sites to monitor scald development during 1997 – 1999 (Period 1), twelve during 2000 – 2001 (Period 2), and twelve during 2003 – 2004 (Period 3). Thirty-eight genotypes and commercial cultivars with different levels of resistance were also evaluated for scald reaction from 2003 to 2004 at these sites. Differentials Abyssinian, Atlas, Atlas 46, Atlas 68, Hudson, Osiris, Kitchin and Turk were resistant to scald at all sites, other differentials including Brier, La Mestita, Modoc and Trebi showed intermediate levels of scald, indicating that the majority of major genes for scald resistance are effective against *R. secalis* pathotypes in Alberta. The differentials showed highly significant correlations in scald severity among all three periods. Period 1 and Period 2 ($R^2=0.86^{**}$) were, however, more closely correlated, compared with Period 1 and Period 3 ($R^2=0.63^{**}$), suggesting the development of considerable variability in *R. secalis* in response to major genes for resistance during 1997 to 2004 in Alberta. Analysis from ten station-years of data classified thirty-eight genotypes and cultivars into three major clusters corresponding to scald reaction: resistant, intermediate and susceptible. Those in Cluster I that were resistant included AC Stacey, Kasota, Mahigan, Manny, Niobe, Seebe and five other genotypes from the Field Crop Development Centre. Eighteen commercial cultivars in Cluster II were intermediate in scald reaction and these included AC Lacombe, CDC Dolly, CDC Earl, CDC Guardian, CDC Kendall, Falcon, Nisku and Peregrine. The three susceptible barley cultivars including Harrington were classified into Cluster III with several malting cultivars including AC Metcalfe, CDC Sisler and Excel, and three feed barleys, AC Bacon, AC Rosser and CDC Candle. The number of cultivars that were classified to be resistant was up from previous studies. A substantial number of feed cultivars were classified to be intermediate in resistance in the present study due partly to the overall moderate level of scald severity during 2003 – 2004 in Alberta. Changes in *R. secalis* pathotypes and conducive weather conditions may increase severity, resulting in breaking down of resistance, as demonstrated in previous study. Given the fact that no malting cultivar was resistant and only one was classified as intermediate in scald reaction, there is a need to incorporate scald resistance genes into malting cultivars for production in Alberta.

Mapping and molecular marker development of scald resistance in ‘Seebe’ barley

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Scald (*Rhynchosporium secalis*) of barley is prevalent in central Alberta, Canada and causes considerable yield and quality losses. Scald can rapidly change in pathotype composition and frequency, thereby making it difficult to develop durable scald resistance in barley. Previous studies have shown that the cultivar ‘Seebe’ carries durable genetic resistance, however, barley breeders have found this trait difficult to transfer into new barley lines. Therefore, we are trying to develop molecular markers for scald resistance from ‘Seebe’. Recombinant inbred lines were developed from the cross of ‘Harrington’ (scald susceptible) and ‘Seebe’ (scald resistant). Progeny of about 175 individual F₂ seedlings were advanced by single-seed descent to the F₈ generation. Disease resistance to a major scald race was phenotyped at the seedling stage in a greenhouse. By utilizing bulked segregant analysis (BSA), resistant and susceptible pooled populations were compared by AFLP analysis. A total of 255 AFLP primer combinations were used to analyze the genetic population and several *EcoRI-MseI* and *PstI-MseI* fragments were found linked to scald disease resistance. We are also analyzing this population with SSR markers. Our goal is to map and identify molecular markers flanking the genes contributing to scald resistance in ‘Seebe’.

Key words: scald resistance, marker development, *Hordeum vulgare*, barley

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Breeding for multiple disease and multiple gene resistance in barley

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Combining genes for disease resistance is very difficult, as most breeding programs can only test for the diseases present at their breeding sites. In addition, pyramiding genes or developing multiple gene resistance is difficult to detect when testing at only one location. Multiple disease and gene resistance involves breeding against more than one pathogen and more than one gene per pathogen. Each pathogen may have several races that are able to attack varieties and render a resistant variety ineffective in a short period of time, presenting a significant challenge to plant breeders.

Over the years the ICARDA/CIMMYT barley program in Mexico has given us an excellent opportunity to screen for multiple gene resistance for Scald in barley and at the same time look at multiple disease resistance to Stripe Rust, Barley Yellow Dwarf Virus, Leaf Rust, and Fusarium Head Blight (FHB). In Canada not only have we screened for Scald, but have also screened for Loose Smut and Covered Smuts as well as Net Blotch (net and spot forms), Spot Blotch, and FHB. Over the last 5 years we have screened over 2000 breeding lines at 4 locations in Canada and 3 locations in Mexico. New combinations of resistance genes have been found with some lines containing genes for resistance to 5 and 6 diseases. We found multiple gene combinations for scald resistance that have 3 or more genes and should give durable resistance to this disease in both countries. In order to classify breeding lines according to resistance gene combinations, we are currently analyzing overall similarity computed from multivariate disease resistance data and matching it to the pedigree.

The best lines will be used in the breeding program in order to rapidly incorporate even greater disease resistance into new varieties for Alberta producers. We will also develop several populations to begin the process of mapping on as many of these genes as possible. Continuation of this research is necessary to anticipate and cope with the changes in disease problems likely to occur in the future. Up to this point in time, stripe rust has not been a problem in Alberta on barley; however, in 2004 this disease was found on barley at Olds, Trochu, Calmar and Lacombe. If this disease continues its move north it will be devastating to Alberta's barley crop. FHB also is not presently a problem in Alberta but seems to be moving west. FHB has cost the barley industry millions of dollars in the Midwest in the United States and in Manitoba in Canada.

Variation in virulence among net blotch isolates infecting barley

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Pyrenophora teres, causal agent of net blotch of barley, is widely distributed throughout North American production areas. Previously, researchers have shown variation in virulence of isolates within and between locations. The purpose of this research was to continue monitoring diversity of isolates in locations of interest to the BARI barley breeding program. Isolates were collected randomly from symptomatic plants from 2000 to 2004. A set of 25 differential varieties was developed based on work of other researchers. Differentials were planted in Rootainers containing Metromix 200 and grown in the greenhouse. Isolates were grown on V8 Juice agar at 18C with 12 hr light per day for 7 days. Plants were inoculated at the 2-leaf stage with a suspension of a single isolate, incubated in the dark at 18C and 100% RH for 24 hr, and returned to the greenhouse. Plants were evaluated for disease on a 0 (R) to 9 (S) scale 7-10 days later. To date, 16 isolates have been characterized on 25 differential varieties. Isolates ranged from the least virulent (PT01/6 from Bottineau, ND), infecting 5 differentials, to the most virulent (PT04/14 from Minot, ND), infecting 20. Of these 25 differentials, only 2 (9839 and CIHO 5822) were resistant to all isolates and 3 (Alexis, Bonanza and Klages) were susceptible to all isolates. There was no correlation of virulence to year or location. This study will continue in order to assist breeding programs developing resistance to net blotch.

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Assessment of artificial inoculation methods and deoxynivalenol levels in barley lines representing various candidate sources of resistance to *Fusarium* head blight

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Fusarium Head Blight (FHB) is one of the most devastating diseases affecting the production of barley and other cereal grains throughout Canada and the world. FHB infection results in drastic decreases in crop yield and severe reduction in grain quality. The most common species causing FHB in North America is *Fusarium graminearum* (Schwab). The fungus produces mycelial extensions that rapidly spread among the florets, leaving shrivelled kernels and floral pieces covered with a pink or white film of mycelium displaying elevated deoxynivalenol (DON) levels. 19 barley lines representing FHB candidate resistance and susceptible sources were point inoculated or spray inoculated in the greenhouse with 40,000M/ml of *F. graminearum* macroconidia. Following inoculation barley plants were kept at 24°C and 95% humidity for 3 days to favour disease establishment and were then returned to normal growing conditions at 21°C without humidity control (45%). 18 days post-inoculation heads were collected and spikelets displaying symptoms of FHB infection were rated for progression of the disease. These lines were also evaluated in the Brandon, MB nursery from 2000-2004. The number of discoloured spikelets produced by point inoculation, indoor spray inoculation, and disease and DON assessment were compared for each of the 19 lines. Barley lines representing candidate sources of FHB resistance or susceptible FHB sources showed varying degrees of symptoms due to fungal infection in the three tests. Barley lines representing more or intermediate FHB resistant sources showed consistent levels of resistance in the three tests. Artificial inoculation methods and DON quantification enable us to rate the level of resistance of the barley lines, with confidence. A higher level of FHB resistance will guarantee lower risks for the farmer associated with crop losses due to reduced grain yield, low quality grain, and DON contamination.

Reactions of barley lines to leaf rust, caused by *Puccinia hordei*

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Resistant barley cultivars are the most economical and efficient means of controlling leaf rust caused by *Puccinia hordei* G. Otth. However, changing virulence in *P. hordei* has rendered ineffective many of the known resistance genes. In this study, isolates 'Race 8', '90-3', '90-15', '89-3', and 'Neth 202' of *P. hordei* were used in the greenhouse to differentiate resistance genes in a collection of 82 selected barley lines. These lines exhibited resistance in the previous tests against isolate 'Race 8' at the seedling stage. Barley line 'C2-02-134-2-2' exhibited low reaction types to all the tested leaf rust isolates, suggesting that in addition to an already introduced resistance gene, *Rph15*, it possesses one or more new resistance genes. The F₂ population of a cross made between 'C2-02-134-2-2' and a susceptible line 'ZA47' which was challenged with isolate 'Race 8', segregated into a 15:1 resistant to susceptible ratio ($X^2=0.853$) based on disease reaction. The 15:1 segregation ratio indicates that 'C2-02-134-2-2' possesses two genes, *Rph15* and a new single dominant resistance gene. In the F₂, the *Rph15* phenotype (00;) was separated from the new resistance gene phenotype (0;12-). To isolate the new gene from *Rph15*, the 10 F₂ plants bearing the new phenotype were transplanted and selfed and the F₃ will be screened for homogeneity of disease reaction. The identification of the new resistance gene(s) and incorporation of them into barley cultivars will add in reducing yield losses due to leaf rust.

The differences in fermentable carbohydrates of major Canadian malting barley varieties and their effects on fermentation

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Abstract

The fermentable carbohydrates of the major Canadian malting barley varieties were monitored during malting and brewing processes. It was observed that the fermentable carbohydrate compositions of the congress wort were varietal dependent, while the fermentable carbohydrate compositions of the finished wort were both varietal and mashing condition dependent. The overall malt modification affected malt carbohydrate composition, and malt's brewing performance and final beer quality.

NanoMash: A novel procedure for research mashing of limited-quantity barley malts

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The ASBC Malt-4 extract analysis method (Congress Mash) provides a standard set of conditions for generating an unhopped wort commonly used to evaluate the malting quality performance of experimental malts. However, the method requires specialized instrumentation and relatively large quantities of malt. These requirements prevent researchers without access to the specialized instrumentation and particularly those with limited sample availability from generating Congress worts for malting quality analysis and other research uses. Use of a commercially available device allows agitation of small volume samples through orbital mixing while heating or cooling them using a Peltier temperature controlled block. Controlling the device to follow the Congress mash temperature profile offers the possibility of conducting a mashing cycle at a significantly reduced scale. Adaptation of standard ASBC wort analysis methodology to microtiter plate and other reduced-scale methodology allows provision of several key parameters for worts generated from size-limited samples. While not intended to supplant standard ASBC methodology, this small-scale mashing protocol significantly lowers the sample requirements and extends the potential for malt analysis to research programs where it may not have been previously feasible.

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Comparison of hull peeling resistance of barley and malt in western Canadian two-row barley lines

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Hull peeling resistance in barley and malt is desired by the malting and brewing industry. A group of 15 two-row malting barley (*Hordeum vulgare* L.) lines and one feed cultivar were used to compare these traits in Canadian two-row barley and determine their relationship to other agronomic traits, including grain yield, kernel plumpness, test weight and kernel weight. Barley samples from standard yield test plots at two sites in each of Manitoba and Saskatchewan during 1999 and 2000 were evaluated for % hull peeling on a weight basis direct from the plot combine (RPWB) and after inducing peeling with an air-blast de-huller (APWB). Micromalted samples were evaluated for % hull peeling of the malt “as is” (RPWM) and after air-blast de-hulling (APWM). Analyses of variance over years and locations indicated that hull peeling of barley and malt were strongly influenced by environmental factors, particularly year. However, the single largest source of variance for RPWM and APWM was the genotypic component, indicating that heritability and response to selection would be higher in malt than barley. Genotypic effects were lowest for RPWB, but still highly significant ($P < 0.001$). Genotype-by-environment interactions were also highly significant, but lower in magnitude. Manley, Stein and CDC Dolly were consistently lower in all hull peeling traits, while TR251, CDC Unity, AC Oxbow and AC Bountiful were significantly higher with TR244 always being the highest. The relative ranking of the barley lines varied somewhat with CDC Unity, AC Bountiful and Merit ranking higher for susceptibility to hull peeling as malt than barley, while AC Metcalfe, Harrington and TR253 were ranked less susceptible as malt. Among hull peeling traits, the highest correlation of 0.95 ($P < 0.001$) was between RPWM and APWM, indicating that RPWM would be adequate for determining hull peeling in malt. The correlation between RPWB and APWB was also high at 0.92 ($P < 0.001$), but RPWB was more variable due to its relatively low values averaging about 1.0% overall compared with 36.8% for APWB. Other correlations among hull peeling traits ranged from 0.82 to 0.92. There were moderate negative correlations between test weight and the hull peeling traits, ranging from -0.55 ($P < 0.05$) to -0.69 ($P < 0.01$). Kernel plumpness, kernel weight and yield were not significantly correlated with any of the hull peeling traits. In conclusion, it should be possible to develop two-row malting barley cultivars with acceptable kernel characteristics and improved resistance to hull peeling of both barley and malt.

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Elimination of barley colour defects in Australia

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Grain colour defects including staining and black point have been a problem for Australian barley growers for a number of years, resulting in thousands of tonnes of malting barley being downgraded each year. Over the last decade, research has been conducted investigating many aspects of these disorders, including objective assessment, biochemical evaluation, crop management, storage effects and resistance breeding. A number of breeding lines have been identified with resistances to both forms of colour defects. The black point tissue has been extracted with high levels of phenolic acids (namely ferulic and coumaric acids) being present suggesting the dark pigmentation may be a large polyphenolic compound. Black pointed grain also has an impact on germination rate. Markers for black point resistance coincide with markers for dormancy and pre-harvest sprouting amylase. The effects on fungal contamination results in a dark staining on the grain. A number of factors impact of the degree of staining including pre-harvest rainfall, timing of the rainfall event and pre-existing level of resistance. Husk content also appears to have an impact on the final appearance of the grain. Markers for husk content also coincide with genetic regions for dormancy and pre-harvest sprouting. The improvement of grain quality at intake can be delivered through two options; 1. the development of barley varieties with resistance to these grain defects as resistance to black point and staining has been shown to be heritable and 2. optimising early harvest strategies and cool-air drying on-farm.

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Characterization of barley tissue-ubiquitous beta-amylase2

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There are two barley β -amylases genes, encoding important starch degrading enzymes. The endosperm-specific β -amylase (Bmy1), the more abundant isozyme in cereal seeds, has been thoroughly characterized. The lesser abundant β -amylase2 (Bmy2), has not been biochemically characterized from any cereal seeds. Characterization of Bmy2 from two commonly grown barley (*Hordeum vulgare* L.) cultivars, 'Morex' and 'Steptoe', was a major objective of this study. The bmy2 cDNAs were sequenced, expressed in *Escherichia coli*, and the recombinant enzymes (rBmy2) characterized. The relative hydrolysis rates of various α -D-glucans and the pH activity optima of 'Morex' and 'Steptoe' rBmy2s were the same and not significantly different from barley rBmy1. The 'Morex' rBmy2 was 7°C more thermostable than the 'Steptoe' rBmy2, determined by differences in their T50 values, and is more thermostable than any reported wild type β -amylase1. Three amino acid differences were identified between the two Bmy2 sequences and the contributions to enzyme thermostability evaluated by site-directed mutagenesis. Examination of mutant enzymes with one amino acid substitution revealed that each of the three residues contributed ~3°C to the thermostability of the 'Morex' wild type rBmy2. Mutant enzymes with two amino acid substitutions contributed ~5.6°C and the triple amino acid mutant enzyme contributed ~8.7°C to thermostability. To date, no quantitative trait loci (QTL) for malting quality traits have been associated with the bmy2 locus. Should an association be discovered, the 'Morex' bmy2 allele, containing D238, M337 and Q362, provides a discrete signature of a thermostable β -amylase2 that could be targeted for marker assisted selection.

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Barley seed osmolyte concentration as an indicator of preharvest sprouting

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This study was conducted to test the hypothesis that barley seed osmolyte concentrations can be used as an indicator of preharvest sprouting (PHS). Osmolyte concentrations from the 2002 Minnesota and North Dakota crops were compared to pearling and other techniques for assessment of PHS. Approximately 30% of the seed evaluated were sprouted. Samples were evaluated for osmolyte concentrations, pearling, and Stirring Number, while smaller subsets were evaluated using other methodologies. Osmolyte concentrations correlated well with pearling ($r=0.822$, $P<0.0001$) and fluorescein dibutyrate staining ($r=0.835$, $P<0.0001$). α -Amylase activity correlated less positively with osmolyte concentration ($r=0.650$, $P<0.0001$). Stirring Number, Falling Number, germination, and accelerated aging had weak negative correlations with osmolyte concentration. It is concluded that seed osmolyte concentration, a relatively simple assay, is a good indicator of PHS in barley as assessed by the pearling method or fluorescein dibutyrate staining.

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Osmolyte concentration as an indicator of malt quality

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This study was conducted to test the hypothesis that malt osmolyte concentrations can be used as an indicator of barley malt quality. Seeds of four 6-row and four 2-row genotypes were steeped for six days at 20°C for 6 days. At intervals of 24 h over the steeping regime green malt from each cultivar was removed and tested for osmolyte concentration, malt extract (ME), diastatic power (DP), α -amylase activity, soluble/total protein (S/T), and β -glucan concentration. Osmolyte concentrations increased most rapidly in days 1 through 3 of germination. After 4 days osmolyte concentrations began to plateau. Significant positive correlations were found for malt extract and osmolyte concentrations in days 1 through 4 and day 6 ($r=0.740$ to 0.942 , $P<0.0001$). Days 2 and 3 osmolyte concentrations correlated well with ME for all days ($r=0.740$ to 0.942 , $P<0.0001$) and α -amylase activity for day 2 ($r=0.771$, $P<0.0001$). Day 2 osmolyte concentration correlated well with days 2 through 6 for β -glucan concentration ($r= -0.702$ to -0.830 , $P<0.0001$). No significant correlations were found for DP and osmolyte concentrations on any day. These data indicate that osmolyte concentrations at early time points in steeping are good indicators of several measures of malt quality at later time points in steeping.

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Relationships among malt fermentability and malt quality parameters under the influence of barley β -amylase heat stable allele

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Starch hydrolysis during germination is achieved by the action of four major enzymes, α - and β -amylase, limit dextrinase and α -glucosidase. This hydrolysis produces fermentable sugars required for yeast nutrition in brewing. Fermentability is a critical quality parameter for brewing that affects the level of alcohol produced by yeast, and is typically assessed in malt extracts by determining the change in specific gravity after small scale fermentation, referred to as apparent attenuation limit. Diastatic power is a measure of the capacity of the malt to degrade starch into fermentable sugars and is primarily determined by β -amylase activity. Although diastatic power is a reasonable predictor of fermentability, it does not always accurately estimate the level of fermentable sugar generated during mashing or the subsequent fermentability of the resultant wort. β -amylase is one of the major proteins found in the starchy endosperm, which rapidly loses activity at mashing temperatures above 55°C. Two alleles with different β -amylase enzyme thermostability are distinguished with the insertion/deletion of a palindromic 126-pb sequence in intron III of β -*amy1* gene. Increased thermostability results in more efficient starch degradation. Barley cultivars with high thermostability β -amylase allele will achieve high levels of fermentability without a good malt modification. On the other hand, barley cultivars with low thermostability β -amylase allele will achieve high levels of fermentability if they achieve high levels of malt modification. The objective of this study was to determine the influence of heat stable β -amylase allele in the relationship between fermentability and quality parameters. We characterized 40 commercial malting barley varieties from different countries. The malt quality data used in the correlations studies come from the average of the results obtained by 50 laboratories. The quality malt parameters analyzed were: Fine Grind Extract, Total Nitrogen, Hartong 45, Diastatic Power, Wort Viscosity, Alpha-Amylase, Friability, Free Amino Nitrogen, Final Attenuation Apparent and Soluble β -Glucans. Significant correlations were obtained from the statistical point of view, but values of R square explained less than 20 % of the variability among the parameters. In general, barley varieties that present high thermostability β -amylase allele showed better qualitative levels of fermentability and malt modification than the varieties with low thermostability β -amylase allele. From these results we conclude that the fermentability is not easily predicted from quality parameters, therefore the use of β -*amy1* allele in barley breeding program is necessary to improve fermentability levels in malting barley grains. More studies are needed to understand all the properties that influence malt fermentability and how they interact.

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Russian wheat aphid resistant barley – cultivar and germplasm release

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RWA continues to be a devastating pest of barley in the high and dry areas of the Western U.S.A. Screening of the entire National Small Grains Collection in Aberdeen Idaho by the USDA-ARS in Stillwater identified 115 accessions with some level of resistance ranging from 2 to 6 on Webster's scale of 1 to 9 where 1 is immune and 9 is dead. Resistant germplasm lines were developed from each accession and two of these lines, STARS 9301B and STARS 9577B were released in 1993 and 1995 respectively. A long term prebreeding project was initiated at the USDA-ARS in Stillwater to develop adapted germplasm lines by bringing multiple sources of resistance into barley cultivars and elite lines of both state and federal barley breeders across the country. These breeders as well as extension personal from several states have been involved in field testing of the 62 prebred germplasm lines now ready for release. A detailed description of these lines and a time table for their release will be presented. Along the way several feed barley cultivars have also been developed. The first RWA-resistant barley cultivar, Burton, has released by USDA-ARS in Aberdeen in conjunction with USDA-ARS in Stillwater and several other cooperators. Burton, a 2-rowed, hulled, spring barley, has shown excellent performance in irrigated and dryland areas both in the presence and absence of RWA. Three, 2-rowed, spring, feed barleys, developed by the USDA-ARS in Stillwater and Aberdeen and which are adapted to the extremely arid conditions of the western high plains are currently in seed increase and planned for release this fall. A new biotype, RWA2, identified in Colorado in the summer of 2003, has been found to damage all currently grown wheat cultivars developed for resistance to the original biotype, RWA1. All germplasm lines and cultivars slated for release from this program have been found to be resistant to RWA2 as well as RWA1.

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BARMS: A new relational database for barley breeding programs

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Breeding programs generate large amounts of phenotypic data on parents, segregating populations and derived experimental lines. There currently are very few commercially available databases that are specifically designed for use in plant breeding programs.

After conducting an initial study of existing database options that were permitted on our corporate computer systems, it was decided that we should develop a proprietary data solution based on the Oracle™ relational database software engine. The development process was done in stages that were user tested and then refined to meet the requirement standards and user acceptance. The Barley Ag. Research Management System (BARMS) went live in February of 2004 and has routinely used by our barley breeding programs since.

One of most difficult problems in interpretation of multi-location, multi-year data from agricultural experiments is separating the relative contributions of genetics (G), environment (E) and G x E interaction. This can be especially critical when comparing ‘un-balanced’ data sets from lines at different stages of trialing that have not been grown in the same number of station x years. One partial solution to such data sets is to adjust all observations relative to one or more check cultivars. Phenotypic data is stored in BARMS in standard units of measure (i.e. Yield in Bu/A, Plant height in cm) and can be queried in that standard format or in alternate units of measure (i.e. Yield in Kg / ha, plant height in inches). Data for all traits can also be retrieved on a 1-99 **RP** (Relative Phenotypic) scale where data is set relative to three known check cultivars. The six-row breeding program uses Morex, Robust and Legacy; the two-row breeding program uses Harrington, B1202 and Merit as the three comparator checks. Data points where an experimental line appears in the same trial with all three of the respective checks will be used to calculate a line RP for that trait on a 1-99 scale. In this form environmental and G x E interactions are minimized and the RP value represents a best linear unbiased estimate of the true genotypic contribution. These RP scores can be used to evaluate lines from un-balanced data sets on a more equitable basis.

We designed a **SELECTION MODULE** in BARMS that permits selections to be made using both independent culling with upper and lower limits on single trait values as well as index selection based on weighted sum of squares of deviation of multiple trait values from defined targets. It is believed that BARMS is the only software program to permit simultaneous use of both selection protocols.

We developed a **CROSS COMPARISON MODULE** that permits multi-trait evaluation of parental phenotypic data in all possible pair-wise combinations as a predictor of future overall success of the resulting progeny of a cross.

We automated our malt quality laboratory equipment to directly upload evaluation results into the BARMS database. This has reduced the manual entry time and increased accuracy of the micro-malting data coming from our laboratory.

Mapping and molecular marker development of seed dormancy in a barley population derived from ‘Samson’ barley

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Wet field conditions just prior to harvest can cause pre-harvest sprouting in barley (*Hordeum vulgare* L.) resulting in significant economic losses especially in barley genotypes with low seed dormancy. Conversely, malting barley varieties with too high dormancy levels can result in inconsistent germination, creating problems in the malt house. Seed dormancy is defined as when viable kernels fail to germinate under optimum conditions of moisture, oxygen, and temperature. Phenotypic selection for sprouting resistance is challenging because the dormancy trait is controlled by multiple genes and influenced by the environment. Developing molecular markers linked to dormancy would be one method of selecting for desirable levels of seed dormancy in barley. Our objective is to identify, map and develop potential molecular markers linked to genes affecting dormancy in ‘Samson’ barley. Several recombinant inbred lines (RILs) were created by crossing ‘Samson’ derived lines, having high dormancy, with hulless barley varieties ‘Falcon’ and ‘Phoenix’. Dormancy levels were calculated using a weighted germination index (WGI) on the RIL population of 239 lines, originally derived from crossing ‘Phoenix’ and ‘Samson’. This phenotyped population is currently being analysed with SSR markers and AFLP analysis. As expected, initial results suggest multiple QTLs throughout the barley genome, with the most apparent marker linkages associated with seed dormancy occurring on chromosomes: 2H, 3H, 4H, 5H, and 6H.

Key words: seed dormancy, marker development, *Hordeum vulgare*, barley

Genotypic variations in preharvest sprouting resistance and seed dormancy in barley

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Seed dormancy is a vital agronomic trait related to seed quality because it determines resistance to preharvest sprouting (PHS). The aim of this study was to evaluate if there are any genotypic differences in spike sprouting and seed dormancy among advanced breeding lines (genotypes) of barley and determine if there is any association between spike sprouting and whole seed dormancy. Five separate tests comprised of 103 advanced breeding lines and registered barley varieties were seeded in the field in 2004 in 8-row plots of 1 x 2.5 m in three replicates arranged in a randomized complete block design. Three intact spikes, mainly from the primary tillers, were evaluated for resistance to sprouting resistance in a rain simulator at 18 °C. Sprouting was rated visually on a 1-5 scale (1= no visible sprouting, 5= 100% sprouted) and ratings were converted to spike sprouting indices (SSI) that took into account the promptness of spike sprouting. The genotypes were designated as resistant (R) to sprouting if they had a SSI range of 3.0 to 4.0; moderately resistant (MR) if 4.1 to 5.0; susceptible (S) if 5.1 to 6.0; and very susceptible (VS) if >6.0. Also whole seeds for each line were tested for seed dormancy based on a weighted germination index (WGI). Continuous variations were observed both in the SSI and WGI among genotypes. There were genotypic differences in tendency for spike sprouting ranging from 3.1 (R) to 7.3 (VS). For the SSI, the hulless barley varieties ranged from 3.5 to 5.9, the 2-rowed ranged from 4.7 to 6.5 and the 6-rowed ranged from 3.8 to 6.5. There were wide variations in WGI ranging between 0 (no seed germination) and 1 (100% seed germination). For the WGI, the hulless barley varieties ranged from 0.2 to 1, the 2-rowed ranged from 0.1 to 0.9 and the 6-rowed ranged from 0 to 0.8. The cultivars Vivar and Xena (feed barley) consistently appeared to have good resistance to spike sprouting. The correlation between the WGI and SSI ($r = 0.37$), although significant ($P > 0.01$), appear to be weak.

Using growing degree days to estimate maturity in small grain cereals

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For over thirty years, maturities of small grain cereals have been estimated at Field Crop Development Centre by using dry-down rates and moisture contents at harvest. Maturity is estimated to occur at 35% moisture content. Indicator plots of each crop type have been used each year at each plot site to determine a linear rate of dry-down based on Julian days. However ever year, there can be problems in getting the indicator plots harvested during the linear phase of dry-down so we wanted to develop a estimate for dry-down that could be used over a wide range of environmental conditions. In previously reported work, standardized rates of dry-down using growing degree days ($GDD = \Sigma[(T_{min} + T_{max})/2]$) and growing season precipitation were developed for barley, spring and winter triticale, spring and winter wheat, and winter rye. In 2004, data were collected to determine the validity of our GDD-based rates of dry-down. We compared GDD-based maturities with maturities based on the indicator plot dry-down rates. For barley, our best correlation ($r=0.80$) between maturities using the two methods ($n=5,181$) was found when the dry-down rate included both GDD and growing season precipitation, however the closest fit based on similar range and mean maturities was found using a mean rate of dry-down based solely on GDD. The 2004 data confirmed over a wide range of environments, that using a standardized rate of dry-down based on GDD was valid. The 2004 data will be incorporated into updated GDD and GDD plus precipitation rates of dry-down, and a final decision will be made on the method of determining maturities from 2005 onwards.

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Twelve years of barley-based rotations

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In 1988 a barley-based rotation was begun at Lacombe. There were eleven four-year rotations that were run for twelve years (three complete cycles). After the 12 years, a uniformity trial was run planting all plots to Niska, six-row feed barley. Soil NO_3N , PO_4P , Na, K, and SO_4S were measured in the fall of 1999, with the only significant rotation effect being found on soil nitrogen levels. When green manure followed three years of triticale N levels were the highest, but when green manure followed barley-canola-winter wheat levels were not significantly elevated. A year of fallow did not elevate nitrogen levels. Protein, acid detergent fiber, neutral detergent fiber, and relative feed value of the biomass harvest after anthesis (about the soft-dough stage) were not affected by rotation. Highest post-anthesis (PA) biomass yields were found when the previous year had been fallow, green manure or alfalfa. The lowest PA yields were following barley (although barley the previous year was not the sole determinant of low yields as other rotations with barley the previous year had intermediate yields). Grain yields and test weights were not affected by rotation. However both kernel weights and percent plumps were positively influenced by barley-barley-canola-fallow, winter wheat-green-manure-barley-canola, and barley-barley-alfalfa-alfalfa rotations; while continuous barley and canola-winter wheat-peas-barley had negative effects on these traits. Grain protein levels were highest following continuous barley, wheat-canola-barley-barley and triticale-triticale-triticale-green manure; and lowest following triticale-barley-peas-barley and barley-winter rye-barley-canola. Rotations that led to high soil N levels did not always translate into high protein levels in the grain; and this combined with higher percent plump and kernel weights may mean we need to rethink our recommendation for malting barley production. Further investigation is warranted.

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Development of winter hulless barley varieties as a high value crop

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Prior to the early 1990's winter barley cultivars released and grown in the U. S. mid-Atlantic region were traditional hulled feed barley types. Traditional hulled barley has been grown for centuries in the mid-Atlantic region on many farms as feed for all classes of livestock. Demand for low-fiber, high-energy grains by the vertically integrated swine and poultry industries, and availability of brewer's distilled grains for beef and dairy industries have resulted in greatly reduced demand for traditional feed barley in recent years. In the mid 1990's, the Virginia Tech Breeding Program realized that survival of winter barley as a viable crop was dependent on development of commercially acceptable winter hulless barley cultivars having high value traits for specific end uses. During the past 10 years, the Virginia Tech barley breeding program has developed hulless lines that yield 314-1129 kg ha⁻¹ higher than initial winter hulless lines developed. Many lines have improved straw strength and grain plumpness and have better resistance to prevalent diseases. Meanwhile, increased interest in the use of hulless barley varieties having high energy and digestibility in manufacturing food and fuel products, as well as feed, has accentuated our desire to develop winter hulless barley varieties having greater marketability in both domestic and foreign markets. Additionally, barley grain contains health-related compounds similar to those found in oats, therefore, adding to its appeal in the health-food sector. The use of barley in ethanol production may soon become a reality and would provide a viable market for hulless barley produced in the mid-Atlantic region. We also have collaborated with nutritionists and chemists to characterize and improve the nutritional and compositional quality of hulless barley via breeding for specific end uses. The breeding program's first major achievement was the release of the winter hulless barley cultivar Doyce in 2003. In collaboration with the USDA-ARS Eastern Regional Research Center, data on chemical and nutritional composition, including protein, starch, lipid and beta glucan concentration, have been obtained on most barley lines in our replicated yield trials. To date, significant progress already has been made in the development of winter hulless barley lines. We have developed more than 3,000 winter hulless barley populations. This year (2005), we will advance over 350 hulless populations and evaluate 325 pure lines in yield tests and select pure lines among nearly 9,000 hulless headrows. Over one hundred advanced winter hulless barley lines are being evaluated in four states (Maryland, Pennsylvania, Kentucky and Delaware). Doyce hulless barley being produced in 2005 will be evaluated in pilot studies for its potential use in ethanol production and as an improved feed component in poultry rations.

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Multiple dominant and recessive marker stock development

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A set of genetic marker stocks for barley, *Hordeum vulgare*, have been developed. Dominant alleles of several genes are in one doubled haploid stock. A master recessive doubled haploid has matching recessive alleles.

Dominant alleles in the master dominant stock with matching recessive alleles in the master recessive: B1p Vrs1 Pre2 Zeo1 Wst7 Btr Alm Pub Kap Hsh Srh Raw1 Rob Wax Nud Lks2.

Several more multiple recessive stocks are available. A genetic male sterile is present in a recessive background, similar to the above master recessive, for each of the seven chromosomes. Also incorporated on the appropriate chromosomes are five surface wax mutants, five dwarfs, and a few other recessive alleles.

Recessive alleles in the chromosomal stocks:

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1H msg1 cer-e ert-b nec1
1H trd
2H msg2 gsh6 eog lig
3H msg5 uzu
3H als
4H msg24 glf1 lbi2 yhd(alm is not present in this stock)
5H msg19 ert-g
6H msg36 gsh4
6H cul2 dsp9
7H msg10 gsh3 brh1
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These barley stocks are moderately early maturing. They were grown and selected in western Canada, with alternate generations in growth chamber and greenhouse.

Reference: 1996 Special Issue - Barley Genetic Newsletter. Vol. 26.

The author is grateful to Mr. Les Shugar for producing the doubled haploid versions of the master dominant and recessive stocks.

Genetic male sterile and xenia assisted reciprocal recurrent selection

Robert I. Wolfe

Two pairs of barley populations were developed to illustrate the potential for genetic male sterile and xenia assisted reciprocal recurrent selection. They carry the following alleles:

- 1a) msg1, Sex1, yellow aleurone, btr1, cer-e, vrs1, nud
- 1b) msg2, sex1, blue aleurone, btr1, rob, vrs1, nud
- 2a) msg1, Sex1, yellow aleurone, btr1, cer-e, Vrs1, nud
- 2b) msg2, sex1, blue aleurone, btr1, rob, Vrs1, nud

These barley lines are spring habit, and have been selected in central Alberta.

The idea is to speed up selection for yield and agronomic performance by completing a full cycle of reciprocal recurrent selection per year.

Unfortunately, the blue aleurone has proven unsatisfactory for this purpose. Its genetics are too complex, and when blue aleuroned lines are crossed onto lines carrying the yellow aleurone, the blue aleurone does not consistently colour up the resulting seeds.

If a practical working system is to be developed the blue aleurone should be replaced by a gene with two easily identifiable alleles having strong xenia penetrance. A possible candidate is Wax wax, perhaps with the closely linked gsh3 for use as a field identifier. Wax wax can be used in hulled barley, whereas aleurone colour could not. Identification of waxy versus starchy seed would be somewhat tedious, but doable. Normal versus yellow starch might also work if it could be inserted into barley as a single gene effect. The Sex1 sex1 gene is acceptable, along with rob as a field identifier.

This noted, following is an explanation of the concept. There are two generations a year, a crossing generation and a yield test. Two spring barley populations are developed, either two-row or six-, and pure for either btr1 or btr2. They must carry a different genetic male sterile, such as msg1 and msg2. In the crossing generation, in a winter nursery, seeds from the two populations are inter-planted close enough to inter-pollinate and far enough apart to produce several tillers per plant. Each population is normally 50% sterile and 50% heterozygous for sterility. Only seed from the genetic male sterile plants is harvested. The seed from each plant is identified as inter- or intra-population seed. Inter-population seed is planted in a hill yield test in the area for which it is to be adapted, with several seeds per hill, each hill being from one plant. The conditions must mimic as closely as possible a farmer's field for selection to be useful. The parent plants from the winter nursery are ranked according to hill performance, and intra-population seed from the best ones sent south for the next crossing nursery.

New elite germplasm can be incorporated and added to each population.

After a few cycles, F₂ seed from the best yield test hills can be entered into the standard breeding system in use in the program.

Isoyield analysis of barley cultivar trials in the Canadian Prairies

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Classification of test sites for cultivar trials into groups with similar within-group site performance and response (isoyield groups) is an important step towards identification of appropriate cultivars that are best suitable for different productivity levels in farm fields. The objective of this presentation is to determine isoyield environments in the Canadian prairies based on the analysis of cultivar trials consolidated from individual provinces for barley (*Hordeum vulgare* L.). Yields for the analysis were taken from 324 replicated trials sown at 84 sites across the prairies during 1995 – 2003. The combined use of regression and cluster analyses of the data normalized for averaging the multi-year unbalanced data led to a stratification of the 84 sites into 13 isoyield groups. A comparison was made of the distributions of the variability among and within groups according to three modes of grouping: isoyield groups, soil zones and agroecoregions. There was more variability among isoyield groups and correspondingly less within the groups than that among and within soil zones and agroecoregions. Similar contrasting pattern existed for the variance components involving genotype-environment interaction (GEI) though the GEI variability was generally small under all three modes of grouping. Relationships of site sensitivity (regression coefficient) and stability (coefficient of determination) with site productivity were shown to be a useful aid for selecting a subset of test sites in an effort to improve efficiency and quality of future cultivar testing. Thus, the isoyield analysis should be a valuable tool for a meaningful subsetting of heterogeneous environments and for a reduced GEI impact in cultivar testing and recommendation.

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Genetic analysis of preharvest sprouting in barley

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Preharvest sprouting (PHS) can be a problem in barley production, especially of malting barley. Rain or even very high humidity from near physiological maturity onward can cause sprouting in spikes. This has very serious consequences for malting grain, since rapid and complete germination is critical. Much information has been gained by studying the genetic control of dormancy (measured as percent germination) in barley. The objective of this study is to determine if the germplasm developed and QTLs discovered in previous research of dormancy can be applied or related to the genetic control of PHS. PHS was measured in this study as ‘PHS score’ based on visual sprouting in mist chamber-treated spikes at 0 and 14 d after physiological maturity and as ‘alpha-amylase activity’ in kernels taken from mist chamber-treated spikes that showed little or no visible sprouting at physiological maturity (0 d). Germination percentage was also measured at 0 and 14 days after physiological maturity. Many QTLs for dormancy have been previously mapped, most of which are minor and inconsistently expressed across environments. Consistently expressed major and minor dormancy QTLs were previously mapped to barley chromosomes 1 (7H), 4 (4H), and 7 (5H) in the U.S. Barley Genome Project Steptoe (dormant) / Morex (non-dormant) doubled haploid mapping population. Evaluation of this population grown in two environments (greenhouse and field) for PHS score revealed QTL regions on all chromosomes, except chromosome 6 (6H) and for alpha-amylase activity on all seven chromosomes from one or both environments. However, many of the QTLs identified were minor in effect. QTL effects for all traits analyzed ranged from 4 to 36%. The two major dormancy QTLs previously identified on chromosome 7, as well as, the minor ones on chromosomes 1 and 4 were confirmed in this study. Some of the PHS score and alpha-amylase QTLs coincide with known dormancy QTLs, but there appear to be QTLs unique to PHS, as well. Some PHS alpha-amylase activity QTLs coincide with known malt-derived alpha-amylase activity QTLs, but some do not. The major chromosome 7 dormancy QTLs detected from this cross are expressed during PHS, but several previously identified minor dormancy QTLs appear to be more important during preharvest sprouting than during after-ripening or after-harvest dormancy conditions. Whereas, the literature frequently equates dormancy and preharvest sprouting, it appears there is some difference in genetic control of these two somewhat opposite traits. Both traits are complexly inherited, but with some overlap and some uniqueness in gene expression. In addition, several relatively major genes seem to stand out in expression with many minor genes presumably interacting or adding to the expression of the two traits. This study continues with two other mapping populations previously analyzed for dormancy. Ultimately, key QTLs will be identified, which should benefit the breeding efforts of both six-row and two-row barley for a suitable balance between the tendencies for preharvest sprouting and dormancy.

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Effects of ethylene in barley (*Hordeum vulgare* L.) tissue culture regeneration

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Ethylene is a gaseous plant hormone that regulates numerous cellular processes from germination to flowering and senescence. It is produced under stress conditions such as tissue culture and can be physiologically significant in-vitro due to enclosed conditions. This study was conducted to determine genotype-dependent ethylene production and its role in regeneration of barley (*Hordeum vulgare* L.) callus. Six barley cultivars were examined and found to produce different amounts of ethylene during culture. The highest regeneration was observed in cultivars generating the most ethylene. Ethylene production was correlated with regeneration rates ($r^2 = 0.90625$). There were no significant genotype by stage interactions for either ethylene production or green plant regeneration. The media was modified by adding the ethylene precursor, ACC (1-amino-cyclopropane-1-carboxylic acid) or the ethylene antagonist silver nitrate (AgNO_3) to the media at different stages of callus culture to determine the effects of ethylene during plant regeneration. Highest regeneration in Morex was observed when AgNO_3 was added to maintenance stages (M-1, M-2) and lowest regeneration when AgNO_3 was applied to the regeneration stage compared to the control. In Golden Promise, AgNO_3 added throughout the second maintenance and regeneration stages showed the highest regeneration compared to control. Regeneration was significantly affected with addition of ACC in Morex and highest when ACC was added at the second maintenance stage. Golden Promise did not show improved regeneration when ACC was added at any time. Regeneration was highest for the control. Further manipulation of ethylene synthesis and/or action will be used to identify critical timing and duration for ethylene to effects on plant regeneration from recalcitrant genotypes. Ethylene exposure for briefer time periods will help pinpoint the specific stages when ethylene should be manipulated.

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Validation of select diastatic power QTL in elite Western U. S. six-rowed spring barley germplasms

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Barley is a major commodity in the western US., especially when utilized for malt production. Spring six-rowed barley germplasm adapted to the western US. is often low in diastatic power (DP), an important malting trait. DP quantitative trait loci (QTL) and linked markers have been identified in a spring six-rowed mapping population grown in western environments, but these QTL have not been validated in elite western six-rowed backgrounds and the QTL are based on restriction fragment length polymorphism (RFLP) markers. The objectives of this study were to validate DP QTL in populations containing elite western germplasm and to identify polymerase chain reaction (PCR) - based markers linked to select DP QTL.

Low DP spring six-rowed cultivars or elite lines were crossed to 'Morex', a high DP 6-rowed barley, or to SM#42, a high DP line of the 'Steptoe'/Morex mapping population. F₁ plants were backcrossed to the adapted, low DP lines two or three times, and segregating lines were developed from the backcrossed populations. The low DP parents and backcross-derived segregating lines were genotyped with PCR-based markers closely aligned to five DP QTL with large effects on chromosomes 1H, 4H, and 7H. Lines homozygous for the PCR-based markers and parental checks were planted in 4-m rows at Aberdeen, ID. Quality analysis to determine DP levels was performed by the Cereal Crops Research Unit, Madison, WI. Nearly all the low DP parental lines had marker alleles that resembled either Steptoe or Morex with regard to DNA fragment size. Two lines from Utah State University had DP-negative marker alleles for all loci except the one on the short arm of chromosome 4H. Some had more DP-positive alleles with a few DP-negative alleles, while others had similar numbers of DP-positive and DP-negative alleles. Also, some heterogeneity within lines was detected. We plan to determine if relationships between marker genotype and DP can be identified in the advanced populations and if other traits are affected by DP marker selection. This study should provide useful information for the development of six-rowed malting barleys in the western U.S.

The barley stem rust resistance gene product RPG1 is specifically degraded upon infection with the stem rust fungus *Puccinia graminis* f. sp. *tritici* pathotype MCC

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Disease resistance in plants, mediated by the gene-for-gene mechanism, involves the direct or indirect recognition by the R-gene product of specific effector molecules produced by the pathogen. This recognition triggers a series of signals regulating an elaborate series of defense mechanisms by the plant. In order to understand the role of the recently cloned barley stem rust resistance gene product RPG1 (Brueggeman et al. 2002) in resistance to the stem rust fungus *Puccinia graminis* f. sp. *tritici*, we investigated the fate of the RPG1 protein in response to infection with the *P. graminis* f. sp. *tritici* avirulent pathotype MCC. Different barley lines with varying levels of resistance were challenged with the avirulent pathotype MCC and sampled at 0, 12, 16, 20, 24 and 36h post-infection. The extracts were immuno-precipitated with an RPG1-specific antibody. The precipitate was subjected to SDS-PAGE, and RPG1 was visualized by western blot analysis. Though the endogenous transcript levels of *Rpg1* remained unchanged upon infection with the avirulent pathotype MCC (Rostoks et al. 2004), the RPG1 protein disappeared to undetectable levels 20-24h post-infection. The disappearance of the RPG1 protein was localized to the infected tissue and did not spread to the adjoining leaves. The RPG1 protein was shown to be stable in cyclohexamide translation inhibited leaves for up to 48 hrs. These results suggest that the localized disappearance of RPG1 protein is due to proteolysis, probably by the avr-gene product. Since the mere absence of the RPG1 protein does not result in resistance, the degradation products or the process of degradation may trigger the signaling resulting in resistance to the stem rust infection.

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Saturation mapping of barley chromosome 2H *Fusarium* head blight resistance QTL

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Outbreaks of fusarium head blight (FHB), caused by *Fusarium graminearum*, within the past two decades have caused large economic losses to farmers in North Dakota and northwestern Minnesota due to the reduced quality of harvested barley by blighted kernels and significant levels of deoxynivalenol (DON), a mycotoxin produced by the fungus. Field practices and chemical control have had limited success, putting greater importance on a genetic approach for control. Two quantitative trait loci (QTL) each for lower FHB severity and plant height, and one major QTL each for DON accumulation and days to heading were mapped with recombinant inbred lines obtained from a cross between CIho 4196, a two-rowed resistant cultivar, and Foster, a six-rowed susceptible cultivar (Horsley et al., submitted). These loci reside in the barley chr. 2H region flanked by the markers ABG306 and MWG882A (bins 8-10). In an attempt to saturate this region with markers, 29 rice chr. 4 BAC clones with synteny to this region were blasted against the barley expressed sequence tag (EST) database. To date, 41 of the ESTs picked by this method have been mapped to this region. Nine other genes, identified based on microarray analysis of the wheat-barley 2HL addition line and comparison to Betzes and Chinese Spring controls (courtesy of Gary Muehlbauer), were also mapped to this region on the Foster x CIho 4196 RFLP map. To date, there are a total of 26 unique loci and 67 markers in this major FHB QTL region on chr. 2. Eighteen markers have been hybridized to the 6x cv. Morex barley BAC library, identifying 131 BAC clones as part of the physical map of the region. Three cleaved amplified polymorphic sequences (CAPS) markers were designed for the major FHB resistance QTL in this region, two flanking and one in the middle, to aid in development of isolines containing fragments of this region from CIho 4196 in a Morex cultivar background.

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Map-based cloning efforts of the barley spot blotch resistance gene *Rcs5*

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The *Rcs5* gene confers seedling resistance to barley spot blotch, caused by the fungus *Cochliobolus sativus*. Spot blotch is a common and economically important foliar disease of barley in the Midwestern United States. Genetic mapping localized the resistance gene between the markers MWG622 and KAJ154 on the short arm of chromosome 1(7H). A BAC clone physical contig was constructed consisting of 4 clones, 053N3, 612G14, 452P9, and 808M17. Subclone shotgun libraries of BAC clones 612G14 and 808M17 were constructed and sequenced by the Arizona Genomics Institute. Sequence assembly resulted in 25 contigs ranging from 1kb to 28.5kb for 808M17 and 7 contigs ranging from 0.6kb to 63.7kb for 612G14. Analysis of the contigs' sequence by a gene prediction program (FGENESH) with limits within monocot genomic DNA yielded 40 putative genes, 22 with protein homology. Of the 22 protein hits, 19 had equivalent Triticeae EST's. The BAC contig overlap between 612G14 and 452P9 as well as the overlap between 452P9 and 808M17 left an unsequenced gap of ~15kb. The BAC 452P9 38kb NotI subclone, TBD001, covers this region and contains markers KAJ108B.2 and KAJ154, which flank a high recombination region with 11 crossovers and potentially the *Rcs5* gene. The subclone TBD001 sequencing is in progress. Physical and genetic mapping of predicted genes will delimit the region of interest. Putative candidate genes will be sequenced from resistant and susceptible cultivars.

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A gene tagging system for *Hordeum vulgare*

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An efficient system for production of knockout plants for functional analysis and gene tagging in cereals would be a significant complement to current cereal genome analyses. *Tos17*, a rice retrotransposon activated by tissue culture, has been successfully used for generation of knockout plants in rice. More recently, it was reported that *Tos17* insertions were predominately located within coding sequences and thus provide a unique system to develop knockout plants at a relatively high frequency. Retrotransposons have the advantage over other transposon systems of yielding stable mutations and low copy numbers which facilitate the identification of genes. Using a novel approach that enables the cultivar-independent regeneration of fertile monocots, transgenic barley T₀ was efficiently obtained, and confirmed by southern blot. Results suggest that *Tos17* is a very efficient system to generate knockout plants in cereals. Under vegetative growth, the integrated rice retrotransposon *Tos 17* is inactive based on the absence of retrotransposase activity in barley leaves and stems. Under tissue culture conditions, the retrotransposase activity was stimulated and readily detected via a reverse transcription-PCR assay. A modification in the number of *Tos17* copies in barley genomic DNA (from callus tissues) was detected. We also regenerated fertile plants C₀, from 4-5 months callus culture, with novel *Tos17* insertion sites. Using real time PCR, we report an increased copy number in C₀ barley genome, and new phenotype in offspring C₁. We will discuss the advantages of this new system for development of knockout plants in cereals and possible impact for genomic studies in barley.

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Unraveling the mysteries of germination using SAGE (Serial Analysis of Gene Expression)

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The processes involved in malting are still somewhat a mystery on a genetic level. SAGE (Serial Analysis of Gene Expression) is a technique that allows rapid, detailed analysis of thousands of transcripts in a cell. The process of SAGE relies on two principles. Firstly, a small sequence of nucleotides from the transcript, called a “tag” can effectively identify the original transcript from whence it came. Secondly, linking these tags allows rapid sequencing analysis of multiple transcripts. By examining the transcripts expressed at any time in the cell it is possible to determine which genes and their related proteins are being expressed at that moment in time. In this study the gene expression profile of germinating (malting) barley is being examined at seven intervals over a time course of 120 hours post steeping. This will be compared to a baseline of dry ungerminated seed. The identification of genes for improved malting quality can be identified and examined using SAGE and ultimately used for commercial improvement.

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