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Chair

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Expanding opportunities for barley food and feed through product innovation

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Barley has a long history of use as both human and animal food and is grown in many countries around the world. In Western countries, barley is primarily used for animal feed and for malting and brewing with very little designated for food use. Over the last two decades there have been a number of important developments that have influenced or have the potential to influence barley utilization in food and feed.

Interest in the use of barley as a food grain has increased primarily because of its reported health benefits. Barley is an excellent source of β-glucan soluble fibre and contains antioxidants, vitamins, minerals, and phytonutrients such as phenolics and lignans. These components have biological activities that can reduce the risk of coronary heart disease, diabetes and certain cancers. As a whole grain, barley can also play a role in weight maintenance. One key development which will have a significant effect on the use of barley as a food ingredient in North America is the pending FDA health claim for coronary heart disease and β-glucan soluble fibre from barley.

Another important development that has the potential to influence the use of barley in food applications is the development of hulless barley cultivars and varieties with low amylose (waxy), zero amylose and high amylose content. Hulless cultivars permit greater ease in milling and pearling with enhanced processing yields and have higher levels of β-glucan. Waxy cultivars typically have higher β-glucan levels than non-waxy types. Although high levels of β-glucan are undesirable in animal feed and malting and brewing applications, it can be advantageous in the development of barley based foods by providing improved functionality and nutritional properties. Thus, the development of a wide range of barley types allows for targeting specific barley cultivars to specific end uses.

Application of novel technologies to isolate and concentrate β-glucan to maintain molecular structure and solubility has resulted in commercialization of barley β-glucan preparations for use in the food industry. In addition, the application of infrared technology has shown the potential to yield whole barley food products with unique end-properties.

In terms of animal feed, barley has the potential to continue to be one of the preferred cereals in beef, dairy and swine rations in Western Canada. Until the late 1990's barley was only used to a limited extent in poultry rations due to the negative effects associated with the viscous soluble fibre, β-glucan, on bird performance. However since that time, an endogenous enzyme, β-glucanase, has been developed that effectively eliminates the anti-nutritional effects associated with β-glucan. This enzyme is now commercially available and it is common practice to use barley in poultry rations when the cost is competitive with other cereals. However, the use of enzymes to reduce the negative impacts of the soluble fibre in cultivars specifically designed for food use which contain exceptionally high levels of soluble β-glucan may not be sufficient thereby reducing the utility of this ingredient in poultry diets unless regular types of barley are used.

One factor that has limited the use of barley in poultry and some swine rations is the high proportion of insoluble fibre from the hull attached to the seed. As a result, hulless barley cultivars have the potential to markedly increase energy content if used as a feed ingredient. Although the development of hulless cultivars could significantly impact the utilization of barley in feed rations both domestically and internationally, there have been difficulties in establishing a market for hulless barley. The primary issue has been the inability to obtain a premium for the product that offsets the reduced yield caused by loss of hulls during harvesting. If this issue can be addressed, hulless barley has the potential to develop into an important market in both feed and food.

Another new possibility which has the potential to increase the use of barley in feed applications is the development of a new type of barley which may reduce phosphorus levels in animal feces. The majority of phosphorus in plants is found in a form that animals are not able to digest. As a result, the diets are supplemented with available inorganic phosphorus and the undigested organic fraction is passed into the feces which can result in environmental issues where intensive livestock production occurs. Although these low phytate cultivars are not currently available on a commercial basis, if they are released for production, they will likely be an attractive feed ingredient given the pressure to reduce the impact of livestock production on the environment.

Although barley is a very popular ingredient in ruminant rations, the starch is susceptible to rapid fermentation in the rumen resulting in digestive disorders if the product is not handled correctly. In addition, rapidly fermented starch can cause depression in milk fat content which is undesirable given the current incentive to increase milk fat content of milk. The recent development of the barley cultivar Valier shows significantly reduced rates of starch fermentation suggesting a possible improvement in the utility of barley as a feed ingredient.

Barley, like all other feed ingredients has inherent variability and this affects the value and the utility of the product. Prior studies have demonstrated the potential to determine nutritional value of barley using Near Infrared Reflectance Spectroscopy (NIRS) but the calibrations were not developed to the extent that they could be used commercially. Recently, a consortium of researchers and industry partners in Canada have initiated a program to develop a commercially useful NIRS calibration so the nutritional profile of individual lots of barley could be rapidly established prior to use. Once this calibration is available in approximately 3 years, both the producers and the end users will have the ability to segregate, value and use the barley based on its' actual value further improving the utilization and value of this important feed grain. Additionally, this technique has the potential to be used to confirm the identity of varieties of grains with unique characteristics increasing the likely hood that novel barley will be identifiable and utilized by the feed industry. It is also possible that this initiative will be useful to the food industry to target selected barley qualities to specific end use applications.

Thus, the use of barley as a food and feed ingredient shows tremendous potential for the future. Development of hulless and novel types of barley with unique characteristics as well as the application of novel technologies has and will continue to have a significant effect on the expansion of barley utilization. Efforts to increase the use of barley need to continue and success can be achieved through innovations made at the breeding, processing and product development level.

Validation of an in vitro analysis to determine energy digestibility of barley for grower pigs

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In vitro analyses will be beneficial to characterize the existing variation in energy digestibility within specific feed ingredients such as grains and to develop procedures predicting nutritional value of grains for swine. Analytical procedures have been developed to determine in vitro energy digestibility and DE content for barley, but have not been validated for their suitability to predict in vivo values. First, 21 barley samples with a range in fiber content (5.7 to 12.1% ADF) and total-tract energy digestibility (51.9 to 78.5%) and DE content in grower pigs were subjected to an existing in vitro analysis in duplicate (Huang et al. 2003). Briefly, the procedure involved subsequent digestions with pepsin (6 hr), pancreatin (18 hr), and cellulase (24 hr), and DM and GE analyses of the barley sample and residue. The in vitro energy digestibility ranged from 63.7 to 82.2% for the 21 barley samples and relative errors for samples ranged from 0.2 to 4.8%. In vitro energy digestibility was strongly related to swine in vivo energy digestibility content (R^2=0.81). Second, a subset of seven barley samples was subjected to quadruplicate in vitro analyses. In vitro energy digestibility ranged from 63.5 to 82.8% for the seven samples and the relative error was 4.2% for the barley sample with a low energy digestibility (63.5%) and ranged from 0.6 to 1.4% for the other six barley samples. For the seven barley samples, in vitro energy digestibility was strongly related to in vivo energy digestibility content (R^2=0.97). In summary, with quadruplicate analyses, in vitro energy digestibility was an accurate predictor of in vivo energy digestibility. In vitro energy digestibility can be successful as the core analytical procedure to calibrate rapid analytical equipment to predict energy digestibility and therefore DE content of barley for grower pigs.

Key Words: swine, grain, analysis

Beta-glucan depleted barley and oat flours as animal feed

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Background

A vital connection exists between the crops and livestock components of agriculture. In Alberta, livestock producers select their grain source for feed based on the cost of the grain and its method of processing, the primary goal of which is to increase energy availability (Owens et al. 1997). The combination of lower price and limitations of climate and soil fertility that impede corn production (Boss & Bowman, 1996) makes barley an economically attractive feed. As a result, barley is used primarily as an energy and protein source in cattle diets and is one of the primary feed ingredients used by the swine industry. Barley varieties are generally plentiful crops and are therefore readily available at a reasonable cost. However, these grains contain relatively high proportions of non-starch polysaccharides (NSPS), especially beta-glucans (~4-7%), which are known for their anti-nutritive properties. In poultry, for example, endogenous enzymes have a limited ability to digest non-starch polysaccharides. Thus their content and composition in the diet can impart significant differences in biological responses and thus influence poultry productivity (Campbell et al. 1989). To control this anti-nutritive effect, enzymes that hydrolyze non-starch polysaccharides are often added to feeds and thus enhance the overall digestibility. Livestock producers generally select varieties low in beta-glucans in an attempt to control for these anti-nutritive properties.

A novel grain fractionation technology has been developed at the Dept. of Agricultural, Food and Nutritional Science, University of Alberta to isolate/concentrate beta-glucan from oat and barley grains in a cost-efficient manner. The technology is patented and now licensed to Cevena Bioproducts Inc., Edmonton, Alberta. Flours produced from these grains undergo alcohol based enzymatic process for beta-glucan isolation/concentration. The beta-glucan concentrate (Viscofiber®) is now commercially available for use in functional food and dietary supplements due to its valuable physiological properties (For more information go to: www.cevena.com). Two major byproducts of the process are crude starch and a blend of hydrolyzed starch and protein mixture. These components are the most valuable by-products of the technology and comprise the largest volume (~80% and ~5%, w/w, respectively) of the raw material weight. While, whole or minimally processed (i.e. flaked) barley and oat grains have been traditionally used in animal feeds, the nutritional value of the aforementioned by-products that are depleted in anti-nutritional factors (i.e. beta-glucan) is not known.

Overall Goals and Objectives

The overall goal of this research project is to evaluate the potential of beta-glucan depleted barley/oat flour (i.e. crude starch) for use in the diets of livestock animals in order to determine their digestible nutrient content and optimal level of inclusion and thereby to provide evidence for inclusion of these byproducts in livestock production. Diets formulated with these ingredients will be compared to commercial feeds. Development of novel applications for these by-products

is important to ensure that the grains are completely utilized and this is critical for the overall commercial success of this technology. Furthermore, since barley and oats are established natural feed crops, utilization of the aforementioned byproducts in feeds would be safe and would not pose any threat to food security. It is also important to note that this research has potential to doubly benefit the agriculture industry, not only by enhancing cereal production and processing, but also by improving livestock production.

Evaluation of the use of beta-glucan depleted barley/oat flour as natural feed ingredient in young Holstein calves

The objective of the first trial conducted in young Holstein calves was to compare the nutritional value of barley and oat starch isolate by-products to a commercial calf grower diet. Calves (n=27, approximately 2-4 months of age, average initial weight: 110 kg ± 3) were randomly allocated to one of three treatment diets (3 calves/diet/pen) and fed *ad libitum* using Cailan gates. Three diets were formulated as specified by the National Research Council (NRC 2000) and contained 25% oat starch (oat starch diet) and 25% barley starch (barley starch diet). In order to allow an appropriate comparison between the commercial and starch test diets, all diets were made isonitrogenous (18% crude protein) and similar in energy content (MCal; Standard – 1.21, Oat – 1.33, Barley – 1.33), which was verified by proximate analysis. Following feed acclimatization and training on the Cailan gate system (approx. 3 weeks), calves were healthy, consumed adequate feed and grew well for the remainder of the trial. On average, calves supplemented for five weeks gained 52 ± 1 kg and had an average final BW of 189 ± 4 kg. Feed intake (FI) averaged 4.95 ± 0.20 kg/d. Despite initial concerns that the high amount of starch in the oat and barley diets would influence calf feed intake, and thus performance, there was no significant effect of diet on any of the recorded performance measures in this study (Table 1). A sub-group of calves that were fed Chromium-mordanted fiber underwent periodic feces collection (4 hours for two days, then at 54, 60, 72 and 96 hours). The appearance of Chromium in feces, and thus the rate of passage of each diet was similar, peaking between 16 and 36 hours and reaching negligible levels by 96 hours. Therefore, based on the results from this initial study, a high proportion (up to 25%) of both oat- and barley-derived starch products can be included in the diets of young calves as an ideal energy source.

Table 1. Body weight and feed intake of calves

Dietary Group	**Standard (g/kg)**	**Oat Crude Starch (g/kg)**	**Barley Crude Starch (g/kg)**
Initial BW, g	136 ± 7[12]	136 ± 5	138 ± 6
Final BW, g	192 ± 8	187 ± 7	190 ± 7
Average Gain, g	56 ± 2	50 ± 3	51 ± 2
Average FI, g/d	5.0 ± 0.53	5.1 ± 0.28	4.7 ± 0.13
[1] Values presented as mean ± SEM.			
[2] There were no significant differences ($p<0.05$) in any performance measure.			

Evaluation of the use of beta-glucan-depleted barley and oat flour as a natural ingredient for chickens

The objective of this trial was to evaluate the digestible nutrient content of beta-glucan depleted barley flour (i.e. crude starch) in the diets of broiler chickens. Since the endogenous enzymes of poultry cannot digest non-starch polysaccharides, the ability of enzyme (Avizyme® 1102; Danisco Animal Nutrition) to hydrolyze beta-glucans and thus enhance the overall digestibility was also examined in this study. Broiler chickens were fed isonitrogenous, isoenergetic diets between 0 and 42 d of age. For each growth period (starter 0-10 d, grower 11-28 d and finisher 29-42 d), a total of 5 diets were formulated according to NRC requirements (NRC 1994), which was confirmed by proximate analysis. Diets included a corn basal diet (#1), a barley flour based diet with (#2) and without (#3) added enzyme, and a barley crude starch-based diet with (#4) and without (#5) added enzyme. The major ingredient (i.e. barley flour, crude starch, etc) in each diet made up approximately 50% of the total diet. Chicks (n=602) were obtained at 1 d of age (initial weight: 44.3 g) and randomly assigned to 1 of 72 pens (8 pens/treatment). Body weight was measured on days 0, 10, 28 and 42. Average daily gain, feed intake and feed conversion efficiency were also determined between 0-10, 11-28 and 29-42 days.

Results of the study (Table 2) revealed that chickens consuming corn-based diets had a significantly higher average body weight, average daily gain and feed intake throughout the course of the trial. In contrast, with the exception of initial values, birds consuming barley flour-based diet had the lowest average body weight compared to all other dietary treatments. Barley-fed chickens had lower average daily gain, but higher feed conversion efficiency for the duration of the trial, which was also reflected at all time points. However, addition of enzyme to barley diets significantly improved average body weight and average daily gain at all time points. Chickens fed with barley crude starch diets were found to have many similar performance parameters to those chickens fed with enzyme-supplemented barley diets.

The poor performance of barley flour-fed chickens may be attributed to the high viscosity and texture of diets containing barley. The high beta-glucan content of barley flour would have substantially increased intestinal viscosity and may have interfered with digestion. In addition, beta-glucan has satiety factors, which may have influenced the amount of feed that chickens in this group were compelled to consume. Alternatively, barley-containing diets had a tendency to stick together within feeders, which may have interfered with the birds' consumption ability (i.e. feed intake). Therefore, an alternate type of feed processing, such as pelleting, may have minimized some of the observed problems in feed handing and feed flow and may improve feed intake. Since feed conversion efficiency was highest in barley-fed chickens, an improvement in feed intake would likely cause increased weight gain. It should also be noted that high inclusion levels (~50%) of the major ingredient were used in this study. Future studies are warranted to determine if performance would differ with different levels of inclusion. Results of this trial confirmed the ability of enzyme supplementation to enhance digestibility, a trend most pronounced in chickens fed barley-based diets. In addition to hydrolyzing non-starch polysaccharides, the enzyme used in this study would have also decreased intestinal viscosity. This would explain the observed increased performance of chicks fed barley-based diets with enzyme. It is important to highlight the fact that chickens fed with barley crude starch diets had generally equal performance to enzyme-supplemented broilers. This finding reinforces the great

potential of this byproduct in livestock formulations to cut down on costs incurred from enzyme supplementation, without suffering any detriment to broiler performance.

Table 2. Performance measures of chickens fed diets differing in major grain

Ingredient[1]	#1 – Corn	#2 – Barley flour	#3 – Barley flour + enzyme	#4 - Barley Crude Starch	#5 - Barley Crude Starch + enzyme
ABW[1] (g)					
D0	43.8[2] ± 0.44	44.5 ± 0.48	44.7 ± 0.39	44.7 ± 0.40	44.3 ± 0.48
D10	231.5 ± 5.43[a3]	147.2 ± 3.98[d]	176.3 ± 5.50[c]	202.3 ± 8.01[b]	186.3 ± 3.32[c]
D28	1110.3 ± 33.53[a]	650.6 ± 26.33[c]	794.3 ± 26.04[b]	825.2 ± 31.93[b]	850.5 ± 18.44[b]
D42	2394.2 ± 33.70[a]	1369.9 ± 46.86[c]	1709.1 ± 64.02[b]	1714.5 ± 50.50[b]	1809.8 ± 73.11[b]
ADG[4] (g/d)					
D0-10	19.7 ± 0.54[a]	10.8 ± 0.38[d]	13.6 ± 0.59[c]	16.4 ± 0.80[b]	14.8 ± 0.30[c]
D11-28	51.7 ± 1.89[a]	29.6 ± 1.34[c]	36.3 ± 1.35[b]	36.4 ± 1.88[b]	38.0 ± 1.16[b]
D29-42	99.9 ± 2.16[a]	55.8 ± 2.21[c]	67.4 ± 3.22[b]	69.9 ± 2.33[b]	72.7 ± 4.56[b]
D1-42	54.8 ± 0.65[a]	30.7 ± 0.99[c]	37.5 ± 1.68[b]	39.1 ± 1.17[b]	38.6 ± 1.34[b]
FI[5] (g/bird/d)					
D0-10	25.0 ± 0.47[a]	20.8 ± 0.84[b]	22.0 ± 0.96[b]	22.0 ± 1.22[b]	21.4 ± 0.33[b]
D11-28	83.3 ± 2.66	81.6 ± 4.93	79.3 ± 3.70	75.9 ± 1.67	84.5 ± 3.00
D29-42	173.3 ± 3.87[a]	134.5 ± 10.61[b]	137.9 ± 8.24[b]	129.3 ± 3.89[c]	152.5 ± 9.92[a]
D1-42	89.7 ± 1.02[a]	76.6 ± 4.16[b]	76.4 ± 3.48[b]	73.4 ± 0.81[b]	79.5 ± 2.33[b]
FCE[6]					
D0-10	1.27 ± 0.03[d]	1.94 ± 0.12[a]	1.62 ± 0.07[b]	1.36 ± 0.08[cd]	1.46 ± 0.05[bc]
D11-28	1.61 ± 0.03[c]	2.79 ± 0.19[a]	2.18 ± 0.07[b]	2.13 ± 0.13[b]	2.24 ± 0.11[b]
D29-42	1.74 ± 0.02[b]	2.40 ± 0.12[a]	2.08 ± 0.15[ab]	1.86 ± 0.07[b]	2.12 ± 0.10[ab]
D1-42	1.64 ± 0.01[c]	2.50 ± 0.12[a]	2.05 ± 0.08[b]	1.89 ± 0.06[b]	2.07 ± 0.07[b]

[1] Average Body Weight
[2] Values presented as mean ± SEM
[3] Within a row, means without a common superscript letter differ ($P < 0.05$)
[4] Average Daily Gain
[5] Feed Intake
[6] Feed Conversion Efficiency

Future Direction and Conclusion

At this stage of the research project, the nutritional value of barley crude starch diets has been determined in young calves and the digestible nutrient content has been determined in broilers. The next phase of the research project is on the verge of beginning. The objective of this trial is to relatively evaluate the digestible nutrient content of barley crude starch in growing pigs. Digestibility will be assessed by fitting 8 pigs with ileal T-cannulas, followed by collection of ileal digesta samples. Results from this trial will be compared to the previous study in poultry, as digestibility of these products is expected to differ. Once the digestible nutrient content of these products has been determined, it will be possible to proceed with more detailed studies. These will include livestock production trials that will utilize these products in nutritionally complete diets that have also been formulated from an economical perspective, thus allowing these diets to

be manufactured and purchased at a reasonable cost. There are also trials being planned to extend the results of previous studies and answer some of the questions these trials have presented. It is still unclear if the texture and form of the diets may have influenced the results of this pilot study. It would be necessary to evaluate if chickens would respond differently, especially in terms of feed intake and weight gain, to a product that undergoes cold pelleting and then crumbling. An additional component of this feed research project currently being planned is the use of the high-quality hydrolyzed protein and starch blend from oat and barley that are produced along with the crude starch products. In conclusion, studies conducted thus far indicate that barley- and oat-derived products have great potential as valuable and cost effective feed ingredients.

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Improvement of barley-based food product color

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Color is one of the most important sensory attributes of food products. A food product with the unacceptable color is not likely to be chosen and eaten by consumers, even it is highly nutritious, flavorful and well texture. While acceptable color of a food varies depending on cultural, geographic and sociological aspects of a given population, certain food groups are acceptable only if they fall within a certain color range. Also, in many cases quality and value of raw materials are judged by their color.

White color of flours from cereal grain is generally preferred to dark color. Moreover, most of the food products prepared from cereal grains should be of bright light color to command high quality. Dark discoloration of abraded barley kernels when used as a rice extender, in soups or in preparation of baby foods has been a serious concern of food industries and a significant factor preventing use of barley in food formulation.

Barley grains contain numerous polyphenols, proanthocyanidins and catechins, which are distributed in the hull, seed coat and aleurone layer. Total polyphenol content of barley, expressed as gallic acid, ranges from 0.2 to 0.4% of grain (Bendelow and LaBerge 1979). PPO has high activity in raw barley (Clarkson et al 1992). The role of polyphenols in brewing, especially their implication in haze formation in beer, was reviewed by Gardner and McGuinness (1977). However, the relationship between polyphenols and discoloration of food products prepared from barley, and the role of PPO on discoloration of barley based products has not been investigated, mainly due to the insignificance of barley as a food.

Barley is increasingly incorporated into the human diet, because of human health benefits, easy availability and inexpensive price. To maintain or even increase consumer's interest in barley foods, and to improve the willingness of food processors to use barley in food product formulations, it is crucial to control discoloration of barley-based food products. Discoloration of barley-based food products may be controlled by the proper selection of raw materials, appropriate processing and use of chemicals. We explored the grain components responsible for the barley food product discoloration, genotypic variation in discoloration as well as phenolics content and PPO activity, and environment effects on the grain components responsible for discoloration of barley food products.

Total polyphenol content and PPO activity

Total polyphenol content of abraded barley kernels was lowest in hulled proanthocyanidin-free barley, ranging from 0.02 to 0.04% (Table I). Hulled proanthocyanidin-containing abraded barley had lower total polyphenol content (0.11-0.18%) than hulless abraded barley (0.19-0.26%). Although proanthocyanidin-containing barleys were abraded by 30% for hulled and 15% for hulless genotypes, abrasion does not necessarily remove the same layers or components

of the kernel because of differences in thickness of outer layers, kernel size and shape among genotypes.

PPO activity of abraded barley grains varied among genotypes, while differences in PPO activity between barley classes were not evident. PPO activity of abraded barley grains ranged from 62.2 to 116.5 units/g in hulled barley and from 63.1 to 106.6 in hulless barley genotypes.

Table I. Total Polyphenol Content and PPO Activity of Abraded Grains of Different Barley Genotypes

Barley Class	Protein (%)	Ash (%)	Total Polyphenol (gallic acid %)	PPO Activity (units/g)
Hulled				
PA + (n=10)	8.0-11.1	0.74-0.96	0.11-0.18	62.2-94.7
PA - (n=4)	8.8-10.1	0.84-0.99	0.02-0.04	84.1-116.5
Hulless				
Regular (n=5)	10.4-15.0	0.85-1.16	0.22-0.26	63.1-106.6
Waxy (n=3)	11.7-13.1	0.99-1.15	0.19-0.21	68.7-79.9

PA +: proanthocyanidin-containing; PA -: proanthocyanidin-free.

Brightness of barley dough sheets

The L* values of barley flour dough sheets measured over time are summarized in Table II. Immediately after preparation, L* value of dough sheets exhibited relatively small differences between classes and genotypes of barley. The rate of L* value decrease during storage was highest in hulless barley genotypes, lower in hulled proanthocyanidin-containing and lowest in hulled proanthocyanidin-free genotypes. Accordingly, differences in L* values of the dough sheets among barley classes and individual genotypes were much more evident at 24 hr after preparation than immediately after preparation. Hulled proanthocyanidin-free barley exhibited the highest L* values (72.2-78.1), followed by hulled proanthocyanidin-containing barley (65.3-69.6) and hulless barley (59.0-63.9). There were also large variations in L* values among genotypes of the same barley class, indicating the complexity of discoloration in processed barley.

Table II. Brightness (L*) of Dough Sheets Prepared from Barley Flours

Barley Class	Brightness (L*)	
	0 Hr	24 Hr
Hulled		
PA + (n=10)	78.0-80.9	65.3-69.6
PA - (n=4)	79.8-82.6	72.2-78.1
Hulless		
Regular (n=5)	74.3-79.3	59.7-63.9
Waxy (n=3)	75.4-77.7	59.0-61.1

PA +: proanthocyanidin-containing; PA -: proanthocyanidin-free.

Relationships between composition and discoloration potential of barley

Correlation analyses between the composition of barley kernels and L* values of dough sheets are summarized in Table III. Total polyphenol content significantly correlated with the L* values of dough sheets. A large variation in L* values of dough sheets among barley genotypes of similar total polyphenol content (Table I) may indicate that, in addition to polyphenols, other factors, including PPO activity and metal ions, may contribute to the discoloration of dough sheets.

Relationships between PPO activity and L* values of dough sheets were not significant. However, protein content and ash content exhibited significant negative correlations with L* values of barley dough sheets in barley genotypes. Protein content may be correlated with an unknown component that affects hardness or the rate of water binding during dough processing. Highly negative correlation between wheat ash content and spaghetti brightness was also reported by Matsuo et al (1982).

Table III. Simple Pearson Coefficients (r)[a] Between Composition and Brightness of Barley Flour Dough

Composition	Brightness of Dough (L*)
Total Polyphenols	-0.910***
Polyphenol Oxidase Activity	0.270
Protein	-0.714**
Ash	-0.469*

[a]*, **, *** = significant at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively.

Genotypic and environmental effects on discoloration potential of barley

Twelve genotypes of barley grown in five environments (location-year combination) were analyzed to determine the relative contribution of genotype and environment on quality traits associated with discoloration potential of barley. Both genotype (G) and environment (E) contributed to significant variation for protein, ash, total polyphenol content, PPO activity and brightness of dough sheets (Table IV). G × E interactions were also significant for all parameters. Analysis of profile plots for all parameters with significant G × E interactions indicated that total polyphenol content, PPO activity and brightness of dough sheets had non-crossover interactions (Figure 1). Non-crossover interaction indicated that the rank of the means for genotypes was unchanged although the magnitude of the differences between genotypes changed among environments.

The ratios of genetic to environmental (G/E) variances showed that genetic factors had a larger influence than environmental factors on total polyphenol content, PPO activity and brightness of dough sheets (Table V). G/E variance ratios were similar in magnitude and nearly evenly balanced for protein and ash content. These results indicate that genetic factors were more important than environmental factors or G × E interactions in determining total polyphenol content, PPO activity and brightness of dough. Therefore, the selection of barley genotypes during the breeding process based on polyphenol content and PPO activity as well as dough brightness, could be the effective way to control discoloration of barley-based food products.

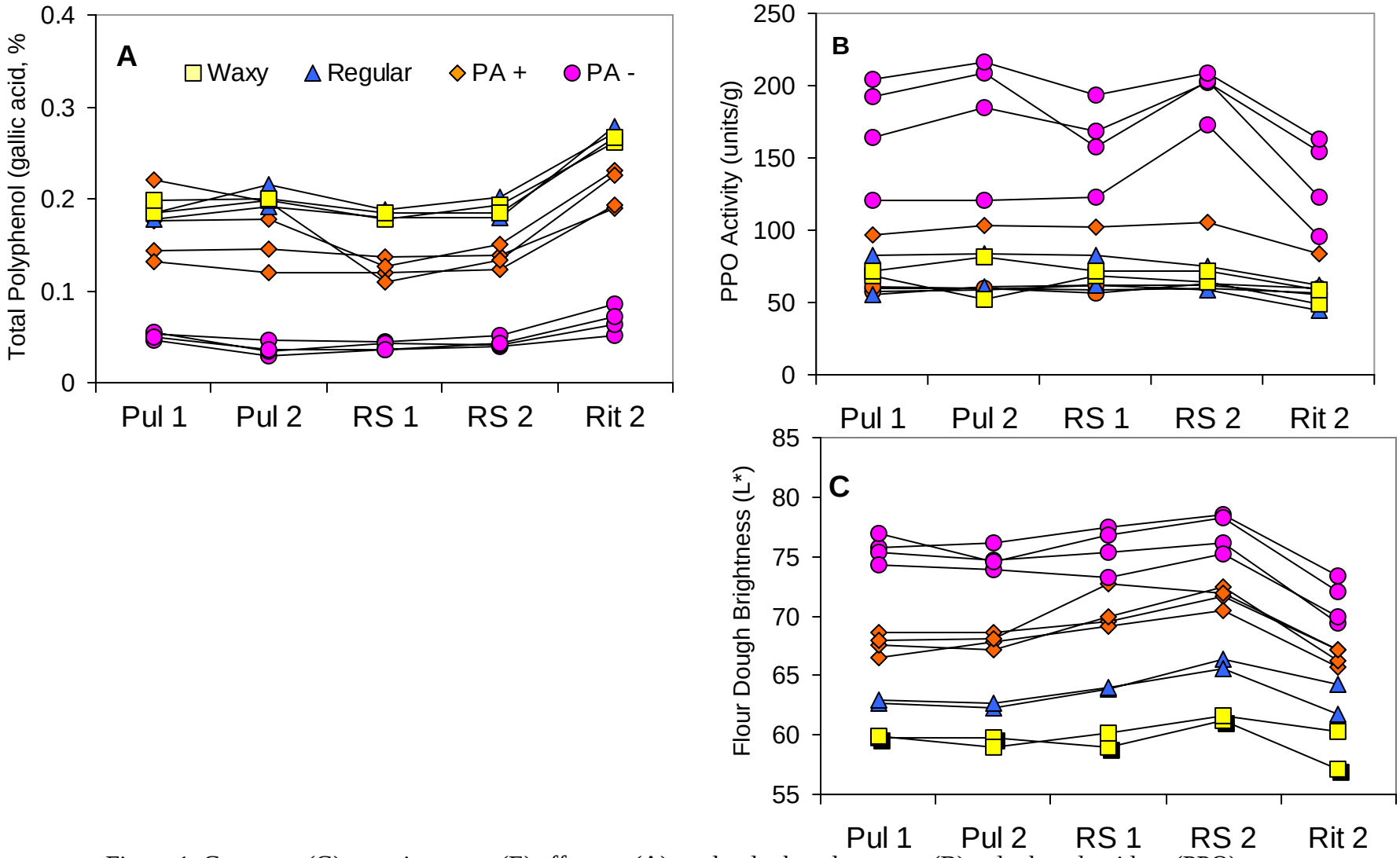

Figure 1. Genotype (G) x environment (E) effect on (A) total polyphenol content, (B) polyphenol oxidase (PPO) activity and (C) flour dough brightness (L*) of barley grown in 2001 and 2002 in three locations. PA + = proanthocyanidin-containing; PA - = proanthocyanidin-free; Pul 1 = Pullman 2001; Pul 2 = Pullman 2002; RS 1 = Royal Slope 1; RS 2 = Royal Slope 2; and Rit 2 = Ritzville 2002.

Table IV. Mean Squares for the ANOVA of Chemical Composition and Discoloration Potential of Abraded Barley[a]

Source of Variation	df	Chemical Composition				Brightness (L*) of Dough
		Protein	Ash	Total Polyphenol	PPO Activity	
Genotype (G)	11	16.2***	0.20***	0.049***	28592***	336.9***
Environment (E)	4	40.9***	0.39***	0.016***	2823***	69.6***
G × E	44	0.7**	0.01***	0.001***	339***	2.5***

[a] ** and *** = P<0.01 and 0.0001, respectively.

Table V. Ratios[a] of Variances Estimated for Genotype and Environment Main Effects and Their Interactions for Chemical Composition and Discoloration Potential of Abraded Barley

	Chemical Composition				Brightness (L*) of Dough
	Protein	Ash	Total Phenolic	PPO Activity	
G/E	0.9	1.2	7.2	24.5	11.7
G/E x E	32.7	18.8	10.6	3.0	12.1

[a]Ratios of genetic to environmental variance components (G/E) and environmental variance to genotype by environment interaction components (E/G × E).

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Food barley development at the Crop Development Centre, University of Saskatchewan

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Food barley development at the Crop Development Centre (CDC), University of Saskatchewan grew out of the longstanding hulless barley development program at the CDC. The food barley sub-project was initially based on crosses made in the mid-1970's with the 95% amylopectin starch genotype "waxy Betzes" from Montana State University. These crosses, aimed at producing hulless waxy barley, had been made with the original intent to produce barley varieties that could be used to provide waxy starch for use in the solution mining system employed in several Saskatchewan potash mines. While this utility has not come to pass to date, the material provided an excellent base from which our food barley R&D efforts have developed.

Because of our joint program efforts on oat R&D, our resultant interaction with the oat food industry, their keen interest in beta glucan and soluble dietary fibre in the 1980's and our knowledge of the tremendous variability in beta glucan content in barley germplasm, especially in the waxy starch type, we were able to foresee a possible increased opportunity for barley in the food industry and embarked upon specific food hulless barley development. Thanks to support from the Saskatchewan Agriculture Development Fund and the Alberta Wheat Pool, Agricore and now Agricore United we have been able to make good progress.

Selection for the waxy type has and is still conducted first by visual inspection of lines from crosses between waxy starch and normal parents to select the waxy segregates. In hulless material this is actually quite easy as the waxy segregates have a much lighter grain colour and, if in doubt, one can cut through the kernel to see if the endosperm is floury. Initial chemical selection was based largely on analysis of acid extract viscosity (AEV) as we did not have affordable access to equipment to analyze large numbers of samples for beta glucan (BG). The strong positive correlation between AEV and BG in waxy starch material allowed reasonable success with this crude screening technique.

However, it is desirable to screen for both BG and AEV in order to develop a diverse selection of high BG varieties with variable AEV for different end-users. Therefore, more recently the CDC program annually screens a significant number of samples for both BG concentration and AEV. BG is determined by Flow Injection Analysis (Aastrup, S. and Jorgensen, K.G., 1988) using a Fiatron Flow Injection analyzer, Oconomowoc, WI and AEV (Greenberg, D.C. and Whitmore, E.T. 1974) is determined using a Brookfield Digital Viscometer Model DV-II. We strongly believe our combined selection for high BG and high AEV is a major reason for the repeated desirability of our materials in various evaluations for food and industrial purposes.

While initially working mainly with the 95% amylopectin waxy starch types the program works on 100% amylopectin starch types (pioneered at the CDC), has an effort dedicated to improvement of high amylose (approx 40%) hulless types and has hulless compound granule or fractured starch types under evaluation and development.

As indicated earlier, the 95% amylopectin waxy type is based on using "waxy Betzes" as the original donor parent. Success to date in the program has been demonstrated by the release of CDC Candle (1995) and CDC Rattan (2003). CDC Candle normally demonstrates BG concentration of 6.5% – 7.0 % and has relatively high AEV. CDC Rattan shows a 0.8 % improvement in BG with similar moderately high AEV (Table 1), and is much improved agronomically being higher yielding, stronger strawed and demonstrating improved threshability at harvest (Table 2).

Table 1. % Beta Glucan and Acid Extract Viscosity (cps) for five CDC varieties and four CDC selections of hulless specialty starch barley at Saskatoon 2000 through 2004.

	%BG					AEV				
Genotype	*2000*	*2001*	*2002*	*2003*	*2004*	*2000*	*2001*	*2002*	*2003*	*2004*
CDC McGwire	5.5	5.3	4.8	5.1	3.9	11	18	6	20	4
CDC Candle*	6.2	6.7	6.5	7.0	6.0	51	93	12	207	13
CDC Rattan*	-----	7.8	7.3	7.7	6.3	-----	235	30	239	18
CDC Alamo**	7.0	7.9	7.9	8.0	6.3	41	207	52	93	19
CDC Fibar**	11.8	9.6	10.0	10.1	9.1	204	347	63	514	56
SR93139*	8.5	9.3	8.5	9.2	7.1	434	>1000	81	578	71
SB94893^	8.5	8.4	8.2	9.6	7.6	46	58	22	220	11
SH99250^	-----	6.8	7.9	8.7	7.3	-----	22	14	34	7
SH99073^	-----	7.7	8.0	9.2	7.0	-----	55	8	94	7

* 95% amylopectin starch, ** 100% amylopectin starch, ^ high amylose starch
Note: lower BG and especially AEV levels in 2002 and 2004 indicate adverse (wet) pre-harvest conditions.

Table 2. Agronomic, BG and AEV data 2001 and 2002 PRRCG, Western Canadian Hulless Barley Cooperative test.

	Yield % McGwire	**Lodging (1-9)**	**Dirty Test Wt. (kg/hl)**	**Clean Test Wt. (kg/hl)**	**% Plump Grain**	**% BG**	**AEV (cps)**
CDC McGwire	100	3.4	74.1	78.6	76	4.7	16
CDC Candle	86	5.5	70.2	76.6	61	6.4	121
CDC Rattan	**95**	**3.0**	**71.3**	**78.1**	**80**	**6.9**	**150**
# Station Years	*25*	*6*	*21*	*27*	*20*	*2*	*2*

Crossing the CDC two row hulless waxy breeding line SB85750 and the six row hulless waxy variety AzHul from the University of Arizona in 1990 to determine if the gene(s) giving rise to the waxy starch in these different germplasm sources was the same gave two interesting results. First was the indication that the genes controlling the waxy starch trait were different based on the fact the some segregates from the cross had normal starch type. More interesting and valuable was the result that some unique segregates had 100% amylopectin starch, a first in barley development (Bhatty and Rossnagel, 1997), and we believe a first in that 100% amylopectin barley starch is the only available native form of pure amylopectin starch.

Of considerable note with this unique waxy type was even higher levels of BG and AEV as demonstrated by the varieties CDC Alamo (released in 1999) and CDC Fibar (released in 2003) (Table 1). CDC Alamo normally has a BG concentration of near 8% with high AEV, while CDC Fibar consistently produces BG concentration > 9% and has on several instance had BG > 10%.

While CDC Fibar has considerably higher BG and AEV, it does not offer any improvement in agronomic performance (Table 3).

Table 3. Agronomic, BG and AEV data 2002 PRRCG, Western Canadian Hulless Barley Cooperative test.

	Yield % McGwire	**Lodging (1-9)**	**Dirty Test Wt. (kg/hl)**	**Clean Test Wt. (kg/hl)**	**% Plump Grain**	**% BG**	**AEV (cps)**
CDC McGwire	100	2.4	74.2	77.8	75	4.7	5
CDC Candle	86	4.9	70.5	76.0	72	6.5	16
CDC Rattan	98	1.9	71.4	77.3	81	7.1	18
CDC Fibar	**78**	**2.9**	**66.0**	**73.5**	**88**	**9.1**	**52**
# Station Years	*14*	*3*	*9*	*11*	*10*	*6*	*6*

Within the 95% amylopectin waxy type, CDC selection SR93139 is of note in that it has BG levels approaching that of CDC Fibar and has exceptionally high AEV (Table 1). These traits may be an advantage in non-food industrial applications.

CDC high amylose hulless materials are represented specifically by SB94893 a two row selection which derives its high amylose starch from the hulled genotype high amylose Glacier. As is the case for high amylose Glacier, SB94893 has starch which is about 40% amylose and it has high BG with moderately high AEV (Table 1). Food industry interest in high amylose types is increasing as interest in "resistant starch" increases, since, relative to amylopectin, amylose behaves like resistant starch. Unfortunately, our experience has been that developing agronomically acceptable high amylose material from the high amylose Glacier background is a definite challenge. While selections like SB94893 are certainly "growable" under normal field conditions, these materials tend to be very tall, weak and relatively low yielding and we have put much less effort into improving these types to date. With increased interest in resistant starch we have recently increased efforts on this type.

Of note are CDC selections SH99250 (2 row) and SH99073 (6 row), both of which, while having no evidence of differential starch type in their pedigrees, have been found to be high in amylose and have elevated BG and somewhat elevated AEV levels (Table 1). As part of our ongoing hulless food barley effort and our annual routine screening of 1000's of selections for BG and AEV we have always been on the lookout for normal starch lines with elevated BG and/or AEV since some potential users have indicated a desire for > BG, but that the waxy or high amylose starch was undesirable for their end-use(s). Based solely on high BG concentration, SH99250 and SH99073 were retained, advanced and increased for just that purpose.

However, since the pedigrees of these two lines gave no evidence either should have altered starch type, they were not evaluated for amylose/amylopectin ratio until relatively recently and, much to our surprise, both selections have elevated amylose levels. While not quite as high in amylose as the 40% amylose derivatives from high amylose Glacier, these selections are definitely not normal 25% amylose starch type, as they consistently demonstrate amylose levels > 35%. Of special interest is that both of these selections have relatively good agronomic performance and are much better field performers than high amylose Glacier derivatives. These selections are currently being further evaluated and crosses have been between them and with high amylose derivatives like SB94893 to determine if the high amylose in these unique

selections is under different genetic control. There is of course an outside chance that combining these materials may also result in selections with amylose > 40%.

While not yet widely used in the food or non-food industries, considerable success has been generated using our 95% and 100% amylopectin hulless waxy material at the research and indeed even at the commercial level. In particular, research level efforts by N. Ames at the CRC, AAFC, Winnipeg; T. Vasanthan and F. Temelli, University of Alberta; M. Izydorczyk and J. Dexter, GRL, CGC, Winnipeg and M. Izydorczyk, J. Li and R. McCaig, CMBTC, Winnipeg have demonstrated unique value for uses in tortillas, high soluble fibre snacks, BG extraction and concentration, noodles and bread products. At the commercial level, InfraReady Products Ltd., Saskatoon has developed a unique, biodegradable, environmentally friendly cat litter, LitterMate™ which is being marketed across western Canada, Cevena Bioproducts, Edmonton has been using these high BG hulless barley materials as the basic feedstock for their BG concentration and extraction process, Sapporo Breweries, Japan have expressed considerable interest in the use of our 100% amylopectin materials CDC Alamo and CDC Fibar for a new food barley venture in Japan, and the Saskatchewan potash industry has again expressed interest in the possible use of these materials in their solution mining process.

In summary we see a good future for specialty starch hulless barley especially with elevated BG (thus soluble fibre) levels, and are confident that our project will serve as a good base for these purposes from a western Canadian plant breeding and barley R&D perspective.

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Session 2 - PATHOLOGY AND ENTOMOLOGY

Chairs
Kelly Turkington and Kequan Xi

Presenters
Flavio Capettini, ICARDA / CIMMYT, Mexico
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Shipra Mittal, North Dakota State University
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International germplasm development for multiple disease resistance

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Introduction

The International Center for Agricultural Research in the Dry Areas (ICARDA), with the headquarters in Aleppo, Syria, is one of 15 centers strategically located all over the world and supported by the Consultative Group on International Agricultural Research (CGIAR). With its main research station and offices based in Aleppo, Syria, ICARDA works through a network of partnerships with national, regional and international institutions, universities, non-governmental organizations and ministries in the developing world; and with advanced research institutes in industrialized countries. ICARDA serves the entire developing world for the improvement of barley, lentil, and faba bean; and dry-area developing countries for the on-farm management of water, improvement of nutrition and productivity of small ruminants (sheep and goats), and rehabilitation and management of rangelands. The Global Barley Enhancement Program has its headquarters in Syria, while the sub-program based in Latin America, targets the developing countries in that region. The development of germplasm with resistance to the main biotic and abiotic stresses has always had the highest priority in the program. Genetic resistance still is the most environmentally-friendly and durable method of control of crop stresses, as well as the only affordable method for low income farmers at different regions worldwide.

Disease Evaluation

To reach our objectives it is very important to have screening environments where it is possible to maximize the response to selection for a determined trait. This many times implies the need to decrease the environmental variation, giving the optimal conditions for disease development (misting, inoculation) or stress expression (drought). This is possible in the environments that the program uses regularly in different parts of the world. In Syria, selection is carried out at key environments where the main diseases in the region can be reproduced. Selection for scald, loose smut, covered smut, barley stripe, powdery mildew, net blotch and root rot are carried out at the headquarters at Tel-Hadya, Aleppo. In Lattakia, selection is also performed for net blotch and powdery mildew, while in Terbol (Lebanon), scald resistance is selected during the winter and powdery mildew and leaf rust resistances during summer. In Haymana (Turkey), scald, net blotch and powdery mildew resistances are selected.

In Mexico, key experimental stations have also allowed the accumulation of resistance to different important diseases in an efficient manner. Toluca and El Batán, during summer time, are key selection hot spots for several diseases. At Toluca, stripe rust, scald, *Fusarium* head blight and BYDV can be selected with highest confidence. Heritabilities for Stripe Rust are usually higher than 95% at Toluca (Vales *et al.*, 2005). The inoculation of all the program segregant material with scald and the natural infection with stripe rust as well as the artificial inoculation of the advanced material with this disease, practically makes all germplasm coming out from the program resistant to these diseases. At Ciudad Obregón during winter season, ideal conditions for leaf rust allow us to confidently select for resistance to this disease in all the

inoculated segregant material. Again, most of the germplasm deployed is expected to be resistant to this disease also.

Collaboration with Advanced Research Institutes and National Research Institutes

The close collaboration with centers of excellence or "Advanced Research Institutes" (ARIs) is fundamental to develop the superior germplasm needed in the target areas, as well as the close contact with the "National Research Institutes" (NARs) is essential to receive input about research priorities. The ICARDA/CIMMYT program for Latin America has had long term collaboration with several programs worldwide. Among the longest and most productive has been the collaboration with the barley program of Alberta Agriculture, Food and Rural Development, headed by Dr. James Helm. For many years, the synergistic interaction which included germplasm exchange, screening and expertise, helped developing superior barleys with resistance to 5-7 important diseases. No less important has been the interaction with the barley program at Oregon State University, mainly regarding stripe rust research. That program under the leadership of Dr. Patrick Hayes, made it possible to map several populations, while carrying out the phenotyping at Toluca. These studies have helped to understand the genetics of the diseases and generated germplasm with pyramided genes for resistance available to all the programs in the region and worldwide. The participation in the now discontinued North American Stripe Rust Nursery, coordinated by Drs. Bill Brown and Vidal Velasco, allowed for the determination of the level of resistance to Stripe Rust present at the different programs in North America, and to use the resistance for germplasm enhancement. The screening of the Australian programs through the PBI of the University of Sydney, under the leadership of Dr. Colin Wellings, also reaches the same objectives with that country. More recently, the collaboration with Bush Agricultural Resources Inc. (BARI) through Dr. Leslie Wright and Linnea Skoglund allowed us to incorporate malting quality in our multiple disease resistant gemplasm. Using their malting barley varieties as templates, in one cycle of breeding it was possible to obtain attractive lines that combined resistance levels to the main diseases at higher level than the parents. In following cycles additional gains in the agronomic and quality traits are expected.

Undoubtedly one of the greatest challenges that the barley programs in the region and worldwide has faced in the last decade has been the epidemic outbreaks of *Fusarium* head blight (FHB). As several of us know, FHB has always been present in the barley cultivated area, but never in the proportions and frequency reached lately. The epidemic patterns appear to repeat in several countries worldwide. After the outbreak in the Midwestern US in 1993, outbreaks of different intensities have occurred in Uruguay, Brazil, Peru, Ecuador, etc. This supports the need to continue intensively working with this destructive disease. Our program has, in several opportunities, linked the NARs and the ARIs in order to facilitate research exchange. Our collaboration within the US Wheat and Barley Scab Initiative has been essential to support our research as well as to keep scientists updated with the latest research advances. Probably as with no other disease has the interaction among colleagues and the synergistic relationships been so important, from the collaborative germplasm screening networks to the brainstorming sessions carried out when working together, from China to the Midwest or Mexico.

Breeding Strategies

ICARDA/CIMMYT - México

Since the early 1980s, several programs have been involved in breeding for disease resistance. The program in Mexico took advantage of collaboration and experience to build the foundation of resistances to the different diseases. Webster *et al.* (1980) screened 18,000 accessions from the world barley collection for scald resistance. They found 273 entries that showed no symptoms. Resistant entries were introduced and screened for virulence in Central Mexico. From the 273, 13% showed susceptibility under those conditions and were discarded.

The national research program at Colombia screened 8,650 accessions for race 24 of *Puccinia striiformis* f. sp. *hordei* (Anonymous, 1984). In Mexico, 285 entries were found to be resistant out of 11,087 accessions screened at CIANO experiment station in Obregón for races 8, 19 and 30 of *P. hordei* . Vivar (1986) in México and Takeda and Heta (1989) in Japan screened 5,000 accessions and found 23 lines with resistance to FHB. The germplasm identified above was used as the starting point for disease resistance in the program.

Resistant germplasm was evaluated against the virulence of the most aggressive pathogens collected and introduced into the US and Europe from hot spots around the world. Work on leaf rust by Sharp and Reinhold (1982) in Montana and Parlevliet in Holland (1977) helped the program identify parents to use in the crossing program. To accomplish the goal of introgressing the resistance of all those diseases into high yielding germplasm, “templates” were developed, first for scald and leaf rust, followed by templates to which stripe rust and other diseases resistances were added. Over a period of 25 years (two growing seasons per year) different diseases were pyramided. Varieties produced appeared to be commonly resistant to scald, leaf rust, stripe rust and stem rust, BYDV, net blotch and spot blotch and since several cases also to FHB. Besides the resistance, the agronomic type made the germplasm attractive enough to be extensively used as cultivars or as source of resistance by our colleagues.

ICARDA – Syria

To target the poor, the breeding philosophy of the project, which evolved during the last 14 years, is based on exploiting specific adaptation through direct selection in the target environments using locally adapted germplasm and sustainable levels of external inputs.

The two major implications of this philosophy were that (1) many varieties were generated by national programs, each adapted to specific conditions, and (2) the superior performance of the varieties developed for low-input and less-favored lands are not dependent on agronomic practices that require large amount of inputs. A breeding program based on this philosophy does not endanger biodiversity, and is environmentally benign.

A fundamental question the barley program has addressed in the last 14 years is why plant breeding has been beneficial to those farmers who either enjoy favorable environments or could profitably modify their environment to suit new cultivars, and it has not been equally beneficial to those farmers who could not afford to modify their environment through the application of additional inputs. Farmers in favorable environments, using high quantities of inputs, are now concerned with the adverse environmental effects and the loss of genetic diversity. Poor farmers in less-favored environments continue to suffer from chronically low yields, crop failures and, in

the worse situations, malnutrition and famine. Because of its past successes, conventional plant breeding has tried to solve the problems of poor farmers living in unfavorable environments by simply extending the same methodologies and philosophies applied earlier to favorable, high potential environments. We have now concluded that difficult environments and resource-poor farmers require a different type of breeding.

Using contrasting sites in NW Syria we found repeatable genotype x environment (GE) interactions of crossover type between the main experiment station and experiment sites managed according to farmers' practices. GE interactions of crossover type are common in the literature, in different crops and in different types of stress environments. We then concluded that selection in high input experimental stations is very effective in generating varieties for favorable environments, but does not allow the identification of the best genotypes for less-favored lands, and promotes genotypes which are in fact inferior in stressful conditions.

Formal breeding has taken a negative attitude towards GE interactions of crossover type, in the sense that only breeding lines with low GE interaction (good average grain yield, across locations and years) are selected, while lines with good performance at some site and poor performance at others are discarded. Because lines with good performance in unfavorable sites and poor response to favorable conditions have a low average grain yield, they are systematically discarded. Yet they would be the ideal lines for farmers in unfavorable locations. Therefore, having recognized the importance of GE interactions of the crossover type, a major conclusion has been that breeding for difficult environments must be based on the exploitation of specific adaptation, and this in turn can only be done by selecting directly in the target environments.

While the application of this philosophy started being successful in Syria with the adoption of three varieties in stress environments, the next question was: how to reconcile the mandate of an international breeding program with the importance of specific adaptation?

The response to this question has been the decentralization of the breeding work. The term decentralization has been used often to describe two fundamentally different processes, namely decentralized selection and decentralized testing.

Decentralized selection is a term first used by Simmonds (1984) and defined as selection in the target environment(s). Decentralized selection has been also termed *in-situ* or on-site selection. In the case of self-pollinated crops it consists in selection of early segregating populations (such as F_2) in a number of locations representing the target environment(s) (climate, soil, farming system and management) the breeding program aims to serve. Decentralized selection becomes selection for specific adaptation when the selection criterion is the performance in specific environments rather than the mean performance across environments.

Decentralized selection is different from decentralized testing, which is a common feature of breeding programs and takes place, usually in the form of multi-location trials and on-farm trials, after a number of cycles of selection in one or few environments (usually with high levels of inputs).

Addressing the issue of resistance to biotic stresses, it is acknowledged that barley is affected by several foliar and root diseases, several insects, nematodes, and viruses. The organisms which can potentially damage a barley crop can be divided in two broad categories, namely those which are specific (either as organism or as a physiological race) to a given country or area, and those which are widespread to several countries.

The overall strategy, once the priority biotic stresses have been identified together with NARs, is to decentralize the work on biotic stresses of the first type to NARs following the development of the necessary expertise, and to concentrate at the headquarters on the second type of biotic stresses. The latter is an ideal ground for collaboration with ARIs.

Within this broad strategy, the work on biotic stresses is integrated in the more general, decentralized approach to plant breeding followed by the project.

In the case of foliar diseases, insects and viruses, the screening of large amount of breeding material, which has represented 90% of the activities in the past, has been gradually reduced to about 10% of the total work on biotic stresses. Eventually, screening was entirely transferred to NARs. Specific pests are tested at hot spots, and information circulated to all collaborators. Sources of resistance are being characterized at the headquarters which focus on the transfer of genes for resistance into the breeding material developed by the decentralized program for specific countries and/or regions. In these cases the national programs receive F_4 families homozygous for the resistance gene(s), but variable for everything else. This is done at the headquarters in the case of genes with non-specific resistance (for example, the genes for resistance to RWA and BYDV), and within five years it will be done routinely with the aid of molecular markers. These first molecular markers assisted selection programs will also be used to train national program scientists.

In the case of foliar diseases, where a large variability exists for physiological races, the responsibility of the headquarter pathologist is the identification of genes which are effective against the virulences of target countries/regions. Sources of resistance for these genes are used in the targeted crosses at the headquarters, but the selection of the segregating populations are done in the target environments. Marker assisted selection will be made available to NARs to increase the efficiency of selection.

Two areas which need expansion are a) scab, root diseases and nematodes, and b) durable resistance and population improvement.

The entire area of durable resistance, and of the consequent changes in the breeding strategies which are needed, are addressed by the barley project, and at least one case-study is being developed to address one of the most variable foliar diseases (powdery mildew) with two alternative strategies, one based on deployment of major genes and one based on the increase of horizontal resistance through population improvement.

Results Obtained

In decentralized selection, the barley project at ICARDA continues to generate genetic variation by maintaining a large crossing program, but selection is carried out by the breeders in the National Programs. At this moment, decentralization of barley breeding is fully implemented in North Africa, Iraq and Ethiopia, and it is gradually being implemented in the Mediterranean highlands in the framework of the ICARDA/Iran Project, and in other countries (Table 1).

Table 1. Countries and regions where decentralized barley breeding has been initiated.

Country/Region	Countries/Area	Status
North Africa	Egypt, Libya, Tunisia, Algeria, Morocco	Fully implemented
Iraq (Baghdad)	Central Iraq	Fully implemented
Iraq (Mosul)	Northern Iraq	Fully implemented
East Africa/Red Sea	Yemen, Eritrea, Tigray	First crosses made in 1998
Ethiopia	Ethiopia (except Tigray)	Use of local landraces fully implemented, first crosses in 1998.
Central Asia		First special nursery in 1997
Turkey		First nursery planned for 1999
Cyprus	Cyprus	First special nursery in 1995, first crosses in 1998
Far East	India, Thailand, Vietnam, China	First special nursery in 1996, first crosses in 1997
Pakistan	Pakistan	First special nursery in 1997
Gulf Countries	S. Arabia, Qatar, Oman	First crosses made in 1992
Ecuador	Ecuador	First nursery planned for 2006

The project in Latin America has been successful in developing useful germplasm adopted by the programs in the main target area, as well as in some other regions worldwide. In Ecuador, all the commercial varieties are directly released from material received from Mexico, or were derived from crosses made with that germplasm, and selected in the country. In Uruguay, Brazil and México, germplasm has been intensively used as a source of disease resistance as well as to improve agronomic types. The same situation occurred with several of the most planted varieties released in Peru in the last 25 years. According to information received from China (Dr. Zhonghu He, CIMMYT representative in China, personal communication), the area planted to barley in 2000 with germplasm developed by the program (either direct introduction or varieties derived from past introductions) account for 40% of the one million hectares cultivated to barley in the country. The key traits for a successful variety in that region is the resistance to FHB, tolerance to barley yellow mosaic virus (BYMV) and consistent high yield potential.

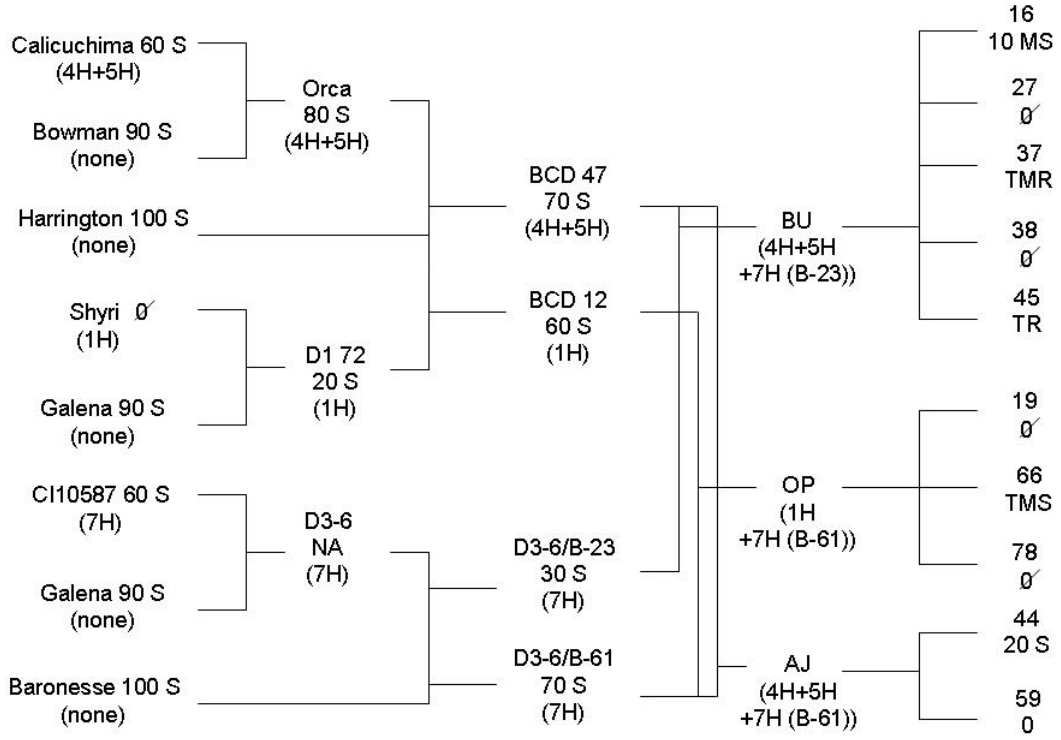
The strategy used to incorporate leaf rust resistance into Shyri, a variety released in Ecuador, may explain the durability of the resistance obtained. The virulence present in that country is capable of overcoming all major resistance genes (Brodny and Rivadeneira, 1996). Shyri was released in 1989 with low symptoms for leaf rust. After two years a race was able to overcome the major gene located on chromosome 1 of the variety (Toojinda et al., 2000), however it

produced reasonable yield despite the presence of symptoms on leaves late in the season. In 1991 Ochoa concluded that Shyri had partial resistance that delays disease development.

In Stripe rust, the collaboration with Oregon State University has been fundamental to understand the genetics of resistance and create germplasm which pyramids resistance genes from different sources. Using resistance sources as Shyri, Calicuchima (a variety also released in Ecuador) and CI10587, mapping studies determined that resistance QTLs were present in the chromosome 1H in Shyri, 4H and 5H in Calicuchima and a major gene was present at the 7H in CI10587 (Picture 1). The importance of this type of study was evident when new resistance patterns were observed in Peru and Ecuador after the year 2000. After more than 15 years of no observed changes, several well known resistant lines appeared as susceptible in that region. The pyramided germplasm allowed us to determine that the resistance QTLs and major gene present at chromosomes 4H, 5H and 7H were susceptible to that putative new race of the disease, while the QTL at Chromosome 1H (from Shyri) was still holding resistance. All the germplasm developed by OSU was planted in those countries, and are expected to collaborate in the development of varieties that still are resistant in the region. Although in North America the old sources still appear as resistant, it is expected that, like has occurred in the past, the possible new race would come to the region and change the resistance patterns of the varieties present there also.

Picture 1.

Pedigree, barley stripe rust severity scores (%) from Huancayo, Peru (2001), and sources of resistance for the AJ, BU, and OP populations. The quantitative sources of resistance are on chromosomes 1H, 4H, and 5H and the qualitative source of resistance is on chromosome 7H.



Pedigree developed by Patrick Hayes, Oregon State University.

Another example of progress in the fight against disease losses has been the case of FHB. When FHB epidemics occurred in the Midwestern US after 1993, the barley program at México had already been working in that disease for several years. That allowed the main source of resistance to become available to the US programs that were re-starting their work with FHB. Several well-known resistance sources like Atahualpa, Shyri, Gobernadora, etc. were rapidly introgressed into the programs. At present, the collaboration is in a more formal fashion, through the USWBSI. This has allowed the testing of high numbers of genotypes every year and participation in the testing network at different locations allows confirmation and sharing of the resistance observed. In the Table 2 we can see some lines developed through the agreement with BARI that have had their resistance confirmed at several locations.

In recent studies of the resistance sources for scald used in the program, slow-scalding resistance (S-SR) appeared to be present in the core material released by the program. Little was known about the inheritance of S-SR. Studies carried out by B. Sorkhilalehloo *et al*. (2001, 2004) showed indications of incomplete dominance for that trait and additive variance was the major portion of total genetic variance for S-SR. The estimates of narrow-sense heritability of S-SR were quite high (0.80-0.98). Such resistance genes with additive effects and high heritabilities, should support successful phenotypic selections for S-SR in early generations, and are promising for pyramiding resistance genes for achieving stable resistance against barley scald using back-crossing methodology. The results also showed that none of the barley genotypes were immune against all the isolates used in the study. However, the cluster of “highly resistant” genotypes contained barleys resistant to the majority of the pathotypes among which there was some malting, hulless, slow-scalding, and differential lines as promising potential sources of stable resistance to scald.

The more than 20-year collaboration with the FCDC in Alberta, focusing on scald resistance and other diseases, has allowed both programs to develop germplasm resistant to all the regionally important diseases. New combinations of resistance genes have been found, with some lines containing resistance to 5 and 6 diseases. Helm *et al.* (2004) determined that these gene combinations for scald resistance should give durable resistance in Canada and México. The classification of breeding lines according to resistance genes combinations is currently under pedigree analysis to determine the relationship for sources of resistance genes. Some of the lines from the collaborative program also showed high level of resistance to FHB in Mexico, Canada, USA and China.

Table 2. Sample of 6-row barley lines with mating quality parents and higher levels of resistance to FHB and other diseases and desirable agronomic traits.

Entry	Cross	FHB						P.hodei	Protein	Yield	PS*
		(%)	(%)	(%)	(%)	(1-5)	(0-5)	(Cobbs)	(%)	(t/ha)	(1-5)
		Hangzhou 2004	Type I Toluca 2004	Type II Toluca 2004	Type I Toluca 2003	ND 2004	Brandon 2004	Obregón 2004	Obregón 2004	Obregón 2004	Mean
254	6B89.2027/CHAMICO	**5.9**	**3.0**	**2.5**	**0.15**			R	13.7	5.4	4.25
77	LEGACY//PENCO/CHEVRON-BAR		**2.3**	**4.4**	**1.52**	3	2.5	60S	**11.0**	**8.1**	1.75
148	LEGACY/3/SVANHALS-BAR/MSEL//AZAF/GOB24DH	**8.2**	**2.0**	10.3	**0.64**			R	14.6	3.9	2.50
147	LEGACY/3/SVANHALS-BAR/MSEL//AZAF/GOB24DH	**11.1**	**1.4**	**4.1**	**1.32**			R	14.3	4.3	3.00
55	LEGACY/4/TOCTE//GOB/HUMAI10/3/ATAH92/ALELI	**6.3**	**1.7**	**4.0**	**0.38**	1	1.5	R	**11.4**	**6.8**	2.25
53	LEGACY/4/TOCTE//GOB/HUMAI10/3/ATAH92/ALELI	**5.3**	**1.4**	**3.9**	**0.13**	2	4	R	**11.3**	**6.4**	3.00
54	LEGACY/4/TOCTE//GOB/HUMAI10/3/ATAH92/ALELI		**1.1**	**4.1**	**1.09**	1	2	R	**12.7**	**6.0**	2.75
60	LEGACY/4/TOCTE//GOB/HUMAI10/3/ATAH92/ALELI		**1.0**	13.3	1.71	1	2.5	R	**11.7**	**5.8**	4.50
65	LEGACY/4/TOCTE//GOB/HUMAI10/3/ATAH92/ALELI		**2.3**	**6.0**	2.14	1	3	R	**12.0**	**6.5**	3.25
73	LEGACY/4/TOCTE//GOB/HUMAI10/3/ATAH92/ALELI		**0.8**	**4.0**	1.80	1	3	R	**10.9**	**6.0**	3.00
	CHEVRON	**3.4**				1	1.5				
	STANDER	9.6	5.15	13.94		3	3	80S	13.0	4.6	3.0
	LEGACY	13.2		6.32				60S	13.0	5.5	3.0

PS = Phenotypic Score 1 = Best; 5 = Worst

Conclusions

Giving support to research programs and producers in developing countries raises challenges that sometimes are not only technical. Despite historical fluctuations in resources available for research, the system implemented has been successful in deploying germplasm and products highly demanded by the customers – either NARs in developing countries or producers in those areas. Successful strategies have to be flexible and adapted to the different and highly variable target areas, and academic recipes most often cannot be directly applied.

The results obtained would have been impossible to reach without the close collaboration with the NARs as well as the ARIs worldwide. Sometimes the roles of our international research programs were to serve as catalysts and facilitators for cooperation among these groups. The unique situation created by the links and networks has allowed the confirmation of the results found in one location and around the world, an approach that is now being validated with other working groups from developed countries (e.g. USWBSI). In addition to the developing areas of the world, the products obtained have also been beneficial to the ARIs and programs present in developed countries.

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Differential response of barley cultivars and accessions to *Rhynchosporium secalis* under field conditions

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Introduction

To ensure an adequate supply of feed many farmers grow barley continuously for several years, and often it is the same cultivar year-after-year. However, continuous production of the same resistant barley cultivar places substantial selection pressure on the scald pathogen, *Rhynchosporium secalis* [Oudem.] J.J. Davis. Research has shown that there is a tremendous amount of diversity in the scald pathogen and therefore potential for rapid changes in the prevalent genotypes of the pathogen in response to the barley cultivars being grown, with some scald races having the ability to attack either individually or in combination several known sources of resistance (Tekauz 1991; Xi et al. 2002, 2003). A recent study demonstrated that 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 (Turkington et al. 2005). One of the cultivars used in the study, Kasota has maintained a relatively high level of resistance to the scald pathogen over a number of sites and years in Alberta (Xi et al. 2003). However, in the cultivar rotation study, although not statistically significant, there was a tendency for greater scald severity when Kasota was grown continuously under barley cultivar monoculture (BCM) compared to its production under barley cultivar rotation or in rotation with triticale. Scald severity on Kasota was very low (<1.0%) in both 1998 and 1999, but had increased to 4.0% on the flag and 7.9% on the penultimate leaf by year 2000 in the BCM treatment. Rotating Kasota with other barley cultivars or triticale maintained scald levels at 1.1% or less on the flag and 1.9% or less on the penultimate leaves.

Single spore isolations were made from symptoms on Kasota leaves from the continuous Kasota treatment (CKT) in the rotation experiment (Turkington et al. 2005) and in the current study were used along with three other *R. secalis* pathotypes derived from specific barley cultivars. Preliminary growth chamber trials indicated that the CKT pathotype produced susceptible reactions on Kasota at the seedling stage. The objective of the current study was to evaluate the occurrence of more virulent scald pathotypes, their pathogenicity on a range of barley genotypes and registered cultivars under field conditions in 2004, and to identify effective sources of resistance in commercial cultivars and accessions. The current study will help to identify the occurrence of *R. secalis* pathotypes with the ability to overcome some of the most effective sources of resistance present in commercially available barley cultivars. Knowledge about variation in pathogenicity of *R. secalis* will also help to identify compatible barley cultivar combinations that can be used as part of short-term cultivar rotation strategies for disease management.

Materials and methods

Thirty-three barley accessions and cultivars with major resistance genes were studied for their differential reactions to *R. secalis* (Table 1). The 4 scald pathotypes used were derived from single spores collected from infected leaf material from each of the barley cultivars Kasota, Seebe, CDC Earl and Harrington collected from several experimental sites. Fertilizer applied to the experimental area consisted of 202 kg/ha of 13-22-22-0, which was broadcast and incorporated into worked pea stubble. Hill plots, consisting of 8-10 seeds planted in a single hole on 0.5 m centers, were arranged in a 4-replicate RCBD for each pathotype. Each pathotype trial was seeded a minimum of 15 m from another. Weed control was accomplished 8 June, 2004 with a tank mix of Refine Extra® at 20 g/ha and Puma Super® at 360 mL/ha. Scald inoculum was prepared from 2-3 week old cultures grown on lima bean agar. Culture plates were scraped using reverse-osmosis water and a hand-held battery operated toothbrush. The spore concentration was adjusted to 10^5 spores/ml and 2.5 L of inoculum for each pathotype was applied as a fine mist using a compressed air sprayer on 14 June and 28 June, 2004. After spores were sprayed onto hills, the cut up agar from the scraped plates from each pathotype was uniformly placed on the respective hills. Hills were rated for percent leaf area diseased (PLAD) using a 0-9 scale (0 = no disease in the lower, middle and upper canopy, 9 = >50% leaf area diseased in lower, middle and upper canopy) on 5 August, 2004.

An analysis of variance of the data was conducted separately for each pathotype using the PROC MIXED procedure of SAS, with block as a random effect and cultivar as a fixed effect (Littel et al. 1996). For significant effects LSD values were derived to allow for further exploration of treatment differences. Treatment effects were declared significant at $P \leq 0.05$. A rating index was also derived from the mean cultivar ratings for each pathotype. Absolute differences in ratings were then calculated among all pathotype combinations and then summed for each cultivar to derive the rating index. A rating index of 0 indicated that the rating for a cultivar remained the same among the pathotypes tested; however increasing index values indicated that ratings changed from one pathotype to the next. Spearman's rank correlations were calculated among the mean cultivar ratings for each of the pathotypes and were declared significant at $P<0.05$. Hudson was included in the present study, but due to its winter growth habit comparable ratings were not possible and data for this cultivar were not included in the analyses.

Results and discussion

Cultivar had a significant influence ($P<0.0001$) on the severity of scald for all pathotypes tested. The trend was for the Kasota pathotype to produce somewhat higher disease severity compared to the other three pathotypes (Table 1). Although not included in the analysis, no symptoms were detected on Hudson when inoculated with the Kasota, Seebe, or CDC Earl pathotypes, while a low level disease was found for the Harrington pathotype (data not shown). The CDC Earl pathotype produced intermediate disease severity, while the trend was for the Seebe and Harrington pathotypes to produce the lowest levels of disease. For the Kasota pathotype, only Seebe and Shyri had relatively low, "resistant-type" reactions, while the highest levels of disease were observed on CDC Dolly, Harrington, AC Stacey, Kasota, Mahigan, Niska, Ponoka, Trochu, and Vivar (Table 1). Among the accessions, Abyssinian, Turk, and Osiris had the lowest levels of disease, while Atlas and API/CM67-B//AGER had the highest level of scald. All other cultivars and accessions had intermediate levels of disease. Different results were observed for

Table 1. Mean scald severity for barley accessions and Alberta cultivars, scald pathotype field study, Lacombe, Alberta, 2004.

	Pathotype and scald severity (0-9 scale)					
Accession/cultivar[†]	Kasota	Seebe	CDC Earl	Harrington	Mean	Rating index
Abyssinian	2.5	2.8	2.3	3.0	2.6	2.5
AC Harper	6.5	5.8	6.8	6.3	6.3	3.3
AC Stacey	9.0	4.5	3.8	3.5	5.2	17.3
API/CM67-B//AGER	8.3	6.5	6.5	5.5	6.7	8.3
Atlas	9.0	4.0	3.8	4.3	5.3	16.0
Atlas46	4.5	2.5	4.0	3.8	3.7	6.3
Atlas68	6.5	4.0	6.3	4.8	5.4	9.0
CDC Dolly	9.0	5.5	4.8	4.3	5.9	15.0
CDC Earl	6.3	6.5	9.0	8.5	7.6	10.3
Duke	5.5	5.5	7.5	7.5	6.5	8.0
Falcon	7.0	6.8	5.5	5.8	6.3	5.5
Gatillo-Bar	7.0	2.5	4.0	3.8	4.3	13.8
Harrington	9.0	9.0	8.8	8.3	8.8	2.5
Jaeger	6.8	4.8	8.8	7.5	6.9	12.8
Johnston	5.8	4.8	6.0	4.8	5.3	4.8
Kasota	8.8	4.3	3.8	3.5	5.1	16.3
Kitchin	4.8	4.0	4.3	3.8	4.2	3.3
Leduc	6.3	6.8	7.5	4.5	6.3	9.5
Mahigan	8.8	3.8	4.3	4.0	5.2	15.3
Manny	5.8	1.0	0.8	2.0	2.4	16.0
Modoc	6.0	6.5	8.0	6.5	6.8	6.0
Niobe	6.3	5.3	9.0	7.8	7.1	12.8
Niska	8.5	7.5	4.3	5.8	6.5	14.5
Osiris	3.8	1.5	3.0	2.5	2.7	7.3
Peregrine	7.0	8.0	7.5	7.3	7.4	3.3
Ponoka	8.5	5.0	3.8	3.8	5.3	15.5
Seebe	3.5	3.3	3.3	2.5	3.1	3.0
Shyri	3.8	3.8	3.5	3.8	3.7	0.8
Trebi	7.3	6.5	7.3	7.3	7.1	2.3
Trochu	7.8	6.5	8.0	6.5	7.2	5.8
Turk	3.3	2.8	5.8	4.0	3.9	9.8
Vivar	7.8	7.5	8.3	6.5	7.5	5.5
$LSD_{.05}$	1.1	1.5	1.3	1.1		
Mean pathotype severity	6.6	5.0	5.6	5.1		

[†] Hudson data not included due to winter growth habit. No symptoms occurred on Hudson with the Kasota, Seebe, and CDC Earl pathotypes, while trace to low scald levels were observed for the Harrington pathotype.

the Seebe pathotype. The lowest levels of scald were observed on Manny, Osiris, Gatillo-Bar, Atlas46, Turk, Abyssinian, Seebe, Mahigan, and Shyri, while the highest levels were observed on Harrington, Peregrine, Niska, and Vivar. All other cultivars and accessions had intermediate diseases levels. For the CDC Earl pathotype, Manny, Abyssinian, Osiris, Seebe, Shyri, Kasota, AC Stacey, Ponoka, and Atlas had the lowest disease levels, while CDC Earl, Niobe, Harrington, Jaeger, Vivar, Modoc, Trochu, Duke, Leduc, Peregrine, and Trebi had the highest. The remaining cultivars and accessions had intermediate levels of disease. For the Harrington pathotype the lowest levels of disease were observed on Manny, Seebe, Osiris, Abyssinian, AC Stacey, Kasota, Shyri, Ponoka, Kitchin, Gatillo-Bar, and Atlas46, while the highest levels were observed on CDC Earl, Harrington, Niobe, Duke, Jaeger, Peregrine, and Trebi. Intermediate scald levels were observed on the remaining cultivars and accessions.

When averaged over the four separate pathotype experiments, cultivars Manny and Seebe had among the lowest scald severities, while Harrington, CDC Earl, Vivar, Peregrine, Trochu, Niobe, and Jaeger had among the highest levels of scald, while the remaining cultivars had intermediate disease levels. Of the accessions, Abyssinian, Shyri, Osiris, Atlas46 and Turk had among the lowest disease severities, while API/CM67-B//AGER, Modoc, and Trebi had the highest scald levels. A greater potential for pathotype specific responses were indicated by the ranking index for a number of cultivars with higher index values, especially for AC Stacey, CDC Dolly, Kasota, Mahigan, Manny, and Ponoka. For example, AC Stacey, Kasota, Manny, and Ponoka tended to have the highest levels of disease with the Kasota pathotype, while CDC Earl, Duke and Niobe tended to have the highest rating when inoculated with either the CDC Earl or Harrington pathotype. Accessions with the highest index values included Atlas, which tended to have the highest rating with the Kasota pathotype. Consistently low ratings over all pathotypes and lower rating index values occurred for Seebe, Shyri, Osiris, Abyssinian, and Kitchin, while consistently higher ratings and lower index values occurred for Harrington, Peregrine, and Trebi.

Results from Spearman's rank correlations suggested that there may be an interaction between pathotype and cultivar. Confounding with location by cultivar interaction effects may be a potential concern; however, all four experiments were planted in the same experimental field and were only spaced 15 to 25 m apart. In addition, seedbed preparations, fertilizer, seeding methodologies, seeding date, and inoculation protocols were similar. Thus, cultivar differences were likely more a function of the interaction of pathotype by cultivar rather than location by cultivar. Cultivar reactions for the Kasota pathotype were not correlated ($P>0.05$) with those from the CDC Earl or Harrington pathotypes, while there was a significant low-moderate correlation with reactions from the Seebe pathotype ($P<0.01$, $r_s = 0.5$). Reactions to the Seebe pathotype were moderately correlated with those from the CDC Earl ($P<0.01$, $r_s = 0.7$) and Harrington ($P<0.01$, $r_s = 0.8$) pathotypes, while reactions for the CDC Earl and Harrington pathotypes were highly correlated ($P<0.01$, $r_s = 0.9$). The CDC Earl and Harrington pathotypes were collected from samples taken from a field area used for scald screening in 2001 and 1999, respectively, and by 1999, scald pathotypes virulent on CDC Earl had already occurred in this area. The Kasota pathotype was taken from the same general field in 2000, but from plots that had been in continuous Kasota for 3 years in a row, while the Seebe pathotype was taken from a screening nursery in Edmonton, AB in 2003.

The current study identified the occurrence of *R. secalis* pathotypes with the ability to overcome some of the most effective sources of scald resistance present in commercially available barley cultivars grown in Alberta. Of particular concern was the Kasota pathotype which produced very susceptible field reactions on AC Stacey, CDC Dolly, Kasota, Mahigan, Niska, Ponoka, API/CM67-B//AGER, and Atlas. In addition, an increased rating was observed on Manny when inoculated with the Kasota pathotype, which is in contrast with other recent trials where Manny has maintained a very good reaction to scald. The scald rating on Seebe inoculated with the Seebe pathotype was low in this trial, whereas other recent observations have shown the occurrence of susceptible-type reactions for Seebe with other scald pathotypes derived from Seebe. Further research with the Kasota and Seebe pathotypes and monitoring of Manny and Seebe reactions are underway. The CDC Earl pathotype produced very susceptible field reactions on CDC Earl, Jaeger, Modoc, Niobe, Trochu, and Vivar, while the Harrington pathotype produced susceptible reactions mainly on CDC Earl. The current study also demonstrated that Abyssinian, Atlas46, Atlas68, Gatillo-Bar, Hudson, Kitchin, Osiris, Shyri, and Turk may possess useful sources of resistance for future Alberta barley cultivars.

Differential responses within and between pathotypes indicated potential compatible barley cultivar combinations that could be used as part of short-term cultivar rotation strategies for disease management. Cultivars that may have potential to produce low to moderate levels of disease when grown in rotation with Kasota include Seebe, Duke, and Johnston, while AC Harper, CDC Earl, Leduc, and Niobe may also have some potential. Low to moderate disease levels may also be possible with Seebe in rotation with AC Stacey, Jaeger, Johnston, Mahigan, and Manny, while AC Harper, CDC Dolly, CDC Earl Duke, Niobe, Ponoka, and Trochu may also have some potential. Other cultivars that may be useful in rotations with CDC Earl include AC Stacey, CDC Dolly, Mahigan, Manny, Niska, and Ponoka.

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Mapping genes for Russian wheat aphid resistance in barley

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Russian wheat aphid (RWA), *Diuraphis noxia* (Mordvilko), is one of the most serious pests of grain crops. Russian wheat aphid infestations reduce grain yield and malting quality of barley. Since it was first identified in Texas in 1986, RWA has caused more than $1 billion in losses in the Western United States. Two Russian wheat aphid resistant spring barley germplasm lines, STARS-9301B and STARS-9577B, were developed by USDA-ARS, Stillwater, OK and released to breeders. Inheritance studies indicated that RWA resistance in STARS-9301B was controlled by two genes, one incompletely dominant and one dominant with epistatic effects. RWA resistance in STARS-9577B was suggested to be controlled by dominant alleles at two loci. The objectives of this project were to confirm the number and effect of genes for RWA resistance from the above mentioned lines, and to map these genes. In a cooperative effort, allelism tests were conducted by the USDA-ARS, Stillwater, OK to determine the number of loci involved. STARS-9301B and STARS-9577B were each crossed to a susceptible spring barley cultivar, Morex, and generations advanced to produce two $F_{2:3}$ populations. From each population, 196 families were used for mapping. Simple sequence repeat (SSR) markers were screened on the $F_{2:3}$ populations. A genetic linkage map was constructed from the data with a LOD of score 3.00 for population developed from STARS-9301B by Morex cross. QTL analysis was conducted to determine the chromosomal locations and effects of genes involved in RWA resistance using MapManager QTX. Two major QTL were found on 1H and 3H chromosome. Work for STARS-9577B is in progress. The genes located for Russian wheat aphid resistance in STARS-9301B and STARS-9577B can be incorporated in to breeding programs where these genes can be transferred to susceptible varieties using linked molecular markers to achieve resistance towards Russian wheat aphid.

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Sequence tagged site markers linked to Septoria speckled leaf blotch resistance genes in barley (*Hordeum vulgare* L.)

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Septoria speckled leaf blotch (SSLB) caused by the pathogen *Septoria passerinii* is a common and important leaf disease in barley in the Upper Midwest and adjacent Canadian provinces. The disease was severe in the 1950s in the north-central region of the United States and Prairie Provinces of Canada (Buchannon 1961; Green and Dickson 1957), with yield losses of 23-38% reported in Canada (Green and Bendelow, 1961). Recent yield losses due to SSLB reported by Toubia-Rahme and Steffenson (1999) are similar to those previously reported in Canada. The importance of SSLB is increased further due to its effects on grain quality such as reduced kernel size and malting quality (Green and Bendelow, 1961). All of the major malting and feed barley cultivars in the Upper Midwest region are susceptible to this pathogen. Development of resistant cultivars is the preferred method to prevent SSLB epidemics. Thus, it is necessary to map the genes controlling the resistance and develop molecular markers for use in screening breeding lines.

To date, three SSLB resistance loci designated *Rsp*1, *Rsp*2, and *Rsp*3 have been identified in CIho14300, CIho4780, and CIho10644 respectively (Rasmusson and Rogers, 1963). The genes, *Rsp*2 and *Rsp*3, are closely linked with about 3.8% recombination (Rasmusson and Rogers, 1963). The molecular mapping work for resistance genes has been published on *Rsp*2 and *Rsp*3 (Zhong *et al*. 2002). Two AFLP markers linked to *Rsp*2 were developed and mapped on the short arm of chromosome 1H(5). Information on map location and molecular markers for *Rsp*1 is still lacking. In addition, the AFLP technique has limited use in marker assisted selection (MAS) because it is laborious, time consuming, expensive, and technically difficult (Neil et al., 1997). Thus, the aim of this research was to identify randomly amplified polymorphic DNA (RAPD) markers linked to *Rsp* genes and convert them into sequence tagged site (STS) markers so that marker assisted selection (MAS) can be used to develop SSLB resistant cultivars in barley.

We developed six mapping populations by crossing the susceptible cultivars, Robust and Foster, with the resistant lines, CIho14300 (*Rsp*1), CIho4780 (*Rsp*2), and CIho10644 (*Rsp*3). Robust comes from the Minnesota barley breeding program and its pedigree is Morex x Manker and Foster comes from the North Dakota breeding program and its pedigree is Robust/6/Glenn/4/ Nordic//Dickson/Trophy/3/Azure/5/Glenn/Karl. F_1 plants were selfed to obtain between 100 and 120 F_2 plants. SSLB phenotypes were evaluated in F_2 plants and $F_{2.3}$ families at the seedling and adult stage in the greenhouse and in the field. Segregation analysis for *Rsp1, Rsp2,* and *Rsp3* genes in two F_2 populations of seedlings in the greenhouse showed an approximate segregation ratio of 3 resistant:1 susceptible in both genetic backgrounds (Table 1). This was confirmed in analysis of the $F_{2:3}$ families from Robust x CIho 14300, Robust x CIho 4780, and Robust x CIho 10644 grown in the greenhouse and field at Langdon and Osnabrock, ND (Table 2).

Table 1. Segregation analysis for Septoria Speckled Leaf Blotch resistance genes *Rsp1*, *Rsp2*, and *Rsp3* in F_2 populations derived from two susceptible cultivars (Robust and Foster) and three resistant lines (CIho14300, CIho4780, CIho10644).

Cross Female (S) x Male (R)	Genes	Resistant plants	Susceptible plants	Expected ratio	χ^2	P^a
Foster x CIho 14300	*Rsp1*	93	25	3 : 1	0.92	0.34
Robust x CIho 14300	*Rsp1*	74	29	3 : 1	0.55	0.46
Foster x CIho 4780	*Rsp2*	93	27	3 : 1	0.40	0.53
Robust x CIho 4780	*Rsp2*	85	23	3 : 1	0.79	0.37
Foster x CIho 10644	*Rsp3*	82	32	3 : 1	0.57	0.45
Robust x CIho 10644	*Rsp3*	83	32	3 : 1	0.49	0.48

[a] $P < 0.05$ = significant deviation from the expected segregation ratio, $P > 0.05$ = fit to the expected segregation ratio of the F_2 population

Table 2. Segregation analysis for *Rsp1*, *2*, and *3* genes in $F_{2:3}$ families derived from Robust × CIho14300, Robust × CIho4780, and Robust × CIho 10644.

Cross	Location	Plant stages	RR[b]	Rr	rr	Expected ratio	χ^2	P^c
Robust x CIho14300	Langdon[a]	Adult	18	44	28	1:2:1	2.27	0.32
	Osnabrock[a]	Adult	19	46	25	1:2:1	0.84	0.66
	Greenhouse	Seedling	18	50	21	1:2:1	1.56	0.46
Robust x CIho4780	Langdon[a]	Adult	25	49	26	1:2:1	0.06	0.97
	Osnabrock[a]	Adult	31	51	18	1:2:1	3.42	0.18
	Greenhouse	Seedling	28	57	27	1:2:1	0.05	0.97
Robust x CIho10644	Langdon[a]	Adult	25	53	27	1:2:1	0.09	0.96
	Osnabrock[a]	Adult	21	51	33	1:2:1	2.83	0.24
	Greenhouse	Seedling	21	52	27	1:2:1	0.88	0.64

[a] Name of field location in ND, USA
[b] RR: Homozygous resistant, Rr: Heterozygous resistant, rr: Homozygous susceptible.
[c] $P < 0.05$ = significant deviation from the expected segregation ratio, $P > 0.05$ = fit to the expected segregation ratio of the $F_{2:3}$ population

To develop molecular markers linked to *Rsp* genes, 480 10-mer RAPD primers (200 from University of British Columbia, and 280 from Operon Technologies Inc.) were used to screen polymorphisms between the two different DNA bulks from resistant and susceptible F_2 plants, and between two susceptible parents and three resistant parents. The RAPD primers that showed a specific polymorphism between a resistant and susceptible bulk were selected to determine the genetic linkage between SSLB resistance genes and markers. Three RAPD markers, $UBC285_{158R}$, $OPAH5_{545C}$, and $OPBA12_{314C}$, associated with *Rsp* genes, were identified using bulked segregant analysis in populations of 100-120 F_2 individuals. Linkage analysis revealed RAPD markers $UBC285_{158}$ (3.8±1.1cM) in repulsion linked to *Rsp1*, RAPD marker $OPAH5_{545C}$ (0.9±1.3) in coupling linked to *Rsp2*, and RAPD marker $OPBA12_{314}$ (2.4cM) in coupling linked to *Rsp3*. A repulsion phase of dominant marker, $UBC285_{158R}$, for *Rsp1* and two coupling phase of dominant markers, $OPAH5_{545C}$ for *Rsp2* and $OPBA12_{314C}$ for *Rsp3*, showed the expected segregation ratio 1 resistant:3 susceptible and 3 resistant:1 susceptible in F_2 plants (Table 3).

For high reproducibility and ease to use, RAPD markers associated with *Rsp* genes were converted into sequence-tagged site (STS) markers, $Rsp1_{158R}$, $Rsp2_{545C}$, and $Rsp3_{314C}$, (Figure 1).

Table 3. Chi-square test of segregation ratios with RAPD markers linked to *Rsp* genes in F_2 populations derived from two susceptible cultivars (Robust and Foster) and three resistant lines (CIho14300, CIho4780, CIho10644).

Cross Female(S) x Male(R)	Genes		RR[a]	Rr	rr	Expected segregation	χ^2	P^b	Genetic[c] distance (cM)
Robust x CIho14300	*Rsp1*	$UBC285_{158R}$	22	81		25.75:77.25	0.55	0.46	3
Foster x CIho14300	*Rsp1*	$UBC285_{158R}$	25	91		29:87	0.74	0.39	4.5
Robust x CIho4780	*Rsp2*	$OPAH5_{545C}$	75		25	75:25	0	1	0
Foster x CIho4780	*Rsp2*	$OPAH5_{545C}$	93		27	90:30	0.28	0.6	1.8
Robust x CIho10644	*Rsp3*	$OPBA12_{314C}$	84		29	84.75:28.25	0.03	0.87	2.4

[a] RR: Homozygous resistant, Rr: Heterozygous resistant, rr: Homozygous susceptible.
[b] $P < 0.05$ = significant deviation from the expected segregation ratio, $P > 0.05$ = fit to the expected segregation ratio of the F_2 population
[c] Genetic distances (cM) were analysed by MAPMAKER, LOD>3.0.

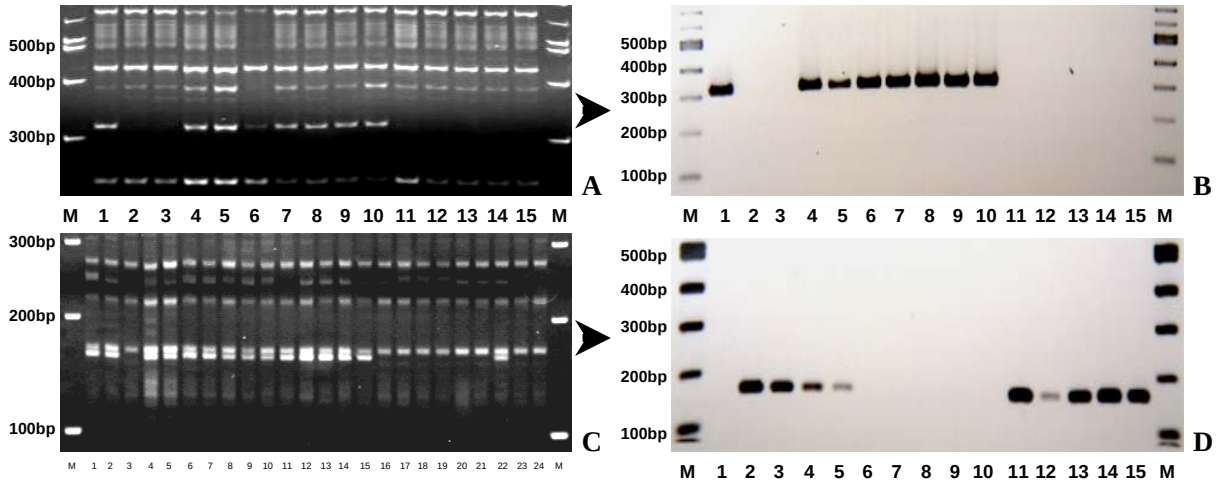

Fig. 1 Identification of polymorphisms associated with SSLB resistance genes, *Rsp2* and *Rsp3*, using RAPD markers (A) $OPBA12_{314C}$ and (C) $UBC285_{158R}$. The RAPD markers were converted into sequence tagged site (STS) markers, (B) $Rsp3_{314C}$ and (C) Rsp_{158R}. Lanes are as follows: (A), (B), and (D) = M, DNA size markers in bp; 1, resistant parent CIho10644 (A and D) and CIho14300 (D); 2, susceptible parent Robust; 3, susceptible parent Foster; 4, F_1 plant of Robust×CIho10644 (A and B) and Robust×CIho14300 (C); 5, F_1 plant of Foster×CIho10644 (A and B) and Foster×CIho14300 (C); 6 through 10, F_2 resistant plants; 11 through 15, F_2 susceptible plants. (C) = M, DNA size markers in bp; 1, susceptible parent Robust; 2, susceptible parent Foster; 3, resistant parent CIho14300; 4, F_1 plant of Robust×CIho14300; 5, F_1 plant of Foster×CIho14300; 6 through 15, F_2 susceptible plants; 16 through 24, F_2 resistant plants.

To determine the existence of STS markers linked to *Rsp* genes, 21 resistant and 16 susceptible barley lines identified as resistant or susceptible by Toubia-Rahme and Steffenson (2004) were evaluated (Table 4). It is not known which resistance genes are present in these lines. Two STS markers, $Rsp1_{158R}$ in repulsion and $Rsp2_{545C}$ in coupling showed the expected presence or absence of bands in resistant lines. However, unexpected results were obtained in 7/18 ($Rsp1_{158R}$) and 9/18 ($Rsp2_{545C}$) susceptible lines. This may be due to our previous finding that the markers are not within, but separated from the genes of interest. The STS marker $Rsp3_{314C}$ linked to *Rsp3*, amplified a band only in the four resistant lines, Bolron, Feebar, Flynn1, and Vaughn. The pedigree of CIho10644 containing *Rsp3* is Feebar/Kindred. The pedigree of Feebar is Peatland/Vaughn, and the pedigree of Vaughn is Mariout/Leiorrhynchium or Club Mariout/Lion. Flynn also comes from a cross between Club Mariout/Lion and Flynn1 is a selection from Flynn.

Thus, the lines CIho10644, Feebar, Vaughn, and Flynn1 which gave a band with the STS marker $Rsp3_{314C}$ contain a genetic background with *Rsp3* in one of the parents. This result gives hope that with further testing the STS marker $Rsp3_{314C}$ can be effectively used in MAS to identify lines containing *Rsp3*. The other line testing positive to $Rsp3_{314C,}$ Bolron, is from a cross Bolivia/Chevron, and has a different genetic background. Bolivia and Chevron need to be tested with the STS marker $Rsp3_{314C}$ and allelism tests done to determine if they contain *Rsp3*.

Table 4. Validation of sequence-tagged site (STS) markers and reaction to SSLB for 24 resistant and 18 susceptible barley lines

Cultivars/Lines	Reaction[a] to SSLB	STS marker		
		$Rsp1_{158R}$	$Rsp2_{545C}$	$Rsp3_{314C}$
Atlas54	R	+[b]	-	-
Atlas	R	+	-	-
Bolron	R	-	-	+
CIho4428	R	-	-	-
CIho4439	R	+	+	-
CIho6398	R	-	-	-
CIho9831	R	-	+	-
Custer	R	+	-	-
Feebar	R	+	-	+
Flynn1	R	+	-	+
Glacier	R	-	-	-
Hor2683-84	R	+	+	-
Hor 9471-87	R	-	+	-
Nomini	R	-	-	-
ND16092	R	+	+	-
PC11	R	-	+	-
PC84	R	+	+	-
Sp.No:1	R	-	-	-
Starling	R	-	+	-
Sussex	R	-	-	-
Vaughn	R	-	-	+
Bowman	S	+	+	-
Carlsberg	S	-	-	-
CIho13581	S	-	+	-
CIho4753	S	+	+	-
CIho592	S	-	-	-
CIho0182	S	-	+	-
CIho2947	S	+	-	-
CIho8096	S	-	+	-
Heimdal	S	-	+	-
Hiland	S	+	+	-
Kindred	S	+	-	-
Olli	S	-	-	-
Supi 1	S	+	+	-
Trebi	S	+	+	-
Velvon	S	+	-	-
ZAU7	S	+	-	-
Robust	S	+	-	-
Foster	S	+	-	-
CIho14300 (*Rsp1*)	R	-	-	-
CIho4780 (*Rsp2*)	R	+	+	-
CIho10644 (*Rsp3*)	R	+	-	+

[a] SSLB inoculation and disease assessment was taken as described by Toubia-Rahme and Steffenson (2004). R: resistant, S: susceptible.

[b] Existence of alleles (+) and absence of alleles (-).

In future work, other RAPD markers linked to *Rsp* genes will be converted into STS markers, with emphasis on *Rsp*1 and *Rsp*2. The evaluation of selection effectiveness in F_3 populations and the allelism test for *Rsp* genes with STS markers will be undertaken. The screening of other resistant sources for resistance genes to SSLB will be performed and validated with the STS markers associated with *Rsp* genes.

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Tuesday, July 19, 2005 – a.m.

Session 3: MALTING AND BREWING QUALITY

Chair
Patricia Juskiw

Presenters
Rob McCaig, Canadian Malting Barley Technical Centre
Berne Jones, USDA
Marta Izydorczyk, Grain Research Laboratory, Canadian Grain Commission
Michael Edney, Grain Research Laboratory, Canadian Grain Commission
Jim Helm, Field Crop Development Centre

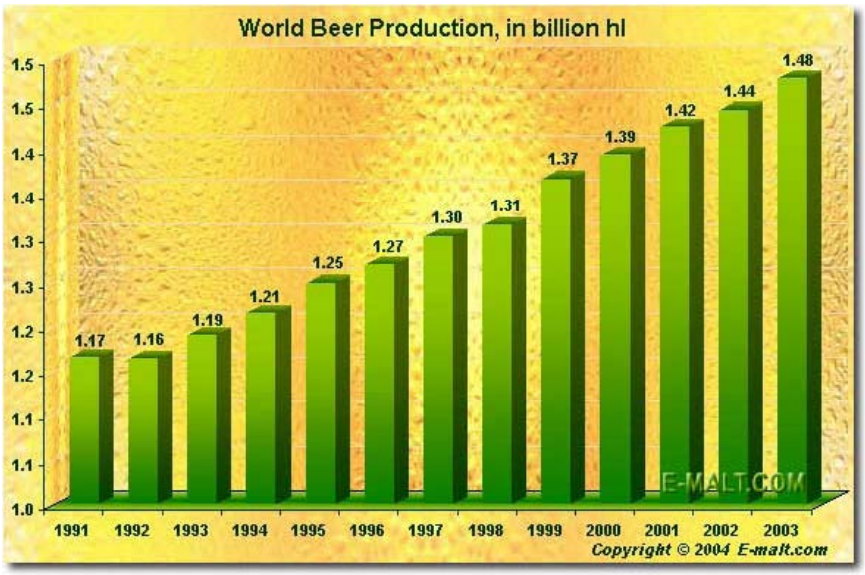
Brewing and Malting: Where are we and more importantly, where are we going?

Rob McCaig

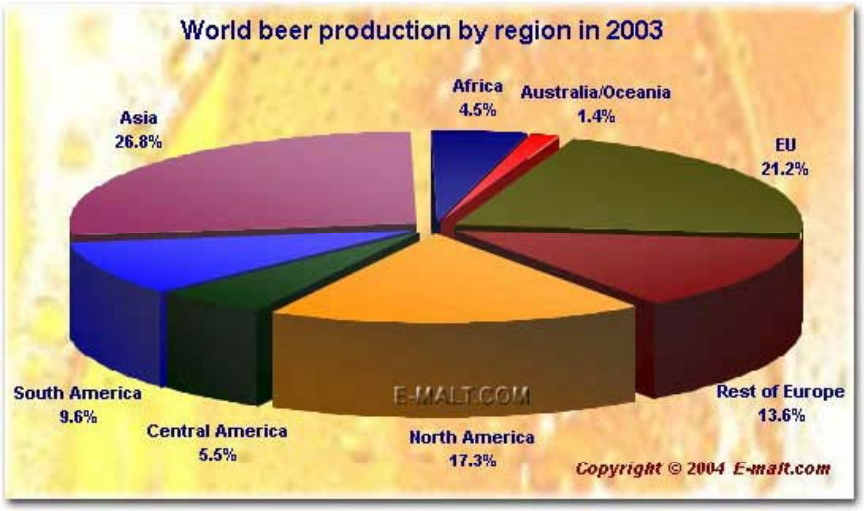
Canadian Malting Barley Technical Centre

Brewing

- There is a tremendous amount of change taking place in the industry
- Most of industrialized nations declining in production, slack being taken up by Asia (China, Vietnam), India, Mexico, Columbia, Russia – growth in these areas approaching double figures
- World beer production growing at a steady pace



- In terms of production area, North America has declined from 24% of beer produced in 1998 to 17% today, while Asia and South America have split that new volume
- China is now number 1 beer producer in the world



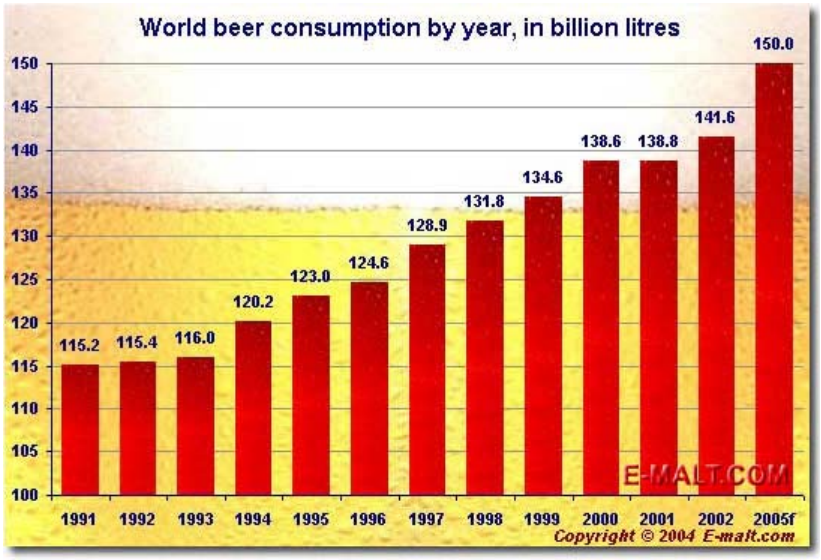
- 64% of the beer produced in Asia is brewed in China, growth is in double digits for past 5 years
- The brewing world is in a major consolidation phase – bigger is better, more economical, global brands

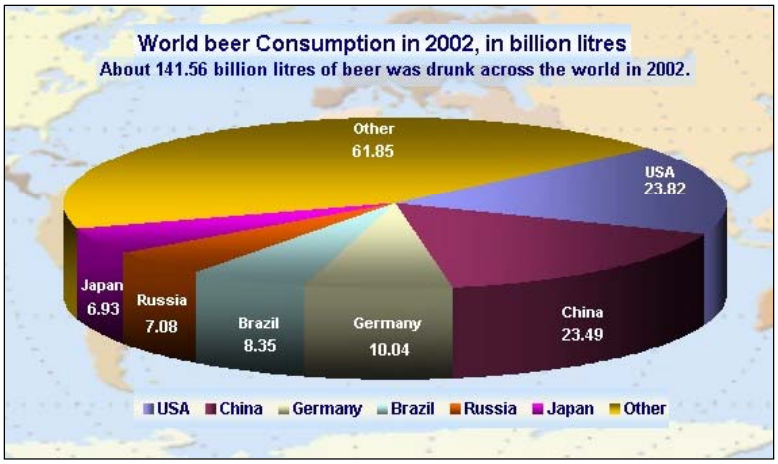
	2002 Global brewers ranking	2005 Global Brewers Ranking
1	Anheuser-Busch 122.4 million hl	Inbev 143.6 million hl
2	SABMiller 110 million hl,	SABMiller 133.5 million hl,
3	Heineken 92.9 million hl,	Anheuser-Busch 128.1 million hl
4	Interbrew 92 million hl	Heineken 94.5 million hl,
5	Carlsberg 68.6 million hl	Molson Coors 49 million hl
6	Companhia de Bebidas das Americas 55 million hl	Carlsberg 35.8 million hl
7	Scottish and Newcastle group 45 million hl	Scottish Courage 32.4 million hl
8	Grupo Modelo 33.4 million hl	Grupo Modelo 33.4 million hl
9	Coors 32.4 million hl	Kirin 30 million hl
10	Kirin 30.7 million hl	BBH 30 million hl

The future "supercompanies and their affiliates"?

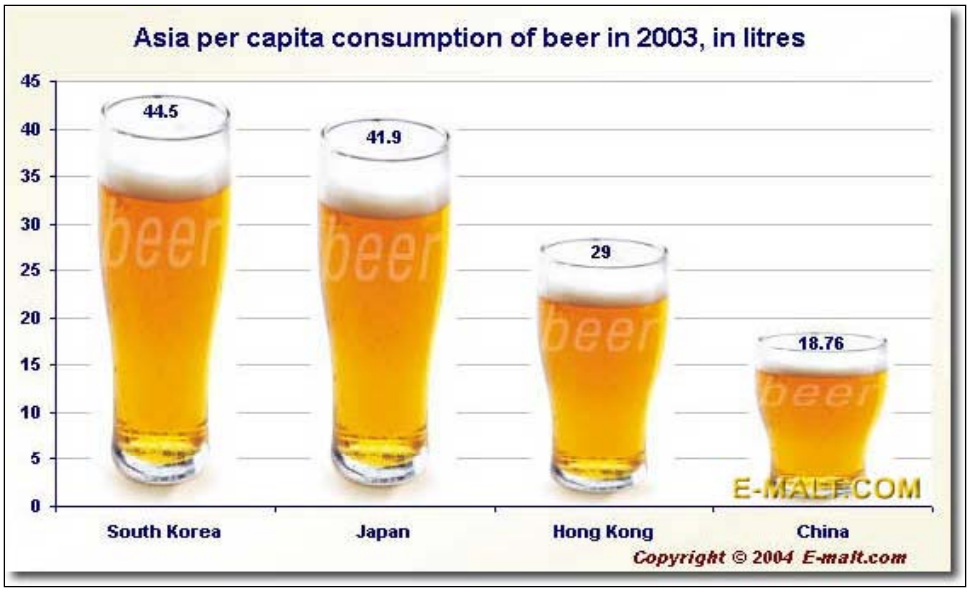
Continent	**Inbev**	**SABMiller**	**AB**	**Heineken**
NA	Modelo Labatt	Miller	AB	Molson Coors
SA	Ambev			Bavaria/Kaiser
Europe	Interbrew Guinness	Scottish Courage	Scottish Courage	Heineken Coors/Bars
Rest of World				
China	Interbrew	CRE	Tsingdoa	Heineken
Asia	Guinness	SAB	Harbin	
Africa	Guinness	SAB/Castal	Fosters	Heineken
Oceania		Fosters		
Total Volume (hL)	330	220	180	180

- World beer consumption continues to increase





- Per-capita volume in China is very low (18 L per annum),



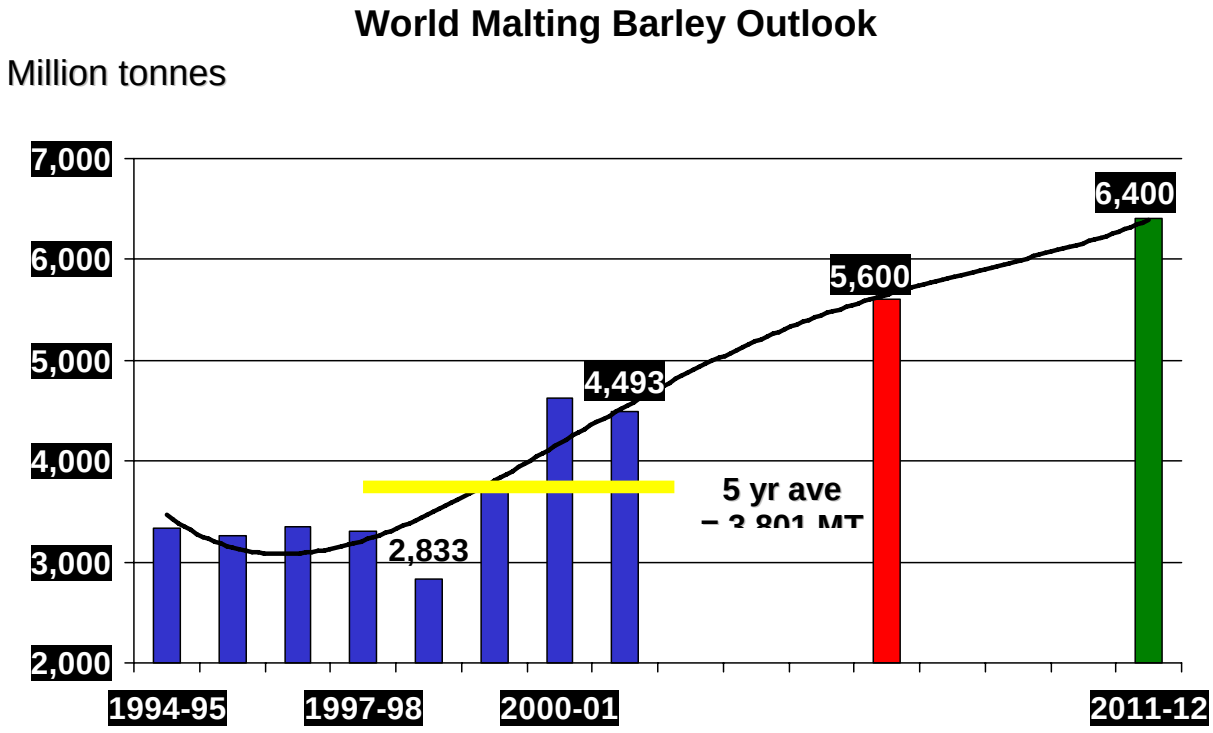
- Many factors affect consumption in the mature markets including:
  - **Demographics**
  - **Lifestyle**
  - **Drinking and Driving**
  - **Growth of Other Beverages**
  - **Price (Taxes)**

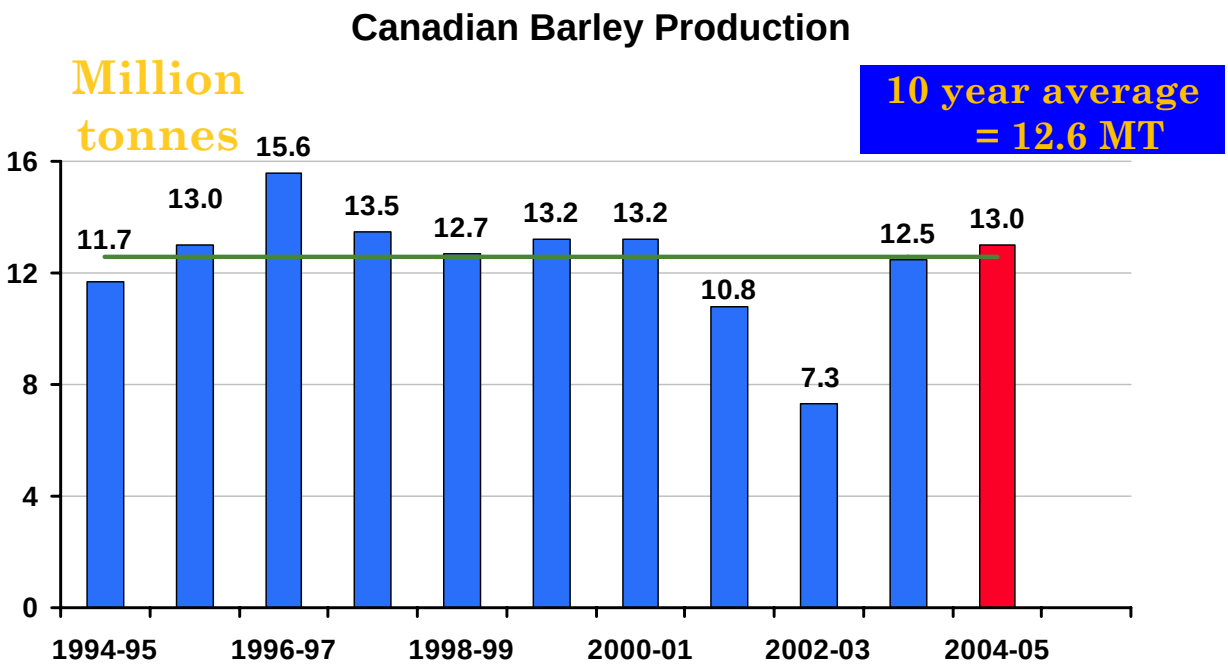
- Aging population trend
- Growth of travel = growth of imports
- Decline in per capita consumption – trend to wine, coolers
- Tax burden on beer
  - Look at Happoshu growth in Japan

- Current and Future brewing trends
  - **Breweries are Traditional Incremental changes – no breakthroughs**
  - **Economic considerations High gravity, more use of adjuncts, hulless barley**
  - **Increased "quality"**
  - **Use of technology – on-line / at-line, NIR, Neural Networks, electronic nose**
  - **Energy use reduction - Wort Boiling**
  - **Environmental Issues - D.E. Replacement**

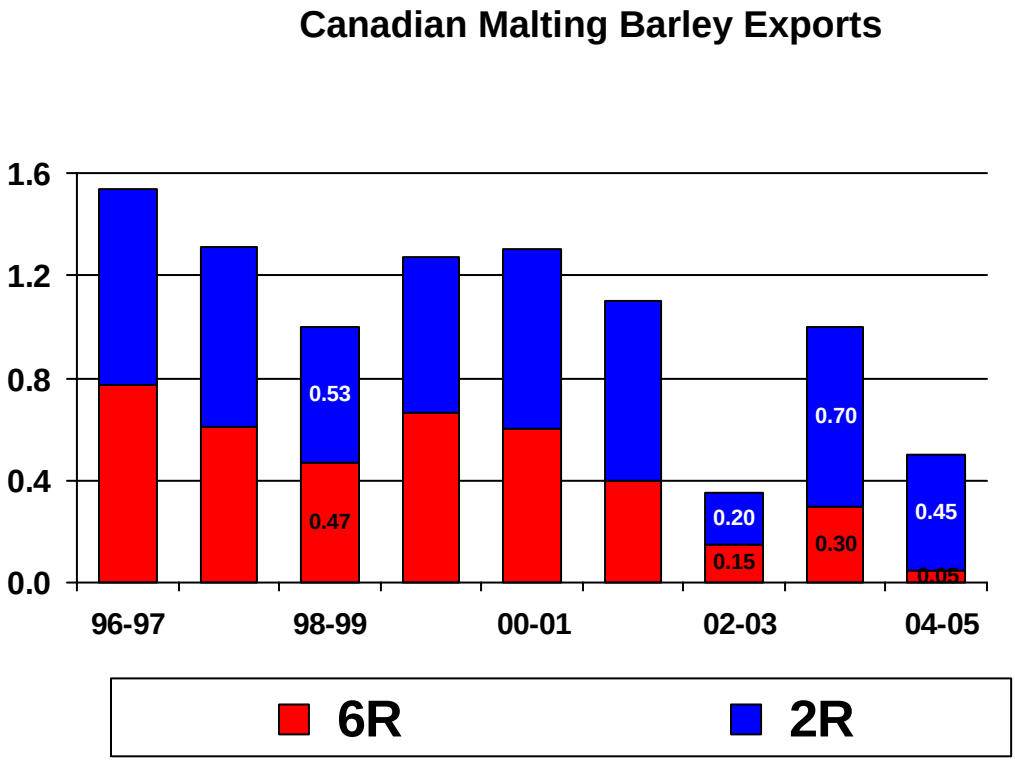
Malting

- Also going through consolidation
- Malting barley trade is increasing yearly, but fluctuates according to the harvest



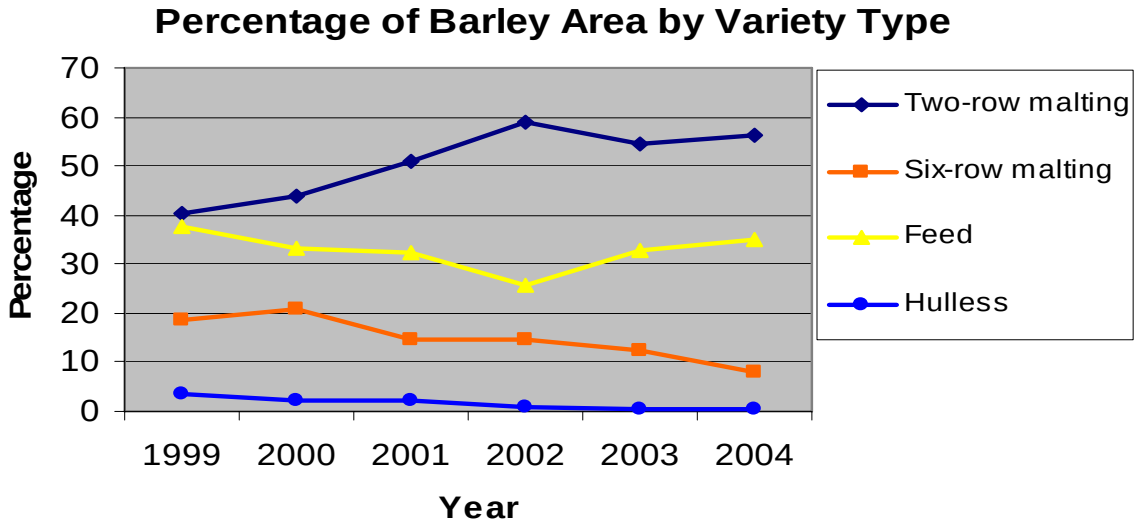


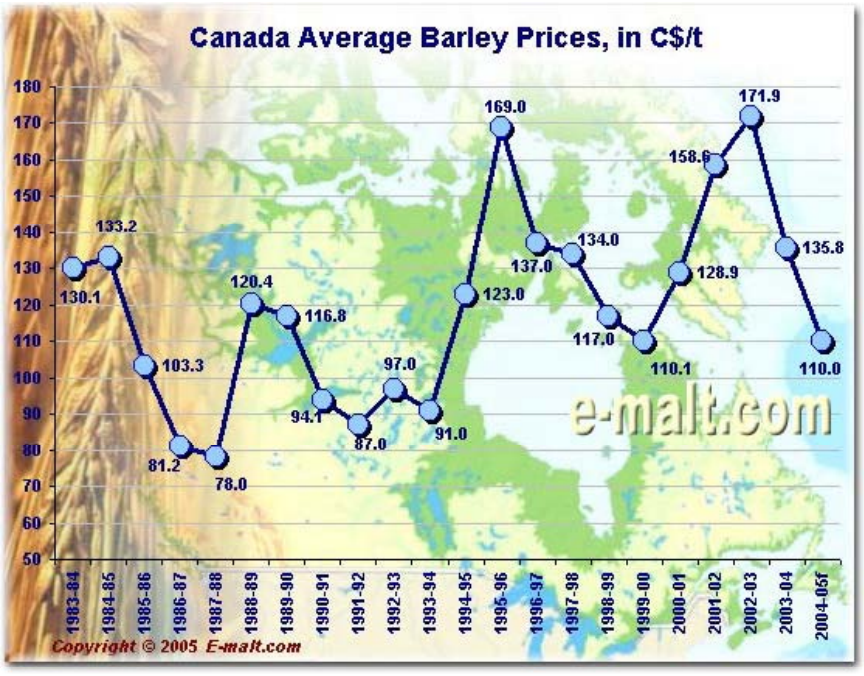
- Selectable average for malting barley in Canada around 2 million tones
- Two-row is taking over, less 6-row
- Six-row mostly goes to the US

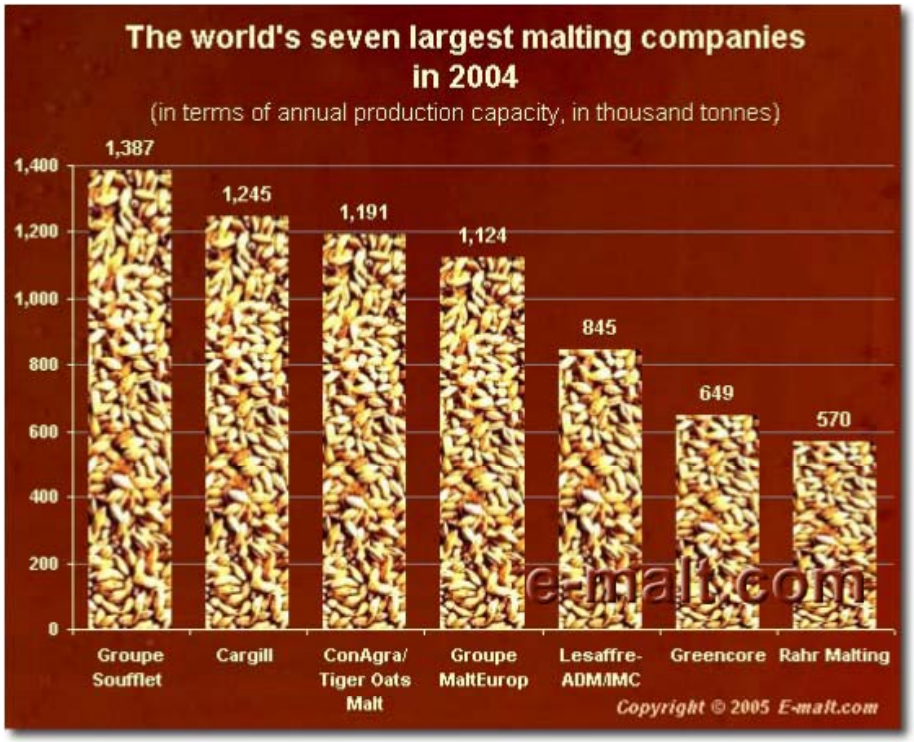


Major Markets for Canadian Malting Barley

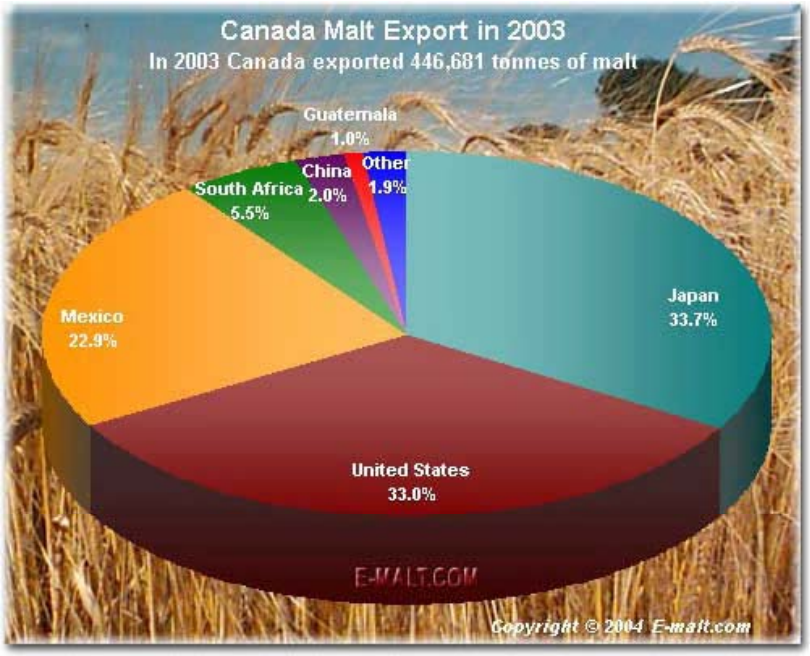
	1997-01	2002-2003	2003-2004	
Canada	1,070	570	981	(90% 2R)
U.S.A.	600	200	420	(58% 6R / 42% 2R)
China	470	43	460	(96% 2R)
S. Africa	20	85	71	(2R Metcalfe)
South America	12	0	124	(2R Metcalfe)
Japan	42	30	22	(B1602 / 2R)
Mexico	51	4	22	(90% 2R)
Total	2,265	932	2,100	







Company	Location	Capacity(MT)
Canada Malting	Calgary, Alberta	260,000
(Conagra)	Thunder Bay, Ontario	130,000
	Montreal, Quebec	80,000
Prairie Malt	Biggar, Saskatchewan	220,000
(Cargill)		
Rahr Malting	Alix, Alberta	140,000
IMC	Winnipeg, Manitoba	92,000
Total		**922,000**



Malting Future Trends

- A search for meaningful analysis
    - Does the current analysis meet the brewers needs?
    - Brewing problems are seldom explained by malt analysis --- the heterogeneity problem
    - Kirin and SAB have specific tests for yeast flocculation
    - Haze prediction?
- No blending to meet specification
- Continuing slow expansion of specialty malts
- Using Biotechnology
    - Speed up breeding cycle
    - Genome mapping – QTL, marker assisted selection
    - New quality characteristics
- More varieties to choose from
- Proprietary varieties
- How many varieties?
- Shorter variety lifespan
- Higher extract - hulless barley, etc.
- Biotechnology solutions to present problems
- A breakthrough GM barley – fusarium resistant?

Industry Future Trends

- Single malt - Single Wort - Single Yeast
- Engineering Solutions to Wort Separation
- Engineering Solutions to Flavour Stability
- Division Between Large and Small Production Units
- Fewer “gimmicks”
- A Functional Food?
    - You are what you eat
    - Beneficial effects of alcohol
    - Antioxidants – polyphenols
    - Medicinal properties of hops

The endoproteinases of barley and malt and their endogenous inhibitors

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Introduction

During the malting and brewing processes, a portion of the barley proteins must be degraded to amino acids and small peptides. Among other problems, if too little protein hydrolysis occurs there will be insufficient low molecular weight nitrogenous compounds in the wort for optimal yeast nourishment. Alternatively, too much hydrolysis will deplete the wort of proteins that are necessary for good beer foam formation, mouth feel, etc. The endoproteinases of barley and malt are the enzymes that initially catalyze the hydrolysis of the insoluble barley storage proteins, and thus play major roles in determining whether or not a given barley variety will be useful for malting and brewing. It is imperative that we understand how, when, and why these enzymes function if new barleys with improved malting quality are to be developed or if malting or brewing methods are to be efficiently altered to produce worts that have improved soluble/ insoluble protein levels. The mixture of proteins, peptides and amino acids that ends up in a wort due to these endoproteolytic activities is termed its 'soluble protein' or 'SP' level.

To understand the protein hydrolysis that occurs during malting and mashing it is necessary to study not only the endoproteinases but also groups of proteins, called proteinase inhibitors, that interact with the proteinases and control their activities. Ungerminated barley contains only relatively small amounts of both endoproteinase enzymes and their protease inhibitors. During the germination process the proteolytic activities increase many fold and the endogenous proteinase inhibitor content increases to a lesser extent.

Barley malt contains multiple representatives of each of the four commonly occurring proteinase types, i.e. those belonging to the aspartic, cysteine, serine and metalloproteinase classes. Early studies on barley and malt indicated that the cysteine and metalloproteinases were probably the main ones responsible for hydrolyzing protein during seed germination and that the grain probably contained endogenous inhibitors that could inhibit the activities of members of both of these enzyme classes. The members of each of the four proteinase types can be specifically inhibited by one of four chemicals. These chemical inhibitors and their target proteases are: E-64 (cysteine proteinases), o-phenanthroline or o-phen (metalloproteinases), pepstatin A (aspartic proteases) and PMSF (serine enzymes).

Enzyme purifications and analyses

Several laboratories have purified and characterized different barley and malt endoproteinases, as listed in Table 1. Of these purified enzymes, some of the cysteine and metalloproteinases were able to hydrolyze hordein protein preparations. The hordeins are the main storage proteins of barley and are presumably the major proteins that need to be hydrolyzed during seed germination and malt mashing. None of the purified aspartic or serine class enzymes hydrolyzed these storage proteins. Using the substrates gelatin and edestin, over 40 proteinase activities were detected using 2-D IEF x PAGE gels.

During these and other purification and characterization studies, nearly all of the enzyme activity analyses were carried out at abnormally low pH values and in the presence of added reducing agents, because these conditions usually yielded maximal activity values. It is now known that these low pH and reducing conditions lead to artificially high cysteine and aspartic proteinase activities and underestimate the serine and metalloproteinase activities. In addition, many of the activity measurements did not yield true initial enzyme reaction rates and

Table 1. Barley/Malt enzymes that have been purified

Enzyme class	# purified, (# distinct enzymes)[1]	hydrolyze hordeins?
Cysteine	5, (3)	Yes
Aspartic	1, plus 4 processing variants	No
Serine	2, (2)	No
Metallo	A group, 3 major and 6 minor forms	Yes

[1] Some enzymes were purified by multiple researchers

often the proteins that were used as substrates were not readily hydrolyzed by members of all of the proteinase classes. For these reasons, many of the endoproteinase activity measurements that have been made with both crude and purified barley/malt endoproteinases have not really been very relevant to what actually occurs during the malting of barley grains and in brewery mashes.

The effects of pH and redox agents on malt proteinases

After it was discovered that the pH values and redox states of mashes strongly affected their proteolytic activities, quantitative studies were carried out to measure how these variables affected the protein solubilization that occurred during mashing (1). Mashing is carried out at about pH 5.9 in North America and the pH inside germinating barley grains is around 4.8, so experimental mashes were carried out at pH values that varied from 5.0 to 6.6. Over this pH range the mash proteolytic activity varied by over 7 fold and the SP levels of the final worts ranged from 4.8% to 7.0%, with the pH 5.9 value being 5.7%, which is a normal value for the varieties tested. This demonstrated several things; 1) that the SP level of a wort can be strongly and easily varied by adjusting the pH of its mash, 2) that any extract or mash proteinase activity measurements that are made at pH values below 5.9 are not relevant to what really happens in a mash and 3) that the rate of protein hydrolysis during malting, at pH 4.8, is probably much slower than what it is during mashing.

The addition of cysteine, a weak reducing agent, to mashes increased their proteolysis rates by over 3 fold and their wort SP levels were raised from 5.5% to 7.3% (1). These effects were negated when oxidizing agents were added to the mashes together with the cysteine, and similar effects were found when stronger reducing agents were added to mashes. This shows that, as with pH adjustments, the presence of redox agents in mashes can strongly shift the SP levels of their worts. Many of the previously measured proteolysis rates, determined in the presence of reducing agents, were probably incorrect. It has been proposed that redox reactions may naturally occur in seeds during germination. If so, that would probably influence the rate at which protein solubilization occurs during both malting and mashing.

How the various malt proteinase classes affect wort soluble protein levels

Based on the early proteinase activity measurements, the dogma had become accepted that the great majority of the protein solubilization that occurred during malting and mashing was due to the cysteine class proteinase activities, with possibly some contribution from the aspartic and metalloproteinases. When the effect of pH and reducing agents on soluble protein levels was noticed, however, it seemed possible that this perceived preponderance of cysteine proteinase activity was an artifact, because these enzymes are the ones that would have been most strongly activated by both the low pH and strongly reducing conditions. The serine and metalloproteinase activities would actually have been reduced under the low pH conditions.

When is the soluble protein of worts released?

In order to ascertain the relative contributions of the malting and mashing steps to the release of SP to worts, barleys and malts were extracted and mashed in the presence and absence of chemical proteinase inhibitors (2). With both Morex (6-rowed) and Harrington (2-rowed) barleys about 32% of the wort SP was already soluble in ungerminated barley grains, 46% was solubilized during malting and the final 22% was released during mashing at pH 6.0. This indicates that while the majority of the protein hydrolysis occurred during malting, almost a quarter of the wort SP was solubilized during mashing, when conditions for altering the proteolytic activities that control its release can be readily regulated.

The contributions of the various endoproteinase classes to wort soluble protein levels

To determine what portion of the final wort SP content was contributed by each of the four protease classes, mashes were carried out in the presence of each of the four class-specific chemical inhibitors (2). The results are shown in Table 2. These show that, under

Table 2. The inhibition of soluble protein formation by class-specific inhibitors during mashing

Protease class	% inhibition[1]			% inhibition, ASBC mash[2]	
	Morex	Harrington	Average	Morex	Harrington
Cysteine[3]	12	12	12	12	11
Aspartic	7	9	8	5	6
Serine	1	4	3	0	3
Metallo	9	14	12	13	16

[1] Average of mashing with 3 malt concentrations.
[2] Average of 3 experiments.
[3] Inhibitors were, respectively, E-64, pepstatin A, PMSF and o-phen.

these standard mashing conditions, the cysteine class proteinases are not the only ones that release SP into the worts. Because of the complexity of these experiments there was substantial variation in the results obtained, but it is obvious that the metalloproteinases released as much SP as the cysteine proteinases, that the aspartic enzymes released SP, but at a slower rate, and that the serine proteases hydrolyzed little or no protein.

These findings correlate fairly well with the results obtained by the researchers who purified and characterized the various barley/malt proteinases. The two enzyme classes whose purified members hydrolyzed barley storage proteins, the cysteine and metalloproteinases, apparently catalyze the majority of the hydrolysis of the storage proteins into SP and the serine proteinases, neither of whose purified forms hydrolyzed hordein preparations, also did not release SP during mashing. On the other hand, even though the one aspartic proteinase that has been studied in depth has only been shown to hydrolyze one non-plant protein, enzymes of this class apparently do hydrolyze proteins during mashing, but at a slower rate than either the cysteine or metalloproteinases. This indicates that there are probably other, still unpurified, aspartic class proteases in malt that carry out this hydrolysis. This hypothesis is strengthened by the fact that electrophoretic studies have demonstrated that several aspartic class proteinases occur in malt. These SP-releasing aspartic protease forms still need to be purified and characterized.

From these recent findings, as well as from the 1970 report that showed that malt contained substantial levels of metalloproteinases, it is obvious that the question of how proteins are solubilized during mashing needs to be reconsidered. The large apparent contribution of the metalloproteinases to the formation of SP shows that these enzymes need to be studied in detail. To date, only a single in-depth study of the barley/malt metalloproteinases has been carried out and very few metalloproteases from any plants have been studied.

Barley and malt proteins that inhibit their endogenous endoproteinases

In the early 1960s it was noted that the addition of unmalted cereal flours to mashes led to worts that would not ferment. This problem was traced to the fact that the worts did not contain enough low molecular weight nitrogenous compounds to support good yeast growth. This low wort nitrogen problem was in turn traced back to the fact that ungerminated wheat and barley both contained compounds that inhibited the abilities of certain of the malt endoproteinases to hydrolyze storage proteins into SP. It was eventually ascertained that these endogenous proteinase inhibitors interfered with the activities of the cysteine class and metalloproteinases.

Metalloproteinase inhibitors. Because the significance of the contribution of the metalloproteinases to SP formation during mashing has only recently been demonstrated, the metalloproteinase inhibitors have scarcely been studied. However, in view of the facts discussed above, which show that these enzymes probably play a major part in SP production, both these proteinases and their endogenous inhibitors obviously deserve to be studied in detail. The malt metalloproteinases have proven to be particularly recalcitrant to purification and characterization and one reason for this could be that they bind to their endogenous inhibitors as soon as extracts are prepared, and are thus rendered inactive.

Cysteine protease inhibitors. Conversely, the inhibitors of the cysteine proteases, the other main enzymes responsible for SP formation during mashing, have been studied in depth and two of them have been purified, identified and characterized in detail (2). Both are proteins that belong to the lipid transfer protein (LTP) family. The major form is apparently a form of LTP1 that has been slightly modified and the other is LTP2. Two other inhibitory fractions have been isolated from barley malt and they also appear to be contain altered forms of LTP1. The amount of the inhibitory LTP1 increases about 3 fold during malting and the protein binds strongly to the cysteine endoproteinases, whose concentrations increase even more strikingly during malting.

The LTP1-enzyme complex is readily broken when heated to 100^{o}C, upon which the enzyme is inactivated and precipitated. The heating does not, however, affect the inhibitory LTP1 molecule, and this characteristic has been used to develop an LTP1-enzyme 'affinity' method for concentrating and partially purifying the endogenous inhibitors of the cysteine and serine proteinases of malt. It has not been possible to dissociate the LTP1- cysteine endoproteinase complex without inactivating the enzymes that are involved.

When added to mashes the purified LTP1 inhibitor strongly inhibited their endoproteolytic activities and lowered the SP content of the resulting worts, so increasing or reducing its concentration in mashes could provide a 'natural' method for altering the SP contents of worts. It is not known whether or not the LTP molecules interact with the endoproteinases inside germinating barley seeds. If so, then altering the natural concentrations of these molecules would presumably also affect the amount of SP that is released during malting. Because the cysteine proteases are very active in producing SP, adjusting the levels of their natural inhibitors should have a significant effect on wort compositions.

Serine proteinase inhibitors. It has been known for many years that barleys and malts both contained a family of proteins that have been called the 'chloroform-methanol', or 'CM', proteins. Because one of these proteins clearly acted as an inhibitor of the bovine serine proteinase called trypsin, these proteins have also been called 'trypsin/α-amylase inhibitors' and it had been proposed that some of them might inhibit the activities of barley serine proteinases. However, none had been shown to affect the barley enzymes. In my laboratory we purified a barley serine proteinase called SEP-1 and showed that barley contained proteins that inhibited its activity. Using the proteinase-inhibitor affinity method discussed above, we purified, isolated and characterized four of the most effective SEP-1 inhibitors and showed that they were all members of the CM protein family. The barley contained additional SEP-1 inhibitors that were apparently less potent and these have not yet been studied.

Because the serine endoproteinases do not appear to directly contribute to the hydrolysis of proteins during mashing, these inhibitors would presumably not affect the SP content of mashes. However, they could affect wort compositions by indirectly controlling the proteolysis that occurs during the germination/malting process, since their true functions in the grain are still unknown.

Summary

Due to the use of inaccurate analytical methods, most of the past dogma that related to the solubilization of proteins during malting and mashing is probably incorrect. The cysteine proteinases do not uniquely control the formation of SP, but share this duty with the aspartic and, to an even greater extent, the metalloproteinases. A good, inclusive study of the malt metalloproteinases needs to be carried out, including a determination of what the metalloproteinase inhibiting compounds of malt are that were reported many years ago. In addition, it always needs to be remembered that it is not simply the presence of the barley/malt endoproteinases that controls the amount, and probably the type, of SP that occurs in worts. By controlling the activities of these enzymes, the various proteinaceous endogenous inhibitors could, and probably do, determine what proteins are really hydrolyzed during mashing and, possibly, during malting.

This report is a greatly condensed version of two papers that are in press/submitted to J. Cereal Sci. (3,4) and any interested parties should consult them when they are published.

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Molecular structure and degradation patterns of endosperm cell walls from barley differing in hardness and beta-glucan and protein contents

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Introduction

The walls surrounding the cells of the starchy endosperm of barley must be effectively degraded during malting if problems with extract yield, wort and beer filtration, and beer clarity are to be avoided. While it is possible to select barley varieties for malting on the basis of low levels of beta-glucans, there is no clear relationship between beta-glucan content and malt quality. Although beta-glucans are the major constituents of the endosperm cell walls, other polysaccharides may also contribute to the overall quality of malting barley. The coexistence of several biopolymers in the cell walls, their spatial organization, and the nature of interactions (cross-linking) among them might contribute to the mechanical strength, permeability, and solubility, and therefore to enzymic susceptibility of cell walls during malting. The influence of composition and properties of the endosperm cell walls on kernel hardness has not been studied in detail, although some relationships between hardness and cell walls have been suggested.

The objectives of this study were (1) to examine compositional and structural differences in endosperm cell wall components derived from barley grains varying in hardness, protein and total beta-glucan contents, (2) to investigate the enzymic degradation of isolated barley endosperm cell walls, and (3) to determine how the differences in composition and morphology of the cell walls influence their solubility, susceptibility to enzymatic hydrolysis, and degradation patterns.

Materials and methods

Three malting barley samples (cv. Metcalfe) were grown in 2003 in 3 different locations in Canada (A: Davidson, SK; B: Hythe, AB; C: Hamiota, MB). Grain hardness was measured with the SKCS 4100 (Perten Instruments Inc., IL). Endosperm cell walls were isolated from a fiber rich fraction obtained by roller milling of pearled barley, followed by pin milling and dry sieving. Wet sieving (with 1% sodium dodecyl sulfate in 70% ethanol), homogenization and sonication were used to purify the endosperm cell wall material (CWM). Monosaccharide and phenolic acid composition was determined by high-performance reverse phase and anion exchange chromatography (HPAEC), respectively (Izydorczyk et al. 1998 and Cyran et al. 2002).

The endosperm cell walls were sequentially extracted with water at 65^{o}C (WE), saturated barium hydroxide (BaE), water (Ba/WE), and 1N sodium hydroxide (NaE) at 25^{o}C. The residue remaining after the sequential extraction was designated RES. The fine structure of beta-glucan was investigated by lichenase digestion and HPAEC (Izydorczyk et al. 1998). Monosaccharide and glycosidic linkage composition was determined by HPLC and GC- MS (Izydorczyk et al., 1998), respectively. Samples were prepared for SEM by mounting them onto aluminum stubs covered with double-sided carbon adhesive discs and allowed to set for 24 h. The mounted

samples were placed in a Hummer VII (Anatech, Ltd.) sputter coater and coated with 50 nm of gold and examined with a JEOL JSM-6400 SEM at 10 KV.

Results and discussion

The endosperm cell walls were obtained from barley samples differing in grain hardness, protein and beta-glucan contents (Table 1). The general morphological features of the inner surface of the walls can be seen in Figure 1. The inner wall surface of sample A appeared deeply pitted with indentations made by small and large starch granules. These indentations were less pronounced in the walls of samples B, and C. Sample C also contained areas with an uneven and folded surface, possibly representing imprints of dense protein matrix rather than starch granules. The thickness of the cell walls ranged from 0.5 to 1.6, 0.5-1.7 and 0.8-2.3 um for samples A, B, and C, respectively.

Table 1. Composition and hardness of barley grains

Sample	Protein %	Starch %	Beta-glucan %	Soluble Beta-glucan %	Arabinoxylans %	Hardness index
A	10.8	61.3	4.2	2.3	5.8	59.25
B	11.8	58.9	4.6	1.7	5.6	68.88
C	17.1	54.8	4.8	2.6	5.4	61.70

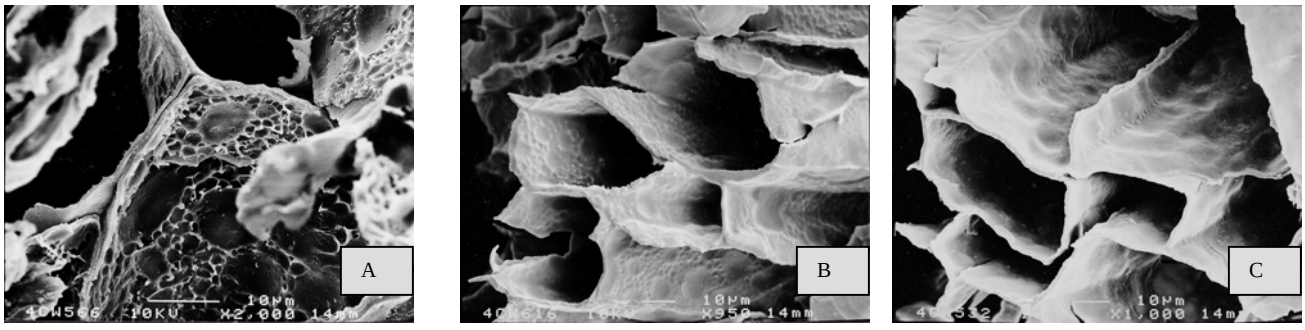

Figure 1. SEM photographs of cell walls isolated from Metcalfe grown in Davidson, SK (A), Hythe, AB (B), and Hamiota, MB (C).

The isolated cell walls contained very little starch (< 1 %) and proteins (~6%) and were built up mainly from glucose, xylose, arabinose, mannose and small amounts of galactose (Fig. 2). The intact endosperm cell walls of sample A contained the least amount of beta-glucans and the highest amount of arabinoxylans and mannose-containing polysaccharides. The walls of sample C contained the highest amount of beta-glucans, in agreement with the highest content of these polymers in the barley grain. The endosperm cell walls of sample A contained the highest amount of phenolic acids (ferulic, coumaric and diferulic), but the arabinoxylans in the walls of sample B were more cross-linked than those in samples A and C (Table 2).

The treatment of the CWM with water at 65^{o}C solubilized mostly beta-glucans, whereas the treatment with saturated barium hydroxide extracted mostly arabinoxylans (Fig. 3). The extract obtained with water after the barium hydroxide treatment contained about 60% beta-glucans and ~35% arabinoxylans. The least soluble extract obtained with NaOH (NaE) and the residue

remaining after all extractions (RES) contained approximately equal parts of beta-glucans, arabinoxylans and mannose-containing polysaccharides.

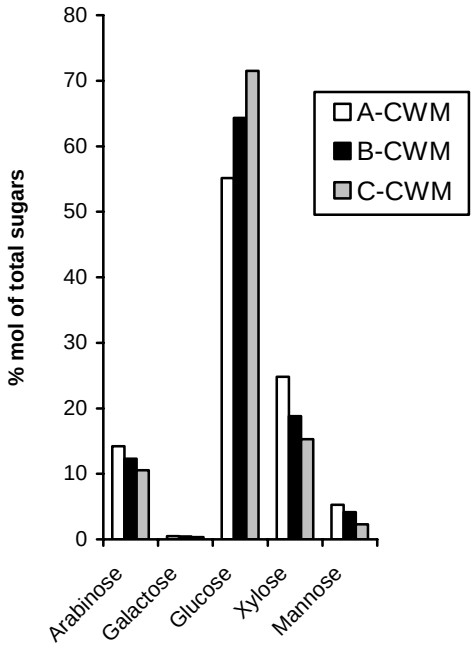

Figure 2. Monosaccharide composition in the CWM.

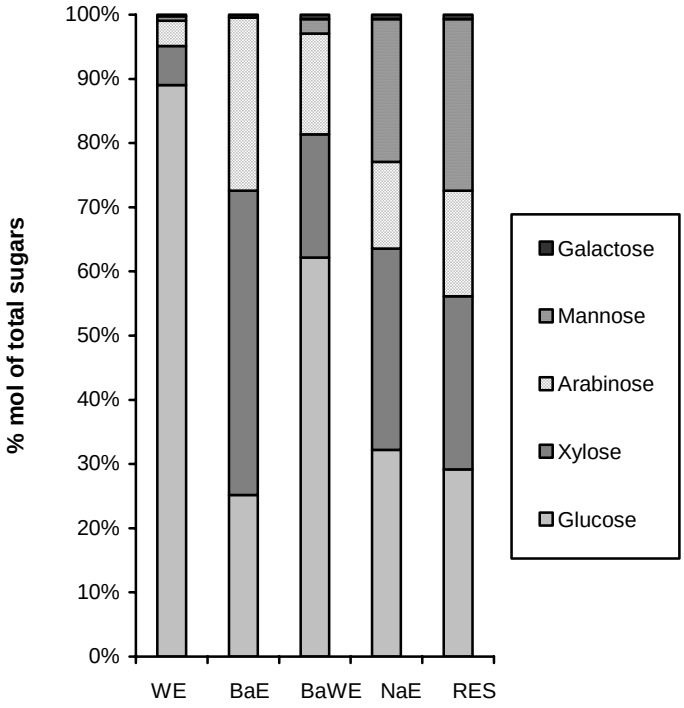

Figure 3. Sugar profile in various extracts

Table 2. Distribution of phenolic acids in CWM

	A	B	C
Total phenolic acids, g/100g CWM	0.34	0.24	0.17
Total FA[a], g / 100g CWM	0.28	0.20	0.15
Total DFA[b], g / 100g CWM	0.0096	0.0092	0.0055
FA/Xyl x 10000[c]	119	128	110
(DFA/Xyl)x10000[d]	2.0	3.0	2.0

[a]ferulic acid; [b]dehydrodiferulic acid; [c]moles of FA per 10,000 moles of Xyl; [d]moles of DFA per 10,000 moles of xylose residues

Following the extraction of the endosperm cell walls with water, the surface indentations due to starch granules could no longer be seen. It appears that the water-soluble beta-glucans may be layered onto the surface of the walls rather than being distributed throughout the wall structure as suggested by Fincher (1975). Following the extraction of arabinoxylans from the water-extracted CWM the definition of endosperm cells disappeared. The NaOH extraction caused further pitting and corrosion of the wall material.

A detailed analysis of oligosaccharides released by lichenase digestion of beta-glucans revealed some differences in the structural features of these polymers among the samples. Beta-glucans in the cell walls of sample B had the highest ratio of 3-O--D-cellobiosyl-D-glucose (DP3) to 3-O--D-cellotriosyl-D-glucose (DP4) and a slightly lower level of oligosaccharides of DP 5-9, representing a more cellulose-like region of the beta-glucans. On the other hand, the DP of longer cellulosic fragments in this sample was higher than in sample A and C. Sample B had the lowest ratio of β-(1→4) to β-(1→3) linkages. Beta-glucans originating from sample C clearly had the greatest amount of glucose residues linked via β-(1→4) linkages, which was confirmed by the highest ratio of β-(1→4) to β-(1→3) linkages and the lowest DP3/DP4 ratio.

Table 3. Structural features of beta-glucans

Sample	Ratio DP3/DP4[a]	DP(5-9)[a] %	Longer cellulosic fragments[b]
A	2.11	7.8	DP 10-24
B	2.28	7.7	DP 10-28
C	2.10	8.2	DP 10-25

[a]found in water-soluble digests from lichenase treatment
[b]found in water-insoluble precipitates released after lichenase treatment

Monosaccharide and glycosidic linkage analyses revealed that arabinoxylans in the cell walls of sample A were less substituted than those in sample B and C (Table 4). Arabinoxylans in the cell walls of sample A had the highest amount of unsubstituted but the smallest amount of doubly substituted xylose residues. The least soluble polysaccharide of the endosperm cell walls, present in the NaE and remaining in the residue, differed substantially from those found in the WE, BaE, and BaWE (Table 5). The linkage analysis revealed the presence of lowly substituted arabinoxylans, beta-glucans with a high ratio of β-(1→4) to β-(1→3) linkages, and the presence of glucomannans and/or(galacto)glucomannans. The highest amounts of lowly substituted arabinoxylans and mannose-containing polysaccharides were found in sample A whereas the highest amount of beta-glucans with cellulose-like features was found in sample C.

Table 4. Structural features of arabinoxylans

Sample	Xyl/Ara[a]	Usub/Sub Xyl[b]	Doubly/Sing Xyl[c]
A	1.74	1.76	0.97
B	1.54	1.46	1.28
C	1.44	1.14	2.43

[a]Ratio of xylose to arabinose residues
[b]Ratio of unsubstituted xylose to substituted Xylp
[c]Ratio of doubly to singly substituted xylose residues

Table 5. Structural features of NaE fraction in CWM of various samples.

	A	B	C
Ratio Unsub/Sub Xylp	2.54	1.98	1.40
Ratio Xyl/Ara	2.8	2.4	2.0
→4 Manp 1→ (%mol)	32	20	12.5
Ratio (1→4)/(1→3) Glc	3.20	3.4	3.9

The enzymic degradation of endosperm cell walls was investigated by treating buffered suspensions of isolated cell wall fragments (previously extracted with water at 45°C) with malt extracts and determining the amount, monosaccharide composition and molecular size distribution of the soluble carbohydrate products. The water solubility of the CWM in 45°C ranged from 24% for sample B, through 27% for sample A, to 41% for sample C. Approximately 20% of the CWM, remaining after the initial water extraction, was solubilized with the malt enzymes. Substantially more beta-glucans than arabinoxylans or mannose-containing polysaccharides were solubilized with water at 45°C (Figure 4). Interestingly, the majority of mannose containing polymers was solubilized during digestion of CWM with the malt enzymes. Almost equal amounts of arabinoxylans (~10%) were solubilized with water and with the malt enzymes. Overall, the solubility and digestibility was lower for sample B than for A and C. Both high- and low-molecular weight (HMW and LMW) materials were released from the CWM during digestion with malt extracts. The monosaccharide analysis revealed the HMW malt digest contained mostly glucose, xylose and arabinose, whereas the LMW malt digest contained glucose and mannose. Differences in the molecular structure of beta glucans and arabinoxylans extracted with water compared to those solubilized with the malt enzymes were also observed.

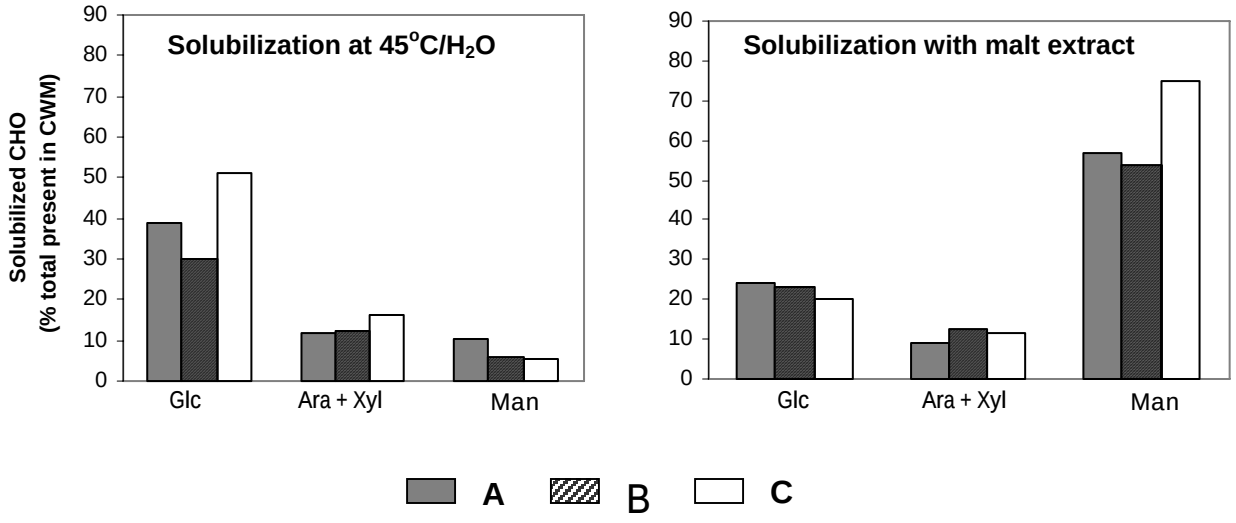

Figure 4. Amount of solubilized carbohydrates during solubilization with water and digestion with malt extract of the CWM.

Acknowledgment

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Amino acid levels in wort and their significance in developing malting barley varieties

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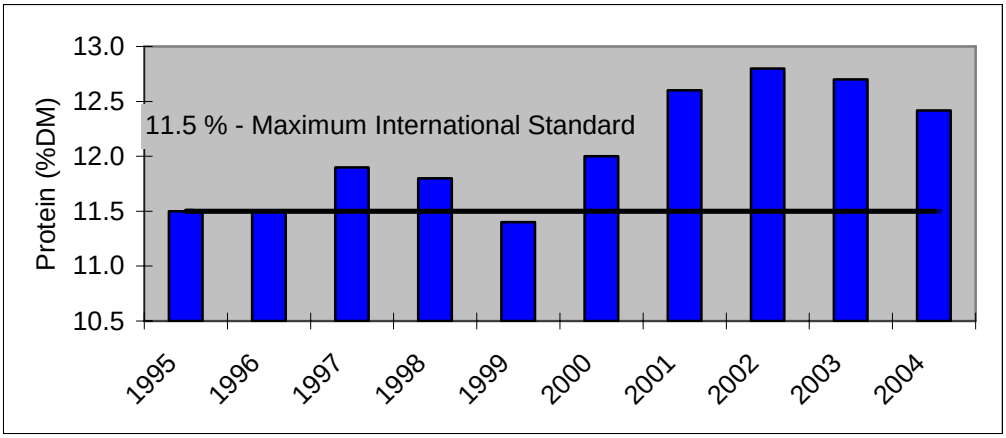
Introduction

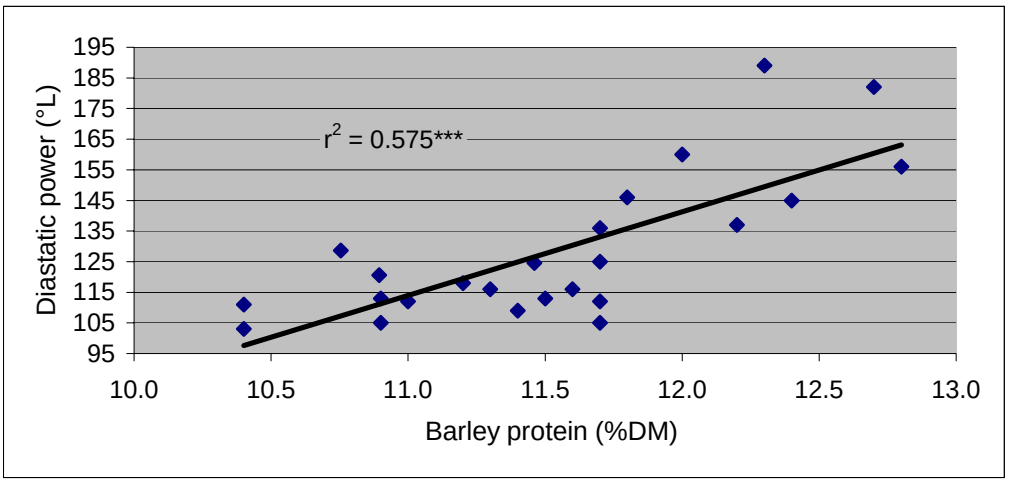
High levels of grain protein are the greatest restriction to increasing the selected malting barley pool in western Canada. International markets traditionally aim for less than 11.5 % protein in malting barley, but barley exported from Canada averaged greater than 12 % over the past 10 years (Fig 1). Higher protein barley is undesirable because of reduced potential fermentable extract which restricts the amount of beer that can be made from a barley's malt. Canadian barley breeders, therefore, are often encouraged to develop varieties with potential for low protein. However, a certain level of barley protein is required to make a quality malt and protein is actually limiting in barley from some parts of the world. Canadian malting barley has a reputation for excellent quality, specifically high fermentability, which is indirectly a result of higher protein barley. Soluble protein in malt, resulting from hydrolysis of barley protein during malting and mashing, contributes to foam retention in the final beer and the amino acids and small peptides resulting from further degradation of the soluble protein, are essential for yeast nutrition during fermentation. Barley protein also provides greater potential to produce adequate levels of starch-degrading enzymes (Fig 2) which are essential for trouble-free fermentations (Evans et al 2003). The higher levels of protein in Canadian malting barley, therefore, contribute to excellent fermentation potential and adequate levels of foam positive proteins but some reduction in protein could still increase the amount of barley selected in western Canada.

Figure 1. Average protein content of 2-rowed malting barley exported from Canada, 1995-2004.



Any initiative to genetically reduce barley protein levels must proceed with caution, especially if extreme changes are expected. Altered barley must still have the potential to provide adequate levels of soluble malt protein for efficient fermentation and adequate beer quality. To ensure adequate protein degradation, breeding programs presently rely on percentage of soluble protein in Congress extract and Kolbach index. These values give some indication of adequate levels of degraded protein for beer foam as well as of nitrogenous nutrients for yeast. Free amino nitrogen (FAN), a measurement of amino acids and peptides, provides more specific information on nitrogenous nutrient status, but is seldom monitored. Fermentability and foam potential are not considered directly in breeding programs.

Figure 2. Diastatic power versus on protein levels in Canadian-grown Harrington barley.



Data source: Langrell & Edney 1995 – 2004

The importance of monitoring free amino acid levels in malts of breeder lines has received limited attention. Levels are seldom even monitored in commercial malts because they are considered to be relatively constant in extracts made from malts of different varieties (Jones & Pierce 1964). These levels are also not considered to be limiting to fermentability in all-malt worts. However, they can be limiting in high-gravity and high-adjunct brewing (O'Connor-Cox & Ingledew 1989) and a recent study found significant relationships between several individual amino acids and fermentability in commercial malts (Yin et al 2004). The present study investigated the importance of monitoring free amino acids, versus other malt quality parameters, for determining fermentation potential in malting barley breeding programs.

Materials and Methods

A doubled haploid population (54 covered and 54 hulless lines) was used to compare levels of free amino acids and indeces of protein degradation to fermentability. The population was produced by anther culture techniques at Agriculture and Agri-Food Canada Brandon from the cross, TR251/HB345. TR251 is a covered breeding line with good malting potential while HB345 is a hulless breeding line with good agronomic traits and the allele for heat stable beta-amylase (sd2H). The 108 lines along with two replicates of each parent, were grown at Hamiota, Manitoba in 2002. Samples of the lines and parents (500 grams) were micromalted in a Phoenix Automated Micromalting machine (Adelaide, SA, Australia) according to the following

schedule: Wet steep 6h, Air rest 2h, Wet steep 4h, Air rest 12h, Wet steep 4h, Air rest 4h, Wet steep 4h, Air rest 4h, Wet steep 4h (steeping at 13°C); Germination 100h (15°C), Kiln 12h @ 55°C, 6h @ 65°C, 2h @ 75°C, 4h @ 85°.

Standard methods of the ASBC (American Society of Brewing Chemists 1992) were used to prepare and analyse fine grind Congress malt extracts. Analysis of free amino acids was based on the method of Garza-Ulloa *et al* (1986) and was performed on a Beckman 7300 High Performance Amino Acid Analyzer (Beckman Coulter, Inc., Fullerton, CA 92834-3100).

A small scale method for measuring apparent attenuation limit (AAL) was used to determine the fermentation properties of the samples. The method incubated 40 ml of EBC wort (European Brewery Convention 1998) with 160 mg dried yeast (Mauribrew lager yeast, Toowoomba, Australia) at 25°C for 24 hours (Logue 1997).

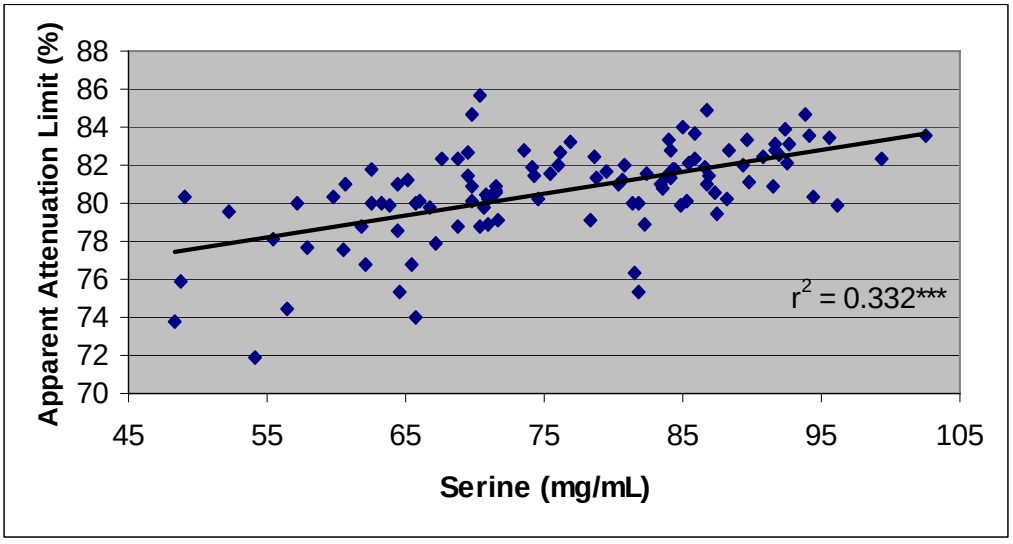
Table 1. Average malt quality of the 108 malt samples studied

	Barley Protein %	Fine Extract %	Soluble Protein %	FAN mg/L	ß-Glucan ppm	DP °L	α-Amylase DU	AAL %	Alcohol v/v%
Average	13.8	82.5	6.33	262	137	185	59.2	80.8	3.5
Maximum	15.8	87.6	7.58	335	466	306	81.1	85.7	3.9
Minimum	12.0	76.1	5.53	202	29	107	21.7	71.9	2.9

Results and Discussion

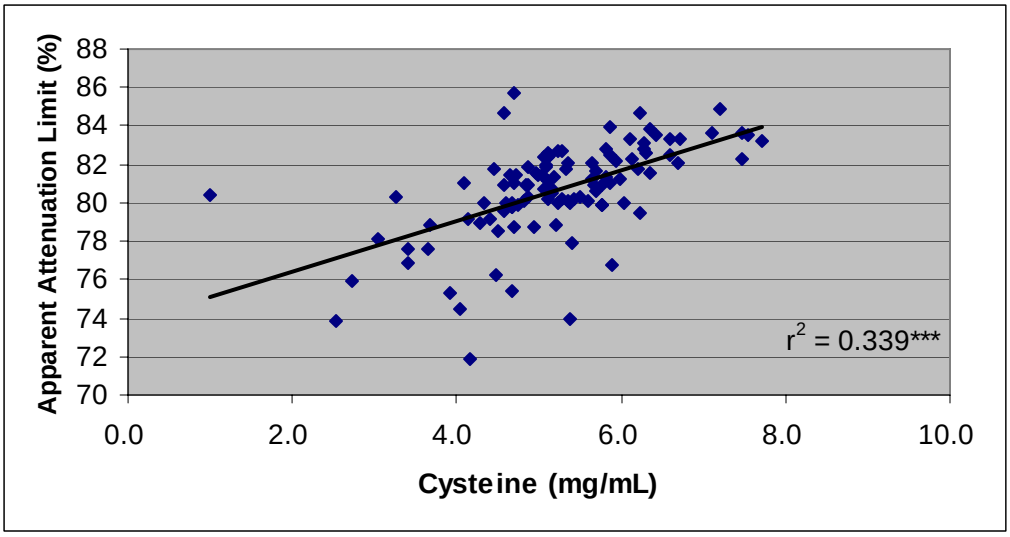
The 108 samples showed a range in quality from very poor to excellent (Table 1) which is similar to what breeders would experience when screening lines for quality in their programs. As a result of the range in quality, fermentability was affected by a number of different malt quality factors, thus, masking, to some extent, direct effects of protein degradation and amino acids on fermentability.

Figure 3. Effect of levels of serine on fermentability of the 108 malt samples studied.



Soluble protein and FAN were found to have insignificant correlations with fermentability (r^2=0.014 and 0.039 respectively). The sum of all individual free amino acid did show a higher correlation coefficient but still insignificant (r^2=0.112). Individual levels of both serine and cysteine were found to correlate highly, significantly with fermentability for the samples tested (Fig 3 & 4). Serine levels also correlated well (r^2=0.441***) with α-amylase levels and levels of both serine and α-amylase were significantly lower in hulless versus covered samples (Edney et al 2004). This suggested that the serine/fermentability correlation was related to α-amylase levels, which are known to affect fermentability, and not serine directly. However, serine has been shown by others (Yin et al 2004) to be related to fermentation which, in combination with the significant cysteine correlation, and to a lesser extent, tryptophan and phenylalanine (r^2=0.186* and 0.174*, respectively), still supported the monitoring of amino acids as an indication of fermentation potential.

Figure 4. Effect of levels of cysteine on fermentability of the 108 malt samples studied.



Conclusions

Percentage of soluble protein in a wort, Kolbach index and FAN levels provided no information on the nitrogenous nutrient status of the worts with respect to fermentability for the breeding population studied. Individual amino acids did explain some of the variability in fermentation, despite masking by other parameters such as levels of enzymes and ß-glucan, suggesting that monitoring of free amino acid could be of benefit. However, the cost of such testing with early generation lines would be prohibitive, although, monitoring at final stages, just prior to commercialization, might be warranted. This would be especially important in altered lines with low levels of barley protein.

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Commercialization of Near Infrared Reflectance Spectroscopy (NIRS) for screening breeding lines in the breeding program and screening grain lots in a malt plant

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Alberta Agriculture Food and Rural Development – Field Crop Development Centre, 5030-50 Street, Lacombe, Alberta T4L 1W8 Canada

Plant breeding is a long-term commitment that puts together the agronomic, disease resistance and quality characteristics into an economically viable cultivar. However, for malt quality, the cost and time required for testing is both expensive and limiting so only a small part of the breeding program can be screened. The malting tests are also destructive and require larger seed samples that are not available in the early generations of a breeding program.

During the commercial malting process the maltster also combines grain lots into the malt house and increases the variability of the grain. A rapid test of the whole grain will allow the variability of the grain to be predicted and controlled before the barley even enters the malt house. This will improve overall quality of the final malt.

The Field Crop Development Centre (FCDC) began a joint project with Canada Malting in Calgary in 1996. The objective was to develop NIRS calibrations for the primary quality factors used by the Malting and Brewing industry to measure malting quality. These included Grain Protein, Fine Extract, Diastatic Power, Alpha-Amylase, Total Malt Protein, Soluble Malt Protein, Wort B-Glucan, Malt Friability, Homogeneity, and Viscosity.

NIRS is an excellent tool to screen large numbers of whole grain samples in a short period of time. For over 20 years the FCDC has used NIRS to screen for feed quality and presently screens over 35,000 samples every year. NIRS is a non-destructive test, requiring as little as 25 grams of seed, allowing the breeder to screen material at a very early stage in the breeding program.

Materials and Methods

The development of the Malting Quality equations were done on breeding samples representing everything from feed barley to the best malting quality available. Samples were selected at the FCDC and scanned on a FOSS 6500 Spectrophotometer. The samples were then sent to Canada Malting. Canada Malting malted these samples in a Phoenix micro malt plant using 150 g of seed. They used a standard cycle in the phoenix plant and the samples were fully modified. Data was sent back to the FCDC to be used for calibration development.

The final research calibration set consisted of approximately two hundred samples per year for five years beginning in 1996. After 2001, samples were added each year in order to strengthen the calibration. In order to insure that we had maximum variation both genetically and environmentally we eliminated redundant samples; therefore, not all samples were used to build the calibration. All the equations developed are based on whole grain, unmalted barley.

The FCDC has successfully used these calibrations to predict malting quality on whole grain samples from the breeding program since 1998. In 2002 be began the process of adding commercial malt samples into the equations. The commercial samples came from three malt houses at Canada Malting in Calgary and from Rahr Malt in Alix, Alberta. The barley samples were taken from lots before malting and the malt analysis came from the same malting company that produced the finished malt.

Results

The commercial malt samples represented a narrow range of variability in the original calibrations. This was expected as these were all malt varieties selected by the malt house. In general the commercial samples contained much more variability within the sample compared to the original research samples. Figures 1, 2, 3 and 4 show the relationship of the commercial malts in the overall calibration sets. By adding them into the calibration set we reduced the accuracy slightly but improved the consistency (Table 1). This translated into slightly different RSQ and Standard Error of Calibration (SEC).

Table 1. Relation between the research calibration based on micro malt data (MM) and the commercial calibration (CM) for the characteristics measured.

Constituent	N	Min Value	Max Value	SEC*	RSQ
Fine Extract - MM	767	73.6	85.5	0.60	0.91
Fine Extract - CM	994	74.0	85.7	0.57	0.91
Diastatic Power - MM	369	68	217	6.72	0.93
Diastatic Power - CM	662	68	224	8.04	0.90
Total Malt Protein - MM	905	6.1	19.0	0.27	0.98
Total Malt Protein - CM	1049	6.6	18.6	0.25	0.98
B-Glucan - MM	262	0	623	32.88	0.93
B-Glucan - CM	495	0	580	34.83	0.91

It is evident to us that the success of the calibrations has been the wide segregating variation we had in the genetic research samples, which allowed us to build upon with commercial samples. Because of the variability in commercial malts which is also increased due to the lot size in the malt house and the variability introduced by both the steeping and killing processes we will see final commercial malts have a greater variance and would expect them to differ from research malts from a pure source. This should not reduce the usefulness of this technology to the malting industry but can only help them to determine how to blend lots to meet optimum quality through there malting process. It also allows breeding programs to screen for malt quality cheaply and quickly at early stages in the breeding program.

Figure 1. NIR Calibration Equation for Total Malt Protein showing FCDC & Conagra Steep Samples.

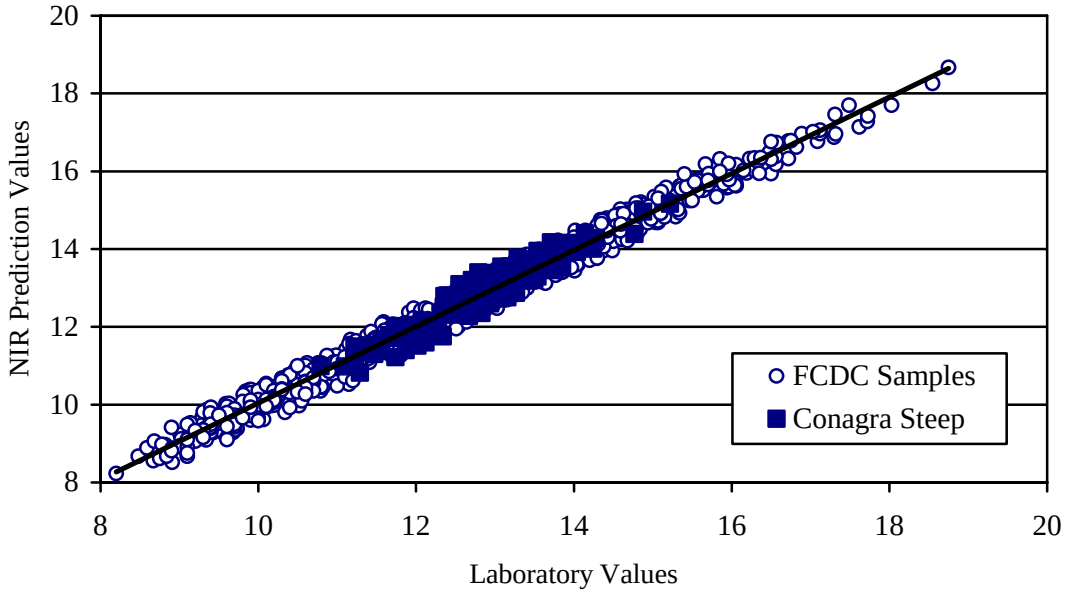


Figure 2. NIR calibration data for Fine Extract showing FCDC samples & Conagra steep samples.

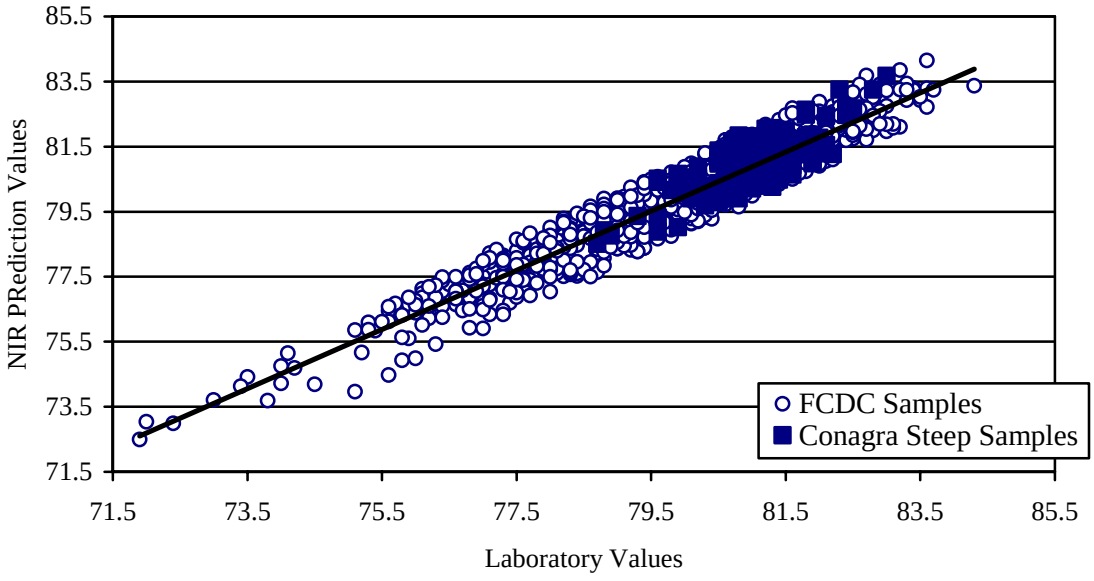


Figure 3. NIR calibration data for Diastatic Power showing FCDC samples and Conagra steep samples.

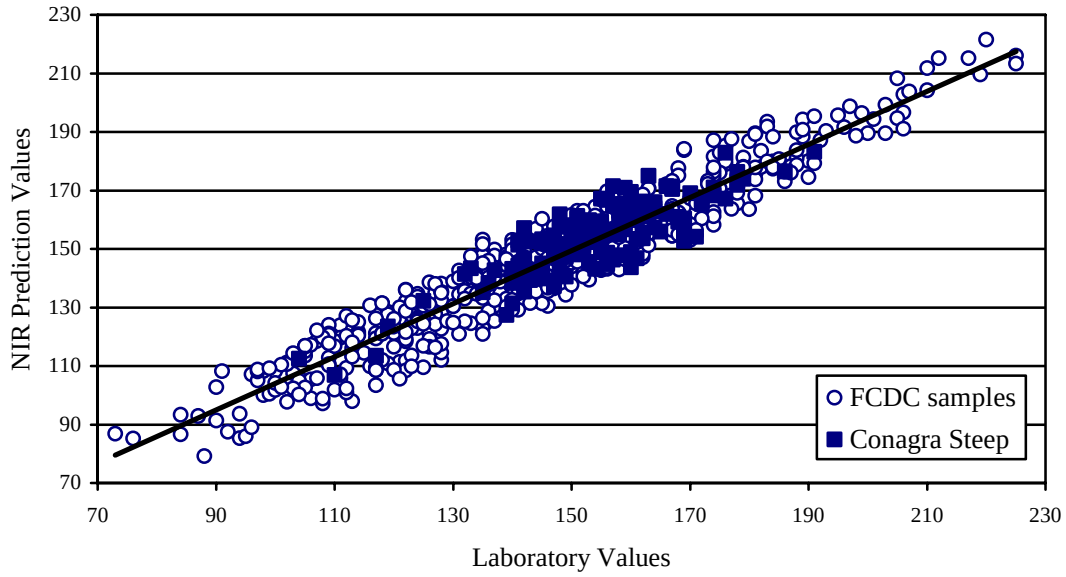
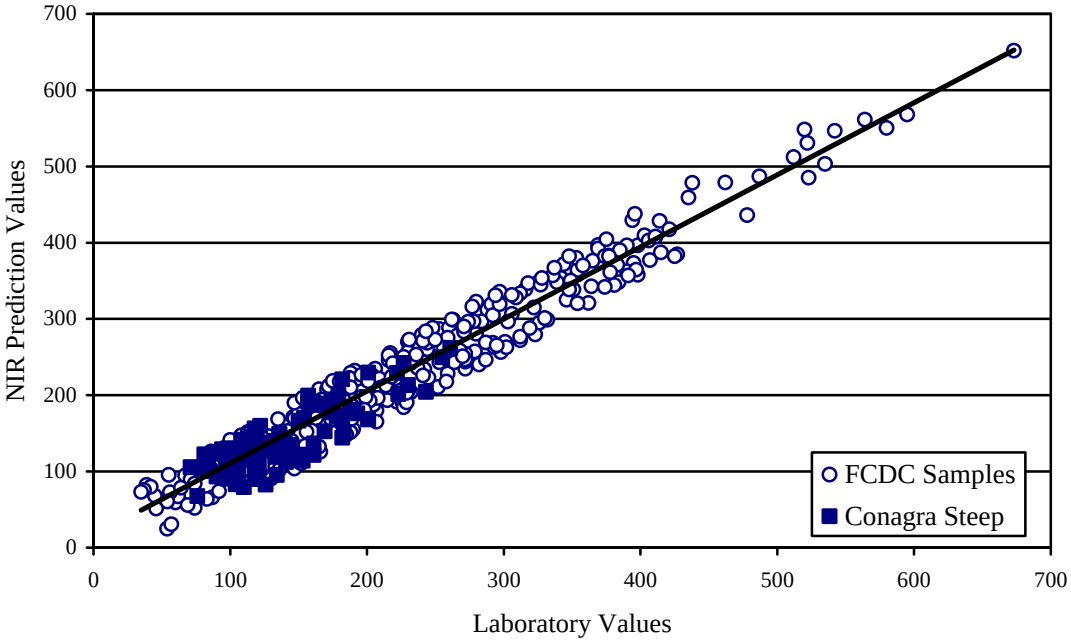


Figure 4. NIR calibration data for Wort B-Glucan showing FCDC samples and Conagra Steep samples.



Tuesday, July 19, 2005 – p.m.

Session 4: BREEDING, AGRONOMY, AND GERMPLASM

Chairs

Joseph Nyachiro and Bill Chapman

Presenters

Dale Clark, Western Plant Breeders
Mike Grenier, Canadian Wheat Board
George Clayton, Agriculture & Agri-Food Canada, Lacombe Research Centre
Anthony Anyia, Alberta Research Council
Mario Therrien, Agriculture & Agri-Food Canada, Brandon Research Centre
Patricia Juskiw, Field Crop Development Centre

Barley ecology and management

Clayton, G.W.[1], O'Donovan, J.T.[2], Irvine, R.B.[3], Harker, K.N.[1], Turkington, T.K.[1] Lupwayi, N.Z.[2], and McKenzie, R.H.[4]

Agriculture and Agri-Food Canada, [1]Lacombe, [2]Beaverlodge, Alberta, [3]Brandon, Manitoba, Canada; and
[4]Alberta Agriculture, Food and Rural Development, Lethbridge, AB

Background and Objectives

Short-term experiments can lead to low input management recommendations that are profitable in the short-term, however, it is doubtful if such practices would continue to be profitable due to carryover effects of uncontrolled weeds and diseases. Many cow/calf producers in central Alberta plant continuous barley for silage and seed, limiting agro-ecosystem diversity and favouring pest outbreaks. The objective of this experiment was to determine the cumulative effects of seeding rate, barley type, rotations and herbicide rate on wild oat management and disease. Barley variety/seeding rate/herbicide rate combinations were seeded in 2003 on previous barley (2001) and canola (2002) stubble to evaluate the rotational and seeding rate impact on reducing input costs and minimizing the risk associated with disease pressure under continuous barley situations.

Methods

Field experiments were conducted at Beaverlodge, Fort Vermilion and Lacombe, Alberta and Brandon, Manitoba in 2001, 2002 and 2003. Two barley varieties, Peregrine (short) and AC Bacon (tall), were seeded at 200 and 400 seeds per m^2 in the continuous barley each year. Barley seeded at 100 seeds per m^2 is equivalent to approximately ¾ bushel, however, this can change every year depending on barley type, variety and seed weight. In addition, hulled barley emerges at approximately 75% of seeds planted, whereas, hull-less barley emerges at approximately 60% of seeds planted. Planting by number of seeds per ft^2 or m^2 ensures the targeted plant establishment. Rotational barley included a canola crop that replaced the short and tall barley in 2002. Herbicide treatments were applied at full, half (H) and quarter (Q) recommended rate. Results from trials at Beaverlodge and Fort Vermilion were similar in nature to those in Lacombe. Only Lacombe and Brandon results are shown.

Results

Lacombe

Weed Biomass and Grain yield

Continuous barley had wild oat biomass that was 2.5 times that of rotational barley. Tall barley with a high seeding rate and a quarter of the recommended herbicide rate significantly reduced wild oat biomass compared to short barley with a low seeding rate and a quarter of the recommended herbicide rate in continuous and rotational barley (Fig. 1a). Wild oat biomass was increased 11 times and 23 times when short barley was grown compared to tall barley at half and a quarter of the recommended herbicide rate, respectively. Grain yield was 8% higher from rotational barley than continuous barley (Fig. 1b). Grain yield increased with the high seeding rate compared to the low seeding rate for both the short and tall barley.

Brandon

Weed Biomass and Grain yield

Tall barley with a high seeding rate and half the recommended herbicide rate significantly reduced wild oat biomass compared to short barley with a low seeding rate and half the recommended herbicide rate. Only tall barley and high seeding rate reduced wild oat biomass at the quarter herbicide rate compared to the other treatments (Barley*seed rate* herbicide rate, $P<0.02$) (Fig. 2a). Wild oat biomass was increased 5.2 times and 1.7 times when short barley was grown compared to tall barley at the half and a quarter of the recommended herbicide rate, respectively. Grain yield was 13% higher from rotational barley than continuous barley, 65% higher from a tall barley than a short barley, and 24% higher when seeding at 400 seeds per m^2 than 200 seeds per m^2 (Fig. 2b). Reducing the herbicide rate reduced grain yield but yield loss was recoverable using half the recommended herbicide rate when combined with the higher seeding rate for both short and tall barley (data not shown).

Disease

Overall, the levels of scald, net blotch and other leaf spots observed at Lacombe and Brandon in 2003 had limited influence on barley productivity. Nevertheless differences among treatments, especially for net blotch, indicate that disease risk can be reduced through crop rotation and cultivar choice. Potential disease increases due to increasing seeding rate can also be countered through the use of crop rotation and/or cultivar choice.

Summary

- Cultivar selection, seeding rate and rotational management provide ecological approaches to managing weeds and disease.
- Integrating a competitive barley type and a high seeding rate is an ecological tool for weed management in monoculture or rotation.
- Pest management strategies are not always compatible. The positive weed management strategies of higher seeding rate had a minor negative effect on disease management.
- Grain yield was positively affected by ecological crop management.
- Continuation of this study will help to explain the relationship between disease pressure (i.e. crop health) and weed management in an integrated crop management (ICM) system.

Acknowledgments

The Alberta Barley Commission and AAFC MII provided financial support. We are grateful to L. Michielsen, B. Pocock, D. Orr, J. Drabble, G. Semach, J. Unruh, and Richard Pacholok for their excellent technical support.

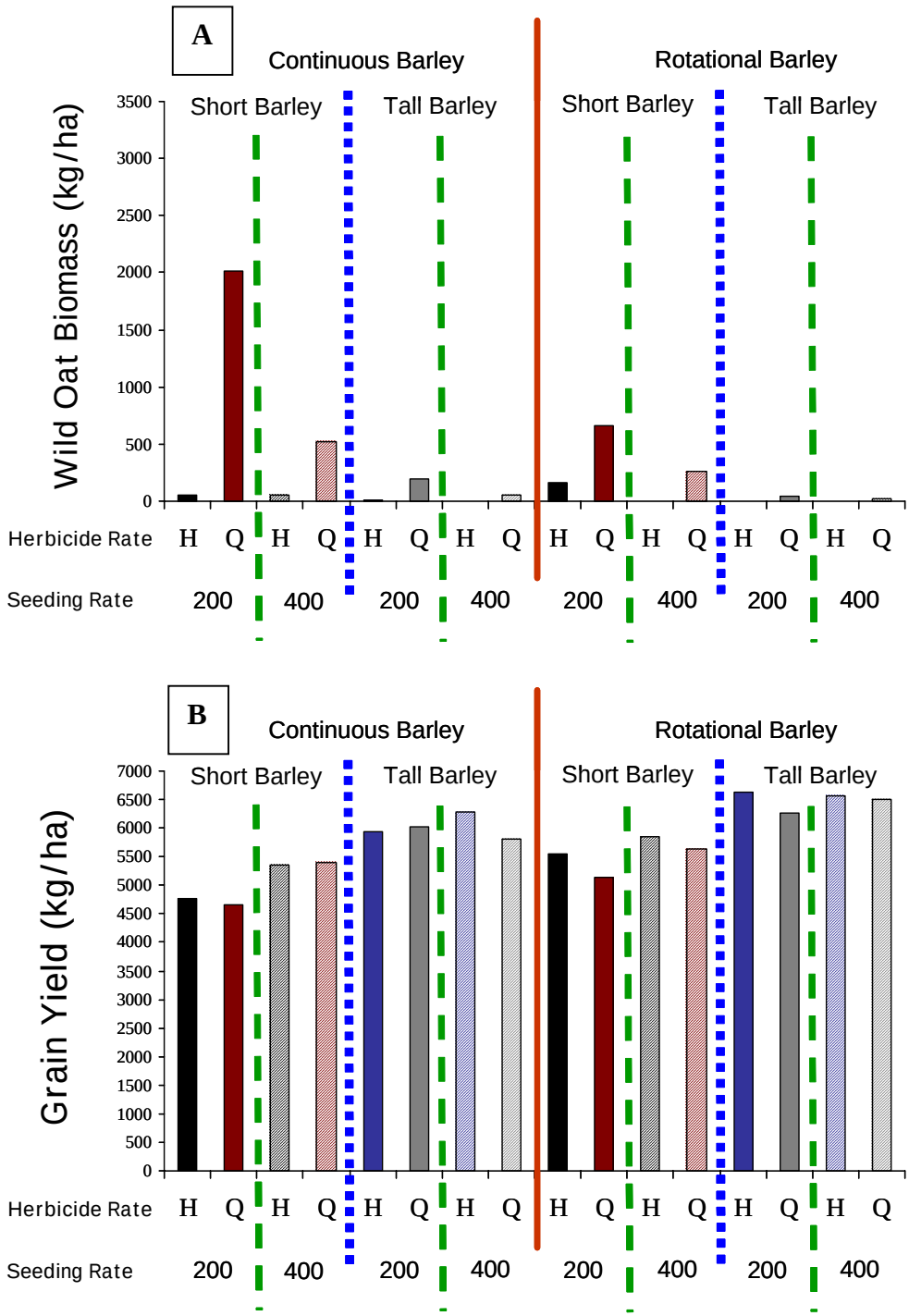


Fig.1. Relationship between barley rotation (barley/barley), barley type (short vs tall), herbicide rate (H=50% recommended and Q = 25% recommended) and seeding rate (200 and 400 seeds planted per $m^{2)}$ on (a) wild oat biomass (kg/ha – 1000 kg = ½ ton/acre) and (b) grain yield (kg/ha - 1000 kg = ~20 bushels) in 2003 at Lacombe, Alberta.

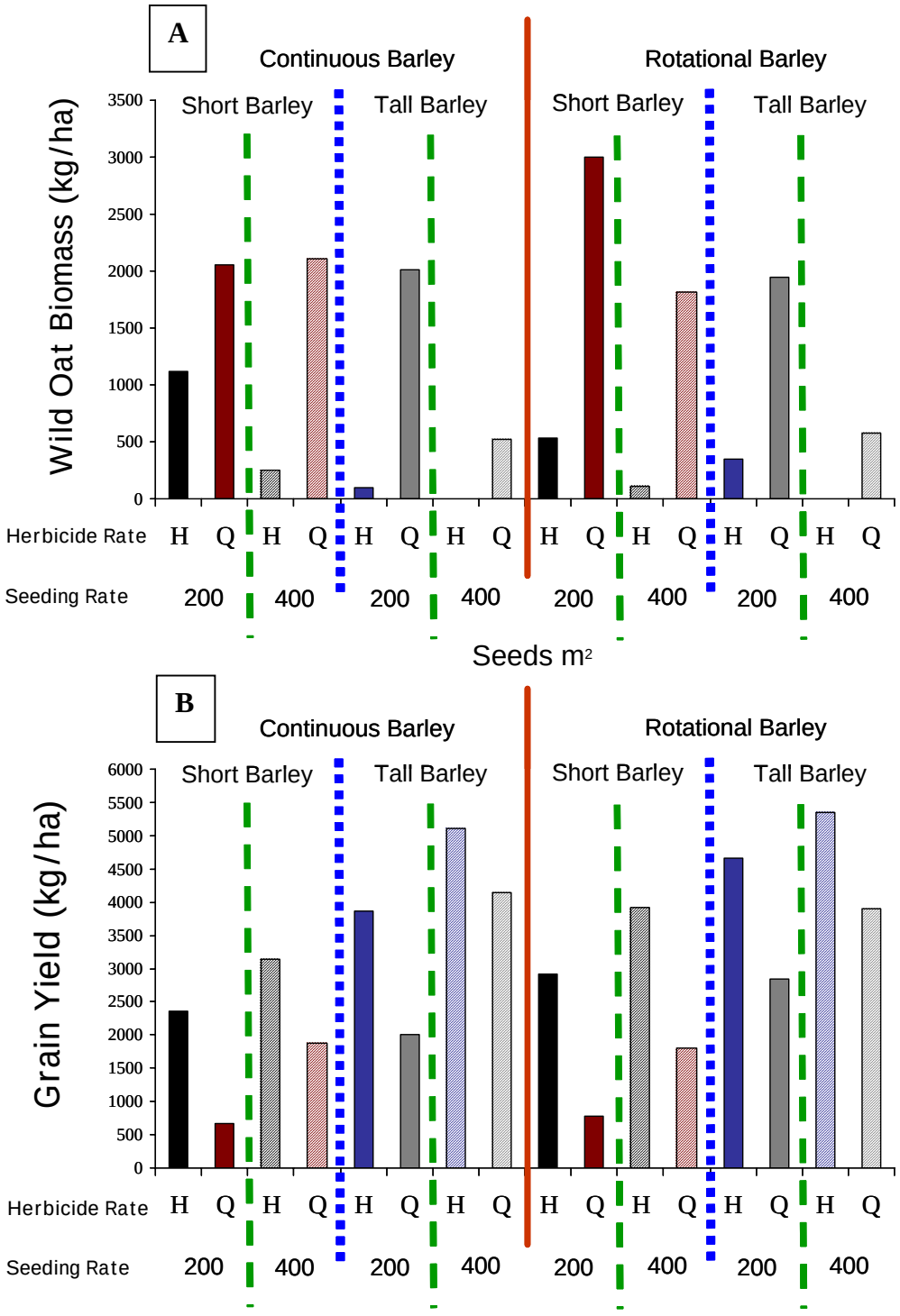


Fig.2. Relationship between barley rotation (barley/barley), barley type (short vs tall), herbicide rate (H=50% recommended rate and Q = 25% recommended rate) and seeding rate (200 and 400 seeds planted per $m^{2)}$ on (a) wild oat biomass (kg/ha – 1000 kg = ½ ton/acre) and (b) grain yield (kg/ha - 1000 kg = ~20 bushels) in 2003 at Brandon, Manitoba.

Carbon isotope discrimination as a selection criterion for improved water use efficiency and productivity of barley on the prairies

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Abstract

This study was done to evaluate the application of carbon isotope (^{13}C) discrimination (Δ) as a selection criterion for improving water use efficiency (WUE) and productivity of barley on the Canadian prairies. Ten genotypes were subjected to drought at the jointing stage to study the relationship between Δ, WUE and barley productivity. Drought caused considerable reductions in aerial biomass and grain yield of all genotypes examined. Significant genotypic variation was found in WUE. Significant correlations were found between Δ, and WUE as well as Δ and aerial biomass and grain productivity, which highlight the potential of Δ (leaves or seeds) as a rapid and reliable method for evaluating WUE and productivity of barley. Genotypes (Manny, Trochu and Seebe) with the highest WUE (low Δ) under drought conditions showed performance comparable to the genotypic average under well-watered conditions. This suggests the potential for improving WUE under drought conditions without yield penalties when conditions are optimum. More research is needed to test this technique under field conditions and to establish a standard protocol that can be used to develop new, improved, water use efficient barley varieties.

Introduction

When pests and diseases are effectively controlled, moisture stress is the major limitation of crop yield across the Canadian prairies. Producers often rely on varieties selected for high yield that are adapted to several environments. Yields, however, do vary within and between locations and years reflecting differences in seasonal distribution and severity of water deficit. In water-limited environments, crop yield is a function of water use, water use efficiency (WUE) and the harvest index (Passioura, 1977). Water use efficiency or water productivity is defined as aerial biomass yield/water use. Crop management or the behavior of various cultivars due to intrinsic differences can influence water use efficiency. Water use efficiency is a trait that has been proposed as a criterion for yield improvement under drought (Rebetzke et al., 2002, Condon and Richard 1992). Breeding for improved WUE has, however been limited for a long time by lack of screening methodology. Farquhar et al. (1982) found that the extent to which C_3 plants discriminate against the carbon isotope ^{13}C during carbon assimilation was related to their water use efficiency.

This study was done to evaluate the use of ^{13}C discrimination as a selection tool for identifying water use efficient and drought tolerant barley.

Materials and Methods

Six 6-row and four 2-row barley genotypes were used for the study. The 6-row genotypes were: AC-Lacombe, Kasota, Manny, Trochu, Tyto and Vivar. The 2-row cultivars were: CDC Dolly, Niobe, Ponoka and Seebe.

The experiment was performed in a greenhouse with photoperiod of 16 hours using natural light supplemented with sodium halide light bulbs. Day and night temperature ranged from 20 to 32°C and 14 to 20°C, respectively while relative humidity was from 10 to 70% throughout the experiments. Large pots (30cm tall by 27 cm diameter) were used for the study. The pots were filled with 8 kg of soil mix containing field soil and peatmoss in a 1:3 ratio. All pots were flushed with 4 L of tap water and allowed to drain for two days before seeding. Tensiometers (Irrometer) were installed in selected reference pots to monitore soil water potential. The 10 barley genotypes were compared under two irrigation treatments which were either well-watered (WW) or water stressed (WS). Six seeds of each genotype were planted per pot, which were thinned to 4 seedlings per pot two weeks after emergence. Fertilizer application was done 3 weeks after seeding at 112 kgN/ha, 39 kgP_2O_5/ha, 85 kgK_2O/ha and 13 kgS/ha equivalents. Each genotype was replicated 4 times and all pots were completely randomised.Water stress (drought) was imposed at the jointing stage by withholding irrigation until the soil moisture content was approximately 10 volume% compared to 30 volume% of the well watered treatments. These moisture levels were then maintained until grain maturity. A 2cm layer of perlite was put on each pot to reduce surface evaporation. Water use was monitored by weighing the pots regularly and replacing the amount of water lost.

At the heading stage, leaf laminas of plants of each genotype were harvested and dried at 70°C for 48 hours. Dried samples were ground to pass a 1-mm sieve and the carbon isotope composition of each cultivar was determined by mass spectrometry. At maturity, plants were harvested and aerial biomass, grain yield and its components were assessed. Seeds of each genotype were sampled and processed for determination of carbon isotope composition. Data were analyzed using SAS, version 10.0 (SAS Institute, Cary, NC) software. A linear correlation analysis was used to examine the mean genotypic relationships between traits using the CORR procedure.

Results and Discussion

Among the 6-row barley genotypes, significant differences were observed within each irrigation treatment in WUE and ^{13}C discrimination. For Δ-seeds and Δ-leaves, extreme cultivars differed by 1.72 and 1.91, respectively, under drought and by 1.61 and 1.22, respectively, under well-watered conditions (data not shown). Among the 2-row genotypes, no significant differences were found in WUE, but ^{13}C discrimination (Δ-seeds and Δ-leaves) was significantly different under both watering conditions. For Δ-seeds and Δ-leaves, extreme cultivars differed by 2.35 and 1.27, respectively, under drought and by 0.94 and 1.15, respectively under well-watered conditions (data not shown).

Among the 6-row barley genotypes, WUE_{DM} was strongly correlated with both Δ-seeds and Δ-leaves under drought (Fig. 1 & 2). Aerial dry matter production (DM) and grain yield were also strongly correlated with Δ-seeds (Figs. 3 & 4). Similar correlations were observed among the 2-row barley cultivars under drought, except grain yield and Δ-seeds, which showed no relationship (Figures not shown).

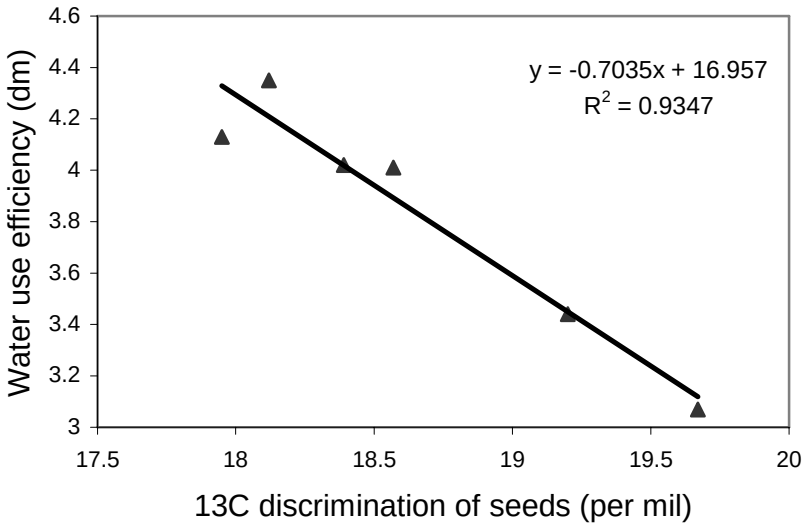

Figure 1: Relationship between ^{13}C discrimination of seeds and WUE (based on dry matter/water use) of 6-row barley under water stress.

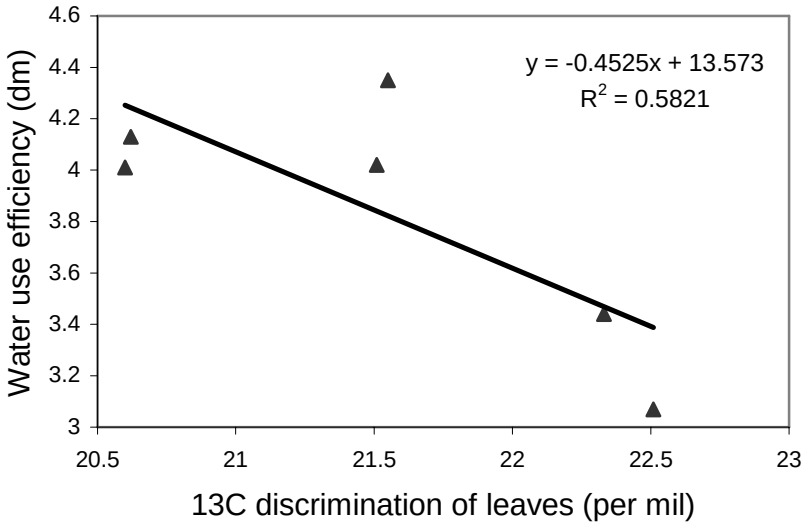

Figure 2: Relationship between ^{13}C discrimination of leaves and WUE (based on dry matter/water use) of 6-row barley under water stress

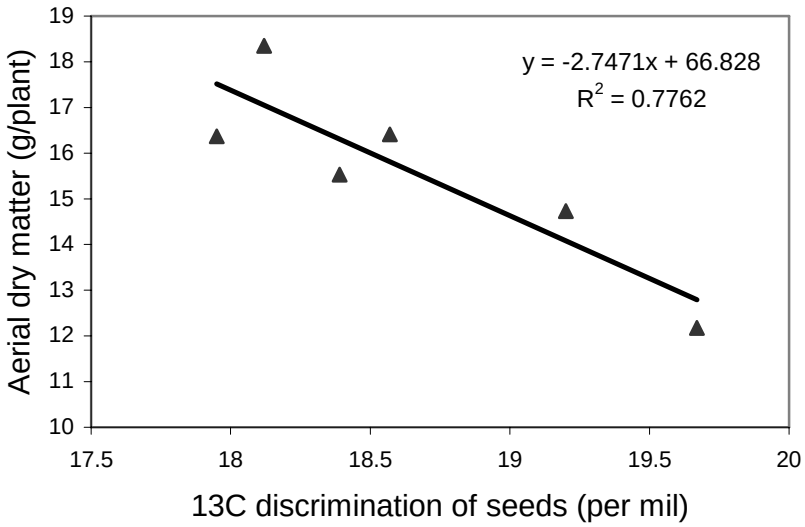

Figure 3: Relationship between ^{13}C discrimination of seeds and aerial dry matter of 6-row barley under water stress

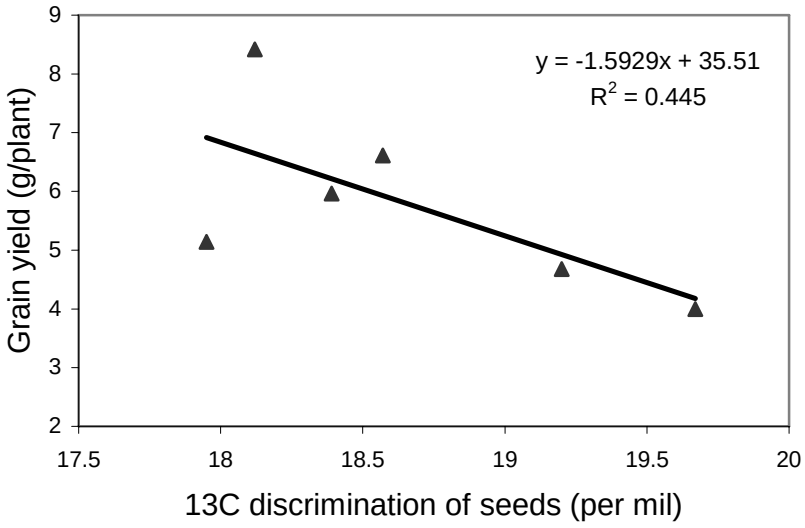

Figure 4: Relationship between ^{13}C discrimination of seeds and grain yield of 6-row barley under water stress

The relationship between Δ and WUE have been studied extensively in several species and the 2 traits have been reported to be negatively associated (Farquhar and Richards 1984, Condon et al., 1990, Read et al., 1991, Ebdon et al., 1998, Teulat et al., 2001, Rebetzke et al., 2002). High correlations have been reported between Δ and aerial biomass or grain yield (Johnson and Bassett, 1991; Acevedo, 1993; Condon and Richards, 1993; Teulat , 2001).

Variation in Δ in cereals is known to arise from variation in photosynthetic capacity as well as stomatal conductance (Condon et al., 1990; Morgan and LeCain, 1991). Some studies have shown that when stomatal conductance is the main source of variation in WUE and when water supply does not impose a major limitation on crop growth, a high WUE may be disadvantageous (Condon et al., 2002). A review by Condon et al., (2002) suggests that improved WUE may be useful in stored-moisture environments where within-season rainfall makes up a relatively small proportion of the total water available for growth.

Results obtained in the present study indicate that significant variation exists in WUE amongst the barley genotypes examined. The strong correlations between Δ and aerial biomass highlight the potential of Δ as a measure of productivity in barley subjected to drought in a greenhouse. There is a need to screen more genotypes and to verify the usefulness of Δ (leaves or seeds) in breeding programs under field conditions.

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Twenty-five years of male-sterile-facilitated recurrent selection in barley

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Using a phenotypic marker-assisted system developed by Falk and Kasha (1982), a Male-Sterile Facilitated Recurrent Selection (MSFRS) program was initiated at the Agriculture and Agri-Food Canada research facility in Brandon, Manitoba, in 1980. The purpose was to develop germplasm that would accumulate additive genetic effects for beneficial traits, including (horizontal) disease resistance and improved grain and biomass yield. After 25 cycles of recurrent selection, involving multiple genotypes from global sources, 4 populations have been developed demonstrating improved levels of disease resistance, to multiple pathogens that are prevalent in the Northern Great Plains, when compared to conventionally bred genotypes. As well, one six-row forage cultivar has been developed, with high biomass production and grazing tolerance, as well as several elite lines showing promise as future forage cultivars. Several hulless lines have also been produced showing markedly reduced levels of deoxynivalenol (DON) mycotoxin, incited by the pathogen *Fusarium graminearum.* The MSFRS program is a low-cost approach to long-term germplasm enhancement that can be applied to direct development of forage barley as well as parental lines demonstrating multiple disease resistance in a single background.

Introduction

In the late 1960's, a number of researchers around the world began to investigate the possibility of introgressing numerous traits in barley using naturally occurring genic male sterility from several sources. Under certain conditions, barley could be made to out-cross in the field in a manner similar to that of rye (*Secale cereale* L.). Hockett and Eslick (1970) observed that enough out-crossing could take place, in barley, to provide for a useful tool in developing Composite Cross populations for use in germplasm improvement and, possibly, direct cultivar development. This approach would be advantageous over conventional crossing by virtue of being able to generate potentially large quantities of hybrid seed, under the right conditions, without much labour input and allow for relatively rapid production of multi-way crosses. In North America, multi-way crosses, for agronomically useful traits, were successfully produced, in the form of Male-Sterile-Derived Composite Cross populations (Ahokas and Hockett, 1981). To ensure that hybrid seed could be easily identified, the male-sterility genes were linked to visual genetic markers, including orange lemma and shrunken endosperm characters (Falk and Kasha, 1982). These developments allowed for practical recurrent selection breeding in the field.

Materials and Methods

In 1980, hybrid seed was obtained from Dr. E.F. Hockett, Montana State University that was produced from a long-standing (20 yr) composite cross population (CC XXXIII) containing the *msg6* male sterile gene. At the same time, elite germplasm containing the male-sterile *msg6*, and the linked genes for orange lemma (*o*) and shrunken endosperm (*sex*), were obtained from Dr. D.E. Falk, Guelph University. Controlled crosses were made in the greenhouse in 1980 and 1981 involving male-sterile and male fertile plants between both the CC XXXIII and marker populations. Marker-tagged male sterile progeny, from this initial cross, were then crossed to 102 elite barley lines varying widely in genetic background and agronomic performance. Each set of

crosses was maintained separately, in isolation from other barley, in the field and allowed to hybridize freely over 3 successive seasons, from 1982 to 1984. This initial work was conducted by Dr. R.B. Irvine, who preceded the author as breeder at the Brandon Research Centre.

From 1985 to 1990, elite lines were introduced to each successful field population with the aim of increasing the level of hybrid seed production in the field and reducing the incident of ergot (*Claviceps purpurea*). Initial hybrid seed set was <1% and incidence of ergot exceeded 10% of all male sterile spikes. By 1990, hybrid seed set was > 5%, on average, and ergot infection was approximately 1% of male sterile spikes. Of the original 102 populations, only four populations were selected that could consistently produce a relatively high level of hybrid seed and acceptably low levels of ergot infection, in an agronomically acceptable background.

Commencing in 1991, cultivars and elite lines were used as recurrent male parents in a MSFRS program using each of the four marker-assisted male sterile populations. After 4 cycles of recurrent selection, male fertile offspring were then placed in head row selection nurseries and selected on phenotype and then evaluated as lines in replicated field trials. During recurrent selection, offspring were subjected natural field pathogens which exerted moderate selection pressure for resistance to multiple pathogens in an environment favouring the development of multiple pathogens. The local environment also favoured selection against lodging in most years.

Results and Discussion

It was noted, since early in the production of lines from the MSFRS populations, that the material tended to be late, seed production tended to be sub-standard, but an abundance in biomass. This lent itself to development of forage type barley for use mainly in silage. Our program also produced forage lines from conventional crosses, in parallel. We then evaluated performance of MSFRS forage lines against conventionally bred lines to determine what advantages, if any, the MSFRS approach would have over the (more labour-intensive) conventional ear-to-row pedigree method.

We first examined level of disease resistance of at least 500 lines from each of the two breeding methods, for resistance to a number of diseases. Table 1 compares the standard (Std) population vs. the Male-Sterile Derived (MSD) population for general leaf diseases in the field (LeafDis), Net Blotch (Nblot) and Spot Blotch (Sblot) in disease nurseries, Fusarium Head Blight (FHB), and the associated mycotoxin deoxynivalenol (DON), in the FHB nursery, Common Root Rot (CRR) in a field nursery, as well as two races of Net Blotch (Net857 and Net858), one race of Scald (Scld1493), and QCCJ Stem Rust (Stemrst), from laboratory inoculation.

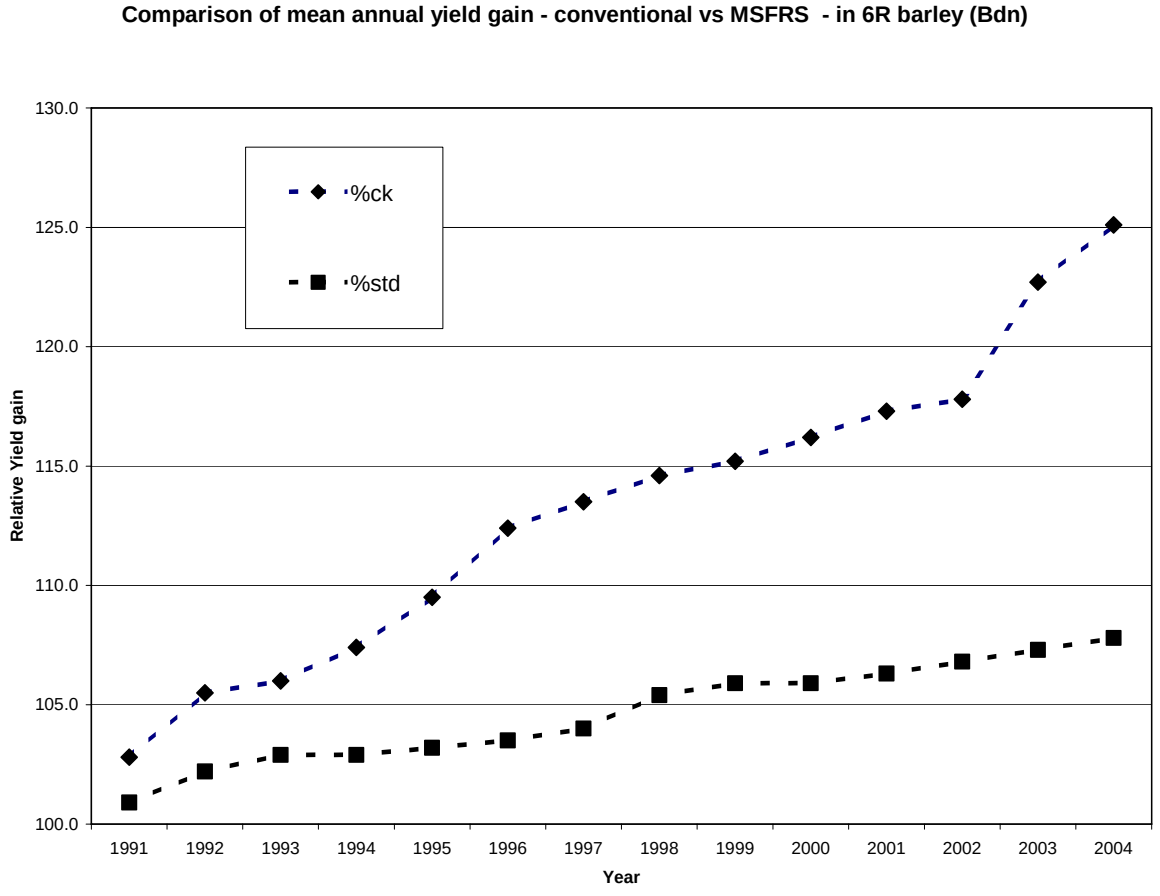
Table 1.
Comparison of disease reactions of approx. 500 standard cross vs. MSD over 4 years (2001-2004):

Entry	LeafDis	Nblot	Sblot	FHB	DON	CRR	Net857	Net858	Scld1493	Stemrst
Std	5.5	4.0	2.7	3.7	29.1	62.4	4.5	8.2	8.8	7.0
MSD	4.8	3.6	2.3	3.4	24.7	61.5	4.4	7.6	8.8	6.4

Results show that, except for Scald, the MSD population consistently had lower disease incidence scores than Std populations selected in the same environments. In the case of FHB, several hulless selections demonstrated very low DON levels that may be released as cultivars in the near future (data not shown), based on their DON levels and overall agronomic performance. Thus, breeding for disease resistance using MSFRS can be advantageous over conventional approaches for some major diseases in barley.

The second advantage for MSFRS is in the development of high biomass forage barley. Figure 1 compares yield gain, vs. the check variety Virden, of conventional vs. MSFRS barley lines over 14 years of testing. By 2004, conventionally bred forage lines produced a yield advantage, over Virden, of approx. 8%, whereas the MSFRS lines averaged a 25% yield gain. This dramatic improvement in yield is off-set by susceptibility to lodging that is generally severe. However, each population has produced a few lines with good to excellent resistance to lodging. One of these lines, tested as FB006, is slated to be released as a cultivar in 2005. FB006 has an average 12.5% yield advantage over Virden with improved forage quality. Several other selections show equal or greater promise.

Fig.1



In conclusion, MSFRS is a very useful breeding tool for long-term germplasm development where multiple disease resistance is desired and direct production of high-yielding forage cultivars is a goal of the breeding effort.

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Measuring phyllochrons in barley to use for seeding date recommendations

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Introduction

A phyllochron is the interval between one leaf appearance and the next, and can be measured using calendar or growing degree days. It is a measurement of plant development that can be used to assess how the plant has responded to environmental conditions or to predict how it is going to respond.

In a previous study on the effects of seeding dates in central Alberta, grain yields of barley varieties were found to decline as seeding was delayed from early May to mid-June (Juskiw et al. 2003; Fig. 1). What was interesting from this study was that there was a link between rapid leaf development (short phyllochron) and ability to have less yield loss under late seeded conditions. The link was independent of maturity of the variety.

The objective of this study was to determine the phyllochrons of barley varieties recently released by Field Crop Development Centre with the potential to use this information to make seeding date recommendations.

Materials and Methods

This study was conducted with plants grown in pots in growth cabinets (Conviron Model PTR15, Controlled Environments Limited, Winnipeg, MB) at 20/15^{o} C, 16/8 h and approx. 450 μ-moles m^{-2} s^{-1}. Pots were filled with Promix BX (Premier Horticulture Inc., Rivier-du-Loup, PQ), a general purpose growing medium of sphagnum peat moss, perlite, vermiculite, limestone and wetting agent. Pots were watered twice weekly with water and once weekly with fertilizer solution. The varieties used in the study were Kasota, Manny, Niobe, Ponoka, Trochu, Tyto, and Vivar. Kasota, Manny, Tochu, and Vivar are six-rowed, hulled feed types. Niobe and Ponoka are two-rowed, hulled feed types. Tyto is a six-rowed, hulless feed type. Five seeds were planted per pot and thinned to two plants per pot at the 3-4 leaf stage. Leaf counts of the main stem were made on Mondays, Wednesdays, and Fridays from emergence to the flag leaf fully emerged. Final leaf counts of the main stem were recorded.

Leaf counts were regressed against Growing Degree Days (GDD, 0^{o} C basis) using Proc GLM of SAS (SAS Institute, Inc., Cary NC). Phyllochrons were determined as the inverse of the GDD regression co-efficient. As well phyllochrons of individual leaves were determined by dividing growing degree days by leaf count for each sampling time.

Results and Discussion

Excellent fits of leaf number versus GDD were found for all seven cultivars (Fig. 2). Differences in phyllochrons of these seven varieties were found (Table 1). Kasota, Trochu and Tyto had relatively rapid phyllochrons; while Ponoka had a slow phyllochron. The values determined in this study were higher than those reported in Juskiw and Helm (2003) that may reflect a slowing

of response under lower light intensities in the growth cabinet versus the field.

Table 1. Phyllochrons and final leaf numbers of seven barley varieties.

Variety	Phyllochron (GDD leaf $^{-1}$)	Final Leaf Number
Kasota	77	8.75
Manny	87	8.00
Niobe	87	9.75
Ponoka	92	9.43
Trochu	74	9.87
Tyto	72	11.00
Vivar	81	9.00

The combination of final leaf number and phyllochron can be used to predict relative maturity (in crop modeling a certain number of phyllochrons are assigned to emergence, head emergence, completion of stem elongation, and kernel filling). At a very simplistic level we used final leaf number times phyllochron to come up with a leaf development duration to see how well this was related to relative maturity based on values from the Alberta Agriculture, Food and Rural Development Agdex100/32 (2005) (Table 2). While Tyto had a rapid phyllochron, when this was combined with its high leaf number, it resulted in a long leaf development duration that was also reflected in its maturity. Kasota combined a rapid phyllochron with low leaf number to have a short leaf development duration that reflected its early maturity. Using phyllochron to estimate maturity would over-estimate the maturity for Niobe; and under-estimate that of Manny and Vivar. Further study is needed to reconcile such differences if phyllochrons are to be used for predictive purposes.

Table 2. Leaf development duration and its relationship to maturity for seven barley varieties.

Variety	Maturity (d)[z]	Duration of leaf development (GDD)	Relationship of maturity/Leaf GDD duration
Kasota	94	670	early/early
Manny	97	700	mid/early (?)
Niobe	97	850	mid/late (?)
Ponoka	100	870	late/late
Trochu	96	730	mid-early/mid-early
Tyto	98	790	mid-late/mid-late
Vivar	98	730	mid-late/mid-early (?)

[z] Maturities from Alberta, Agriculture, Food and Rural Development (2005).

While there was good fit of the linear regression of leaf number versus GDD, when phyllochrons were estimated using the leaf count at each sampling time, we found that the phyllochrons for the

first two leaves were often slower than for subsequent leaves (Fig 3.). There were two distinct patterns: 1) six-rowed cultivars had slow initial phyllochrons followed by more rapid initiation of subsequent leaves; and 2) two-rowed cultivars, especially Niobe, had more rapid initiation of the first two leaves followed by slower initiation of subsequent leaves. What effect these differences in initiation rates would have on competition needs further study.

As a final point, we took our long term yield data from 1998 and 2002 at Lacombe and Stettler and compared yield differences between early and late May planting dates for the varieties under study (Fig. 4 and 5). This was just a quick look to see if a recommendation based on rapid phyllochrons would be valid. The late planting was generally before the end of May at either location and would not be considered extremely late, so the data is of limited value. Our recommendation based on phyllochrons would be that Tyto, Trochu and Kasota would be the varieties of choice. However Trochu had one of the greatest drops in yield from early or mid-May plantings to the late May planting. Overall, Ponoka, Tyto and Vivar had the least yield reductions with the later planting. While we would like to make a clear-cut recommendation to plant Tyto, Trochu or Kasota when faced with late seeding, further field work is needed to confirm or refute the phyllochron, late-seeding yield response relationship.

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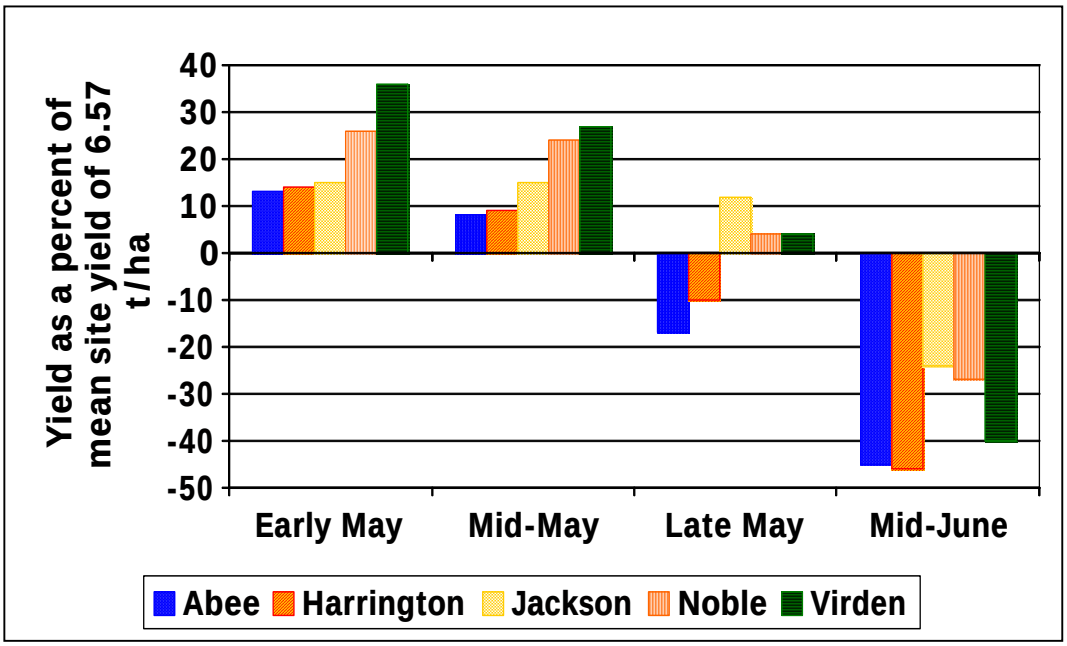

Figure 1. Effects of seeding date of relative yield of five barley varieties (from Juskiw and Helm 2003).

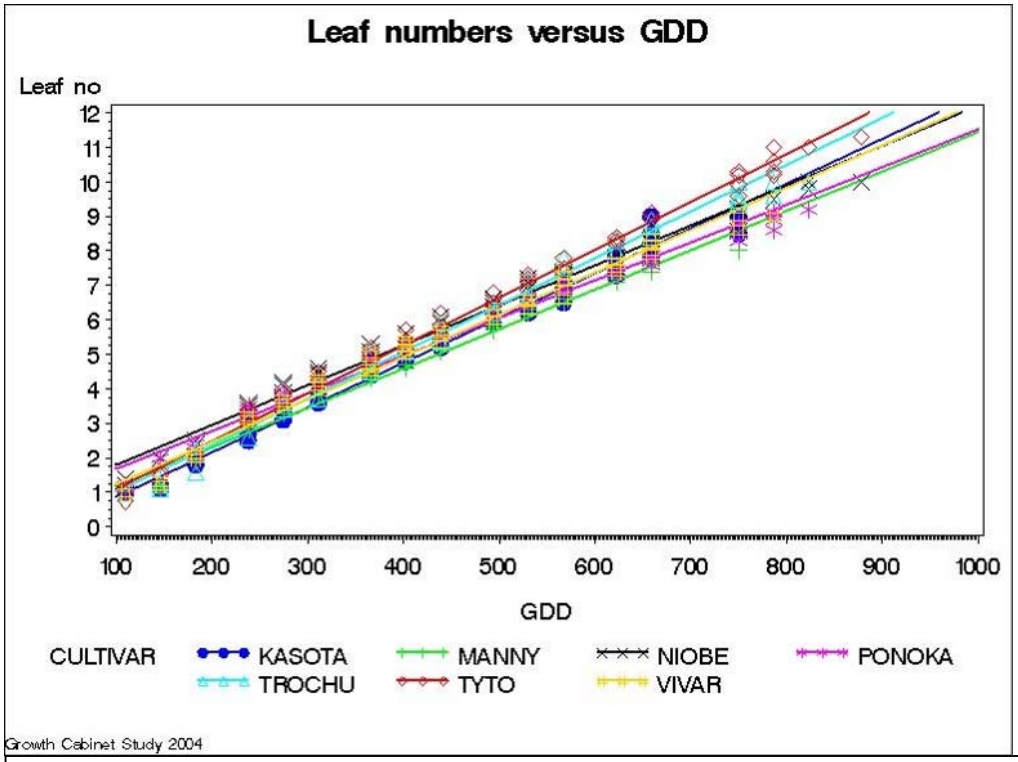

Figure 2. Regression of leaf counts against accumulated GDD for seven spring barley varieties.

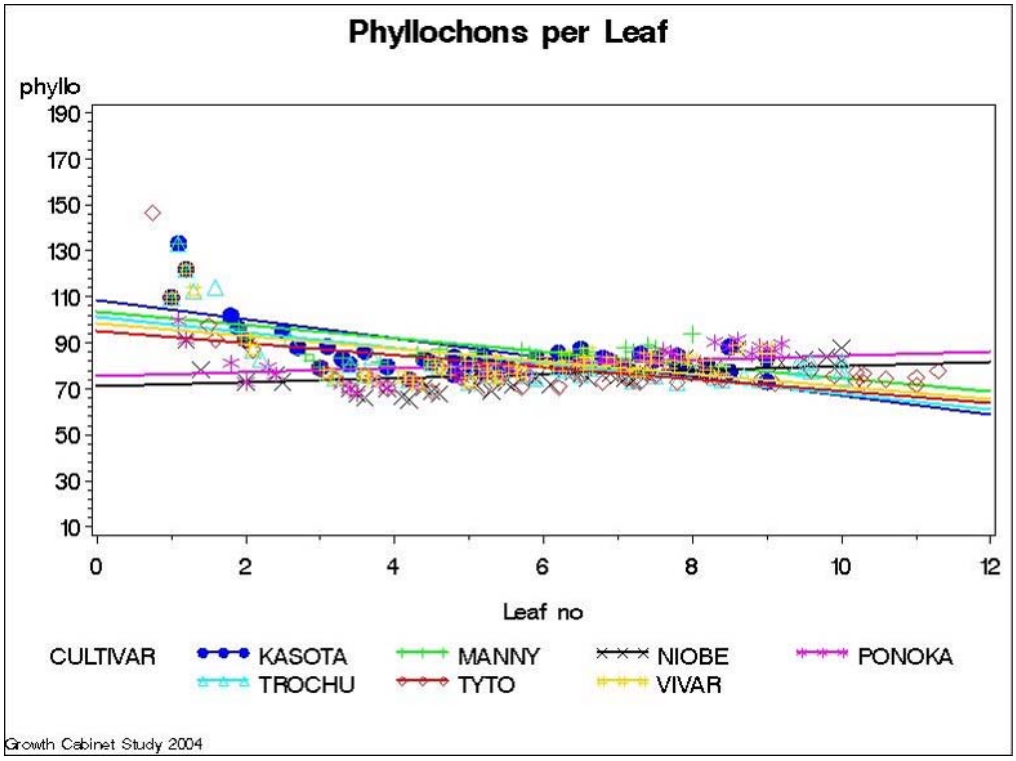

Figure 3. Phyllochron estimates for individual leaves for seven spring barley varieties.

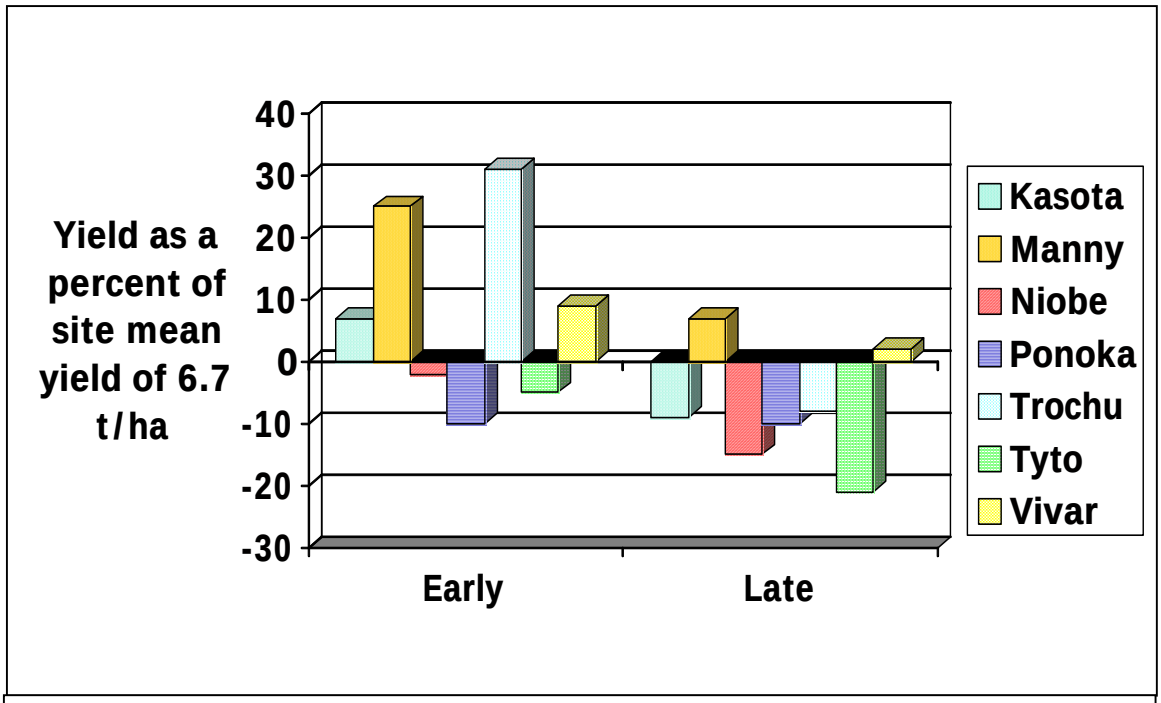

Figure 4. Yield response to seeding dates at Lacombe from 1998 to 2003 (where early was the first week of May and late was the last week of May).

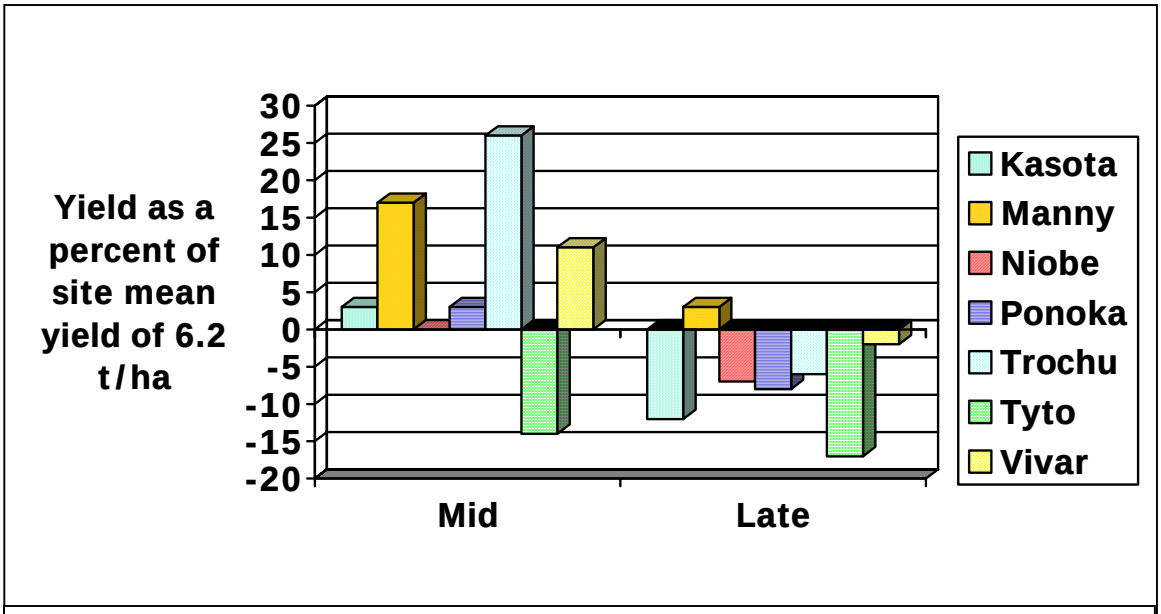

Figure 5. Yield response to seeding dates at Stettler from 1998 to 2003 (where mid was the second week of May and late was the last week of May).

Wednesday, July 20, 2005 – a.m.

Session 5: BIOTECHNOLOGY AND GENOMICS

Chair
Jennifer Zantinge

Presenters
Gary Muehlbauer, University of Minnesota
Peter Eckstein, University of Saskatchewan
Nora Lapitan, Colorado State University
Tajinder Grewal, University of Saskatchewan
Kavitha Madishetty, University of California Riverside
Andris Kleinhofs, Washington State University

Applications of GeneChips for barley improvement

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Abstract

The use of RNA profiling has recently become a powerful tool to examine genome-wide transcript accumulation. The commercial release of the Barley1 Affymetrix GeneChip probe array has provided the resource to conduct RNA profiling of 22,439 barley genes in a single experiment. We have focused primarily on using the Barley1 GeneChip to (1) physically map barley genes to chromosomes; (2) to examine the RNA profiles in barley infected with *Fusarium graminearum*, and (3) as a proof of concept for targeting markers to genomic regions. In this article, we will describe these applications of the Barley1 GeneChip and discuss some of our results.

Introduction

High-throughput RNA profiling technologies are useful tools for examining the expression of thousands of genes in parallel. Traditionally, gene expression studies have relied on methods and technologies that examine one to a few transcripts at a time. Thus, RNA profiling technology provides a substantial increase in the number of transcripts compared to more classical methods. In 2003, the barley1 Affymetrix GeneChip probe array was fabricated and provided a new resource for barley geneticists to conduct high throughput RNA profiling experiments in barley (Close et al., 2004). This article summarizes the development of the barley1 GeneChip, and applications to barley research and improvement.

The barley1 GeneChip

A USDA-IFAFS grant to a group of U.S. barley geneticists (Andris Kleinhofs, Timothy Close, Roger Wise, Rod Wing and Gary Muehlbauer) provided the funding to develop RNA profiling technology in barley. The genomics company Affymetrix (Santa Clara, CA), which specializes in the development of GeneChip probe arrays, was chosen to develop this resource for the barley research community. The design of the Barley1 GeneChip probe array was based on approximately 350,000 barley expressed sequence tags (ESTs) developed through an effort of barley geneticists in the U.S. (R. Wing, A. Kleinhofs, R. Wise, and T. Close), Scotland (R. Waugh), Japan (K. Sato), Finland (A. Schulman) and Germany (A. Graner). These barley gene sequences were condensed into an exemplary set of sequences for the GeneChip design. The finished product was the Barley1 GeneChip probe array, which represents 22,439 barley genes and thus provides the resource to examine transcript accumulation of all of these genes in parallel (Close et al., 2004).

The 22,439 genes are represented on the Barley1 GeneChip in the form of 22,439 probe sets. These probe sets are comprised of 11 matched and mismatched pairs of 25-mer oligonucleotides. Most of the oligonucleotides were designed from the 3' end of each exemplar sequence (Close et al., 2004). Hybridization of labeled RNA to each probe set is determined and raw numerical

values representing the amount of transcript accumulation are obtained. These values can be examined with a variety of computer programs and statistical packages to address specific questions relating to transcript accumulation.

Each GeneChip experiment results in a tremendous amount of data. To house these data, to conduct data analysis, and to provide a resource for future comparative analysis the MIAME (minimum information about a microarray experiment) compliant BarleyBase (http://barleybase.org/; Shen et al., 2005) database has been established. BarleyBase is an on-line public repository for raw and normalized expression data for Affymetrix GeneChip data. Currently, data from multiple Barley1 GeneChip experiments are housed on this site.

Uses of microarray technology

There are multiple uses of microarray technology including: (1) examining the response to abiotic and biotic stresses; (2) high-throughput gene mapping; (3) determining gene expression patterns associated with malting; (4) identifying tissue-specific gene expression; (5) determining gene expression differences in defined mutant backgrounds; (6) gene cloning; and (7) targeting markers to genomic regions. In this article, we will discuss our work with the Barley1 GeneChip to (1) physically map barley genes to chromosomes; (2) to examine the RNA profiles in barley infected with *Fusarium graminearum*, and (3) as a proof of concept for targeting markers to genomic regions.

Results And Discussion

High-throughput physical mapping

We developed an approach to utilize the Barley1 GeneChip to physically map large numbers of barley genes to chromosomes. We are using the wheat-barley addition lines to assign barley genes to chromosomes. These disomic chromosome addition lines were developed through wide hybridization between the donor Betzes barley (*Hordeum vulgare* L.) and the recipient Chinese Spring wheat (*Triticum aestivum*) (Islam et al., 1981). These genetic stocks contain all 21 wheat chromosome pairs and a single chromosome pair from barley. Wheat-barley disomic addition lines have been developed for six of the seven barley chromosomes including 1(7H), 2(2H), 3(3H), 4(4H), 6(6H) and 7(5H), and ditelosomic addition lines harboring 13 of the 14 barley chromosome arms have been generated (Islam et al., 1981). Our objectives were to use the wheat-barley addition lines in combination with the Barley1 GeneChip to assign barley genes to chromosomes. The basic idea is as follows: transcripts detected in Betzes and the addition lines, but low or no detection in Chinese Spring were derived from Betzes and the barley gene encoding the transcript was assigned to a specific donor barley chromosome.

We examined transcript accumulation in seedling tissues of Betzes barley, Chinese Spring wheat and wheat-barley chromosome addition lines carrying barley chromosome 2H, 3H, 4H, 5H, 6H, or 7H. By examining only those transcripts that were detected in Betzes and one or more of the addition lines, we identified 482, 331, 352, 392, 246 and 421 transcripts in the addition lines carrying barley chromosome 2H, 3H, 4H, 5H, 6H and 7H, respectively. Based on these results, we assigned 2,224 genes to barley chromosomes. Our results were validated through extensive genomic PCR and by *in silico* comparisons to the wheat and rice genomes. We found that our physical map positions were highly syntenic with the wheat and rice genomes and that our

genomic PCR results were consistent with our GeneChip interpretations. We also examined transcript accumulation in ditelosomic addition lines carrying the long and short arm of chromosome 6H and assigned 139 and 105 genes to chromosome 6HL and 6HS, respectively. The chromosome 6H ditelosomic addition line results validated the location of 244 out of the 246 genes assigned to chromosome 6H. Therefore, we have substantially increased the number of genetic markers for use in marker-assisted selection, map-based cloning and for scaffolds for full-genome sequencing. Our results show that this is an efficient method to physically map barley genes to chromosomes.

Fusarium head blight of barley

Fusarium head blight (FHB) of barley is caused by *F. graminearum* and related *Fusarium* species. FHB is a major disease problem for barley growers in the United States and in the barley growing regions of the world (Parry et al., 1995). Trichothecenes mycotoxins, such as deoxynivalenol (DON) are produced by the fungus during infection and accumulate in the harvested grain grain. Barley grain containing measurable levels of DON results in reduced malting quality. Therefore, our goals are to understand the interaction between barley and *F. graminearum* with the intent to identify genes that provide resistance to FHB. Our approach is to use the Barley1 GeneChip to gain an understanding of the interaction between barley and *F. graminearum* during infection and to use the gene expression data to direct marker development for FHB resistant QTL-containing regions of the genome.

Transcript accumulation in Morex during Fusarium graminearum infection

Four replications of spikes from the FHB susceptible barley cultivar Morex at 1, 2, 3, 4, and 6 days after *F. graminearum* and water inoculation and a fifth replication at 1 and 3 days after *F. graminearum* and water inoculation were sampled for RNA isolation. RNA profiles were examined at these treatment/timepoints using the Barley1 GeneChip. Three hundred and fifty seven transcripts were differentially expressed between *F. graminearum*-and mock (water) inoculated barley spikes at one or more time points. The differentially accumulating transcripts were placed into two subgroups. One subgroup of 182 transcripts was identified based on the presence versus absence test of transcripts between *F. graminearum* and mock-inoculated spikes and referred to as qualititatively-induced during infection. The other subgroup of 175 transcripts was identified as significantly induced between *F. graminearum*- and mock-inoculated barley spikes and referred to as quantitatively-induced during infection. The transcript accumulation from all detected genes was greater in the *F. graminearum*-treated plants, there were no transcripts that were down regulated in this experiment. These transcript accumulation patterns were validated via RNA gel blot analysis

Examination of the transcript accumulation profiles resulted in the following three major observations. (1) There are three major stages of disease progression: an early stage between 0-2 days after inoculation (dai), an intermediate stage between 2-4 dai; and a late stage between 4-6 dai. (2) Most of the induced genes were identified at 3 dai during the intermediate stage, indicating that this is an important host response timepoint. (3) We observed upregulation of the tryptophan biosynthetic pathway. This observation demonstrates a specific biochemical host response to infection. These observations provide the theoretical basis for a better understanding of the plant response to infection.

Transcript accumulation in contrasting alleles at the Chromosome 3H DON accumulation QTL
To identify potential genes that are involved with FHB resistance and markers that are linked to a DON accumulation resistant QTL, we examined transcript accumulation in a barley near-isogenic line (NIL) pair carrying resistant and susceptible alleles at the DON resistant chromosome 3H (BIN 6) QTL. The DON resistant QTL was identified in the Fredrickson/Stander recombinant inbred line population (Smith et al., 2004). An NIL pair carrying resistant and susceptible alleles at the chromosome 3 DON QTL was provided by Kevin Smith (University of Minnesota).

We used the Barley1 GeneChip to examine transcript accumulation in plants carrying the resistant and susceptible alleles at the chromosome 3H DON QTL at 48 and 96 hours after inoculation. We identified seven genes that are differentially expressed in the lines containing the differing alleles at the barley chromosome 3H QTL. These transcript accumulation differences were due solely to genotype not the treatment. No genes were identified that exhibited differential transcript accumulation between the contrasting alleles due to *F. graminearum* infection.

Based on the allelic differences in the NIL pairs carrying the resistant and susceptible alleles, some of the 7 differentially expressed genes may map to the chromosome 3H QTL region. We mapped two of the seven genes on the Fredrickson/Stander mapping population in the chromosome 3H DON accumulation QTL region. Our results show that the Barley1 GeneChip can be used to identify allelic differences that can be converted into genetic markers that target specific regions of the genome.

Impact of trichothecene accumulation on barley gene expression
F. graminearum infection of barley results in the fungus synthesizing trichothecene mycotoxins. These mycotoxins are a major detriment to grain quality, especially for grain intended for use as malt. In wheat, the ability of *F. graminearum* to synthesize trichothecenes increases the virulence of the fungus (Proctor et al., 1995). Loss-of-function mutations in the *Tri5* gene, the first committed step in the trichothecene biosynthetic pathway, results in the inability of the fungus to synthesize trichothecenes and a reduction of virulence on wheat. To determine the host response to trichothecene accumulation in barley, we examined the transcript accumulation profiles in Morex barley inoculated with a wildtype strain of *F. graminearum*, the *Tri5* mutant and water at 48 and 96 hours after inoculation.

Examination of the transcript accumulation data revealed three classes of genes that respond differentially to trichothecene biosynthesis. We identified 37 genes that were only expressed in barley during *Tri5* mutant infection (no trichothecene accumulation), and 96 genes that were only expressed during wildtype infection (trichothecene accumulation). We also identified 27 genes that are statistically significantly upregulated in wildtype-infected plants versus *Tri5* mutant infected plants. These results show that there are genes that are specifically upregulated and downregulated during trichothecene accumulation. Further analysis and annotation of the genes is ongoing.

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Molecular characterization of barley for variety description and identification

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In Canada, the protection of a barley variety under Plant Breeders Rights requires the candidate variety be shown to be distinct, uniform and stable (DUS). To demonstrate that a variety meets these requirements, the variety is described by a series of morphological/botanical characteristics. The combination of phenotypic characteristics unique to a variety becomes the legal basis to assess its distinctiveness, uniformity and stability. The limitations to the current system are many. Phenotypic descriptions need to be determined by experienced personnel at varying times throughout the season, and need to be duplicated over at least two field seasons. The process is often long and expensive. Since descriptions are comparative in nature (to two or three reference varieties chosen by the Plant Breeder) the descriptions are often subject to interpretation and may be described differently in subsequent evaluations. If variety identity is challenged, the material needs to be grown either in the field or a growth facility along with the reference varieties included at registration, and the characteristics may appear different when grown under different conditions.

Since the advent of DNA fingerprinting technology, the opportunity exists to replace the current phenotypic description of a plant variety with molecular characterization. Several technologies exist that could be serve the purpose, all with inherent advantages and disadvantages. The advantages of all of these technologies however are the non-subjective nature of the descriptive data, and the stability of molecular data.

To demonstrate that molecular characterization can be used to describe a new variety, a project on 23 hulless barley varieties was begun. Hulless barley was chosen as the "model" because of the simple genetics of this crop species, the relatively small number of varieties in this class registered in Canada, and Breeder Seed of all varieties was readily available. The project investigates the ability of AFLP technology to establish distinctiveness and uniformity of molecular data within a variety. AFLP analysis as chosen as a model because it allows for the generation of many bands (examines numerous loci) at one time providing an overall "picture" of the variety.

Materials and Methods

DNA was extracted from 4-5 seeds from Breeder Seed of each variety using a CTAB based protocol (Procunier *et al*., 1991). AFLP DNA fingerprinting was performed according to the standard protocol of Vos *et al*. (1995), on 500ng of genomic DNA. DNA templates were prepared using *Eco*RI and *Mse*I restriction enzymes, and amplified with primers having three selective bases. Amplified fragments were separated on 6% denaturing polyacrylamide gels (50 cm length) and visualized by staining with silver nitrate.

Results and Discussion

Twenty-three hulless barley varieties are currently registered in Canada and originate from five institutions (see below).

Variety	Institution
AC Bacon	Agricuture and Agri-Food Canada, Brandon, Manitoba
AC Hawkeye	Agricuture and Agri-Food Canada, Brandon, Manitoba
CDC Silky	Crop Development Centre, Saskatoon, Saskatchewan
Falcon	Field Crop Development Centre, Lacombe, Alberta
Jaeger	Field Crop Development Centre, Lacombe, Alberta
Peregrine	Field Crop Development Centre, Lacombe, Alberta
Tyto	Field Crop Development Centre, Lacombe, Alberta
AC Alberte	AAFC, ECORC, Ottawa, Ontario
CDC Dawn	Crop Development Centre, Saskatoon, Saskatchewan
CDC Freedom	Crop Development Centre, Saskatoon, Saskatchewan
CDC Gainer	Crop Development Centre, Saskatoon, Saskatchewan
CDC McGwire	Crop Development Centre, Saskatoon, Saskatchewan
CDC Speedy	Crop Development Centre, Saskatoon, Saskatchewan
Condor	Field Crop Development Centre, Lacombe, Alberta
Merlin	Western Plant Breeders, Bozeman, Montana
Phoenix	Field Crop Development Centre, Lacombe, Alberta
Tercel	Field Crop Development Centre, Lacombe, Alberta
CDC Alamo	Crop Development Centre, Saskatoon, Saskatchewan
CDC Candle	Crop Development Centre, Saskatoon, Saskatchewan
CDC Fibar	Crop Development Centre, Saskatoon, Saskatchewan
CDC Rattan	Crop Development Centre, Saskatoon, Saskatchewan
HB803	Western Plant Breeders, Bozeman, Montana
HB805	Western Plant Breeders, Bozeman, Montana

AFLP analysis was performed using three selective primer combinations. On average, each combination amplified 50 to 60 bands per sample. Since gels were silver stained, some of the bands were likely complementary strands of the same fragment, therefore the number of loci actually tested is less than this number. Of the three randomly chosen selective primer combinations tested, each combination alone was able to amplify a set of fragments and generate enough polymorphism to distinguish the 23 varieties. Thirteen bands were required to uniquely characterize the varieties using primer combination E35-M49 (Figure 1), nine bands and 12 bands were required for primer combinations E38-M61 and E37-M62 respectively. These sets of bands constitute the “discriminatory set” for that primer combination. In all cases, the bands included in the “discriminatory set” were major bands that amplified strongly and consistently from amplification to amplification and gel to gel. Minor bands, which are especially prevalent with silver staining, and are inconsistent from one amplification to the next, were not considered. In all three test cases, additional polymorphic bands were available but not required. These bands may be included in the “discriminatory set” as required when new varieties are put forward. Conversely bands presently used in the set may be removed as the polymorphic bands are read in different combinations and varieties are de-registered.

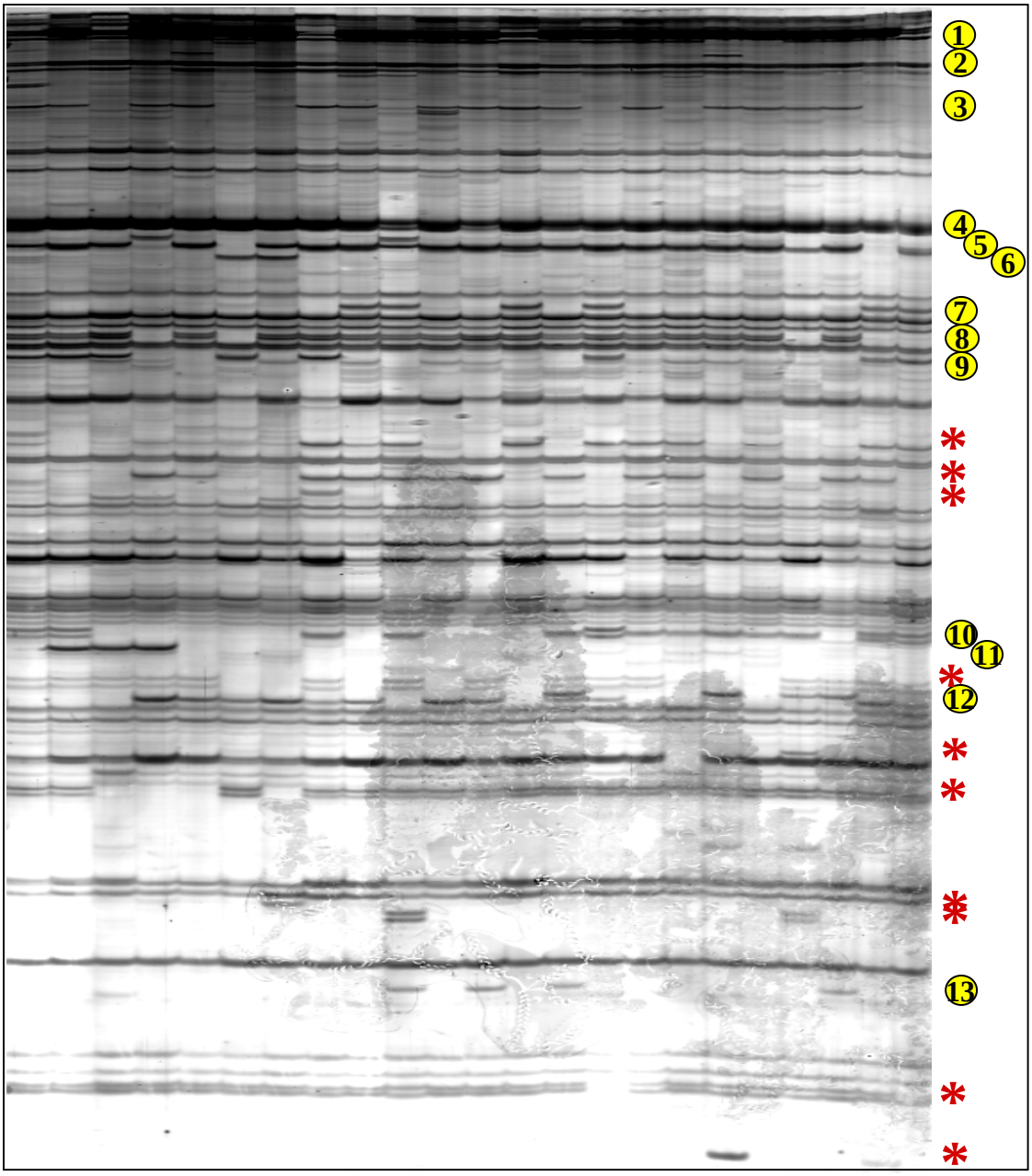

Figure 1. AFLP generated banding patterns using primers E35-M49 on DNA templates from 23 hulless barley varieties. The circled numbers indicate the bands required to distinguish all 23 varieties. Bands marked with an asterisk identify additional polymorphism.

The accurate identification of varieties by a series of bands requires that a variety is uniform for a given banding pattern. Most barley varieties are purified for phenotype based on evaluation of a number of individual rows of plants (approximately 200), the seed for each row originating from a single spike. While the resulting Breeder Seed is essentially homogeneous for visual phenotypic characteristics, DNA fingerprinting has the ability to detect heterogeneity that cannot

be identified visually. Many varieties therefore may not be uniform for their DNA banding patterns. We examined intra-varietal uniformity in the candidate variety CDC Cowboy. Of the 200 Breeder Seed long rows tested, banding uniformity was high, with little variation in overall banding pattern and no variation amongst major bands. The small amount of variation detected among relatively minor bands could be accommodated by disregarding these bands and not including them in the discriminatory set for this variety.

For future candidates, the variety could be purified for a given molecular characterization at the time of Breeders Seed production. This principle was demonstrated in the candidate oat variety CDC Weaver. Two hundred long rows were assessed using AFLP primer combination E37-M62 which is able to efficiently discriminate between several oat varieties. Our analysis revealed that 12 rows showed variation at one or more of nine polymorphic loci, all major bands that could be used to constitute the discriminatory set for oat. The seed of these 12 rows was discarded and is not represented in the Breeder Seed. Of the 12 molecular discards, five rows varied at multiple loci, and were part of six molecular discards that would also have been discarded based on visual phenotype. In addition, one locus (major band) was segregating (nearly 50:50) amongst the rows. The even segregation of this band amongst the long rows necessitated that the banding pattern could not be purified for this locus without possibly changing the character of the candidate variety. This band therefore could not be included in the discriminatory set for this variety.

Applying the same principle to CDC Cowboy, five rows should have been discarded on the basis of banding pattern and one of these rows was one of seven rows eliminated from the Breeder Seed based on phenotype. The small number of discards necessitated by banding pattern variation is unlikely to change the overall agronomic or quality profile of the variety.

While we have been able to demonstrate that AFLP technology is able to establish varietal distinctness and uniformity in barley (and hexaploid oat with a more complicated genome), several other genotyping technologies could be used. Those best suited are likely to be microsattelite (SSR) and single nucleotide polymorphism (SNP) analysis. The ability of all of the technologies to establish distinctness among large groups of cultivars, and uniformity in previously registered varieties needs to be considered. In addition, stability of the banding patterns, where variation in banding pattern may be due to seed purity issues, mutations over time, or errors intrinsic to the fingerprinting technology itself will need to be addressed. Nonetheless, DNA based identification systems can easily meet the standards set by the current system and merit further investigation.

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Transcriptional profiling of gene expression during malting in barley

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Abstract

Malt is a major raw material for the production of beer, and during the malting process barley grains are germinated under strictly-controlled conditions. Malting is a complex process that involves many enzymes. Four enzymes known to be important in malting are α-amylase, β-amylase, α-glucosidase, and limit dextrinase. The goal of this project is to isolate specific gene sequences and allelic variants of genes involved with the malting process. This includes known genes as well as undiscovered genes. To investigate the determining factors of malting quality, RNA expression patterns in different stages of micromalting (i.e, steeping, germination, kilning) in the 6-row cultivar 'Morex' was studied through hybridization of RNA against the 22K Barley1 Affymetrix GeneChip probe array. A subset of candidate genes that appear to be important in malting was identified. Expression patterns of these genes were then compared among the 6-row cultivars, 'Morex' and 'Legacy', and the 2-row cultivars, 'Harrington' and 'Merit.' Genes that were differentially expressed between 2-row and 6-row cultivars, as well as among individual cultivars were identified.

Introduction

DNA arrays have been successfully utilized in plants to help decipher biochemical pathways involved in complex traits. Two recent studies investigated pathways involved in the responses of *Arabidopsis thaliana* against infection by cucumber mosaic virus strain Y *(1)* and barley against attack by *Blumeria graminis* f. sp *hordei (2)*. Both studies identified genes of unkown function which appear to be important in the plant's defense response against the pathogens.

Malting quality of barley involves several traits that show quantitative variation *(3)*. The number of QTLs (>150) that have been associated with malting quality phenotypes indicate the involvement of many more genes than the four major genes known to be important in seed germination and malting. Based on the hypothesis that the observed differences at the trait level are due to differences in the expression of the underlying genes, cDNA array technologies could be deployed to monitor gene expression in different genotypes and to identify genes contributing to complex traits such as malting *(4)*. Based on an analysis of 1400 ESTs, between 17 and 30 candidate genes were identified for each of six malting quality parameters analyzed (*4*). These genes include well known malting related genes, as well as others with unknown function. This study was conducted to identify candidate genes that may be important determinants of malting quality in barley using the Barley 1 Gene Chip probe array containing 22,792 barley genes *(5)*. There were two specific objectives: 1) to identify genes that are highly regulated during malting in the cultivar 'Morex', and; 2) identify genes that show expression level polymorphisms among four malting cultivars.

Materials and Methods

Plant Material and Micromalting

Four barley cultivars were used: 'Harrington', 'Legacy', 'Merit', and 'Morex'. One hundred grams of seed from each cultivar were micro-malted at Busch Agricultural Resources, Inc., Fort Collins, CO. Three sets of all cultivars were separately germinated. Samples for 'Morex' were collected at 4 stages: 1) steeping (14^0C for 48h), 2) Day-2 (48h germination, 20^0C) malting; 3) Day-4 malting ((96 h germination, 20^0C) and 4) after kilning (22 hrs). For the other three cultivars, samples were collected at Day-2 and Day-4. Dry seed was used as control.

RNA Extraction and Hybridization

Total RNA was prepared from a bulk of 5 seeds per sample using TRIzol Reagent (Gibco BRL Life Technologies, Rockville, MD) and tested for quality by denaturing gradient gel electrophoresis. Isolated total RNA samples were processed as recommended by Affymetrix, Inc. (Affymetrix GeneChip Expression Analysis Technical Manual, Affymetrix, Inc., Santa Clara, CA). All starting total RNA samples were quality assessed prior to beginning target preparation/processing steps by running out a small amount of each sample (typically 25-250 ng/well) onto a RNA Lab-On-A-Chip (Caliper Technologies Corp., Mountain View, CA) that was evaluated on an Agilent Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA). Single-stranded, then double-stranded cDNA was synthesized from the poly(A)+ mRNA present in the isolated total RNA (10 ug total RNA starting material each sample reaction) using the SuperScript Double-Stranded cDNA Synthesis Kit (Invitrogen Corp., Carlsbad, CA) and poly (T)-nucleotide primers that contained a sequence recognized by T7 RNA polymerase.A portion of the resulting ds cDNA was used as a template to generate biotin-tagged cRNA from an *in vitro* transcription reaction (IVT), using the BioArray High-Yield RNA Transcript Labeling Kit (T7) (Enzo Diagnostics, Inc., Farmingdale, NY). Fifteen µg of the resulting biotin-tagged cRNA was fragmented to strands of 35-200 bases in length following prescribed protocols (Affymetrix GeneChip Expression Analysis Technical Manual). Subsequently, 10 ug of this fragmented target cRNA was hybridized at 45°C with rotation for 16 hours (Affymetrix GeneChip Hybridization Oven 640) to probe sets present on the Barley1 GeneChip probe array. The GeneChip arrays were washed and then stained (SAPE, streptavidin-phycoerythrin) on an Affymetrix Fluidics Station 450, followed by scanning on a GeneChip Scanner 3000.

Experimental Design and Data analysis

To identify genes that are highly regulated during malting (Objective 1), RNA from 4 different stages of micro-malted 'Morex' and dry seed were hybridized onto the Barley 1 GeneChip probe array. Three replications per time point were conducted. For comparison of gene expression profiles among cultivars, RNA from Day-2 and Day-4 from 'Legacy', 'Harrington' and 'Merit' was hybridized to Barley 1 GeneChip array. Two replications per genotype/time point were performed. The data were quantified and analyzed using GCOS 1.1.1 software (Affymetrix, Inc.) and/or ArrayAssist's gcRMA (Iobion Informatics, Inc.) using default values (Scaling, Target Signal Intensity = 500; Normalization, All Probe Sets, and Parameters, were set at default values). Statistical analysis was done using limma (Linear models for microarray data) (Smythe et al., 2005, http://bioinf.wehi.edu.au/limma) and hierarchical clustering and Bioconductor software *(6)*

Quantitative RT-PCR
Quantitative RT-PCR was done using the QuantiTect® SYBR® Green RT-PCR system (Qiagen Inc., Valencia, CA, USA) and the Cepheid Smart Cycler (Cepheid, Sunnyvale CA, USA). Primers were designed using the specific barley sequences on the Barley1 GeneChip probe array.

Results and Discussion

Transcript profiling of genes expressed during different stages of malting in barley
In order to better understand malting and possibly discover novel genes involved in this process, we employed the Affymetrix Barley 1 GeneChip probe array for transcriptional profiling of gene expression during malting. We began by looking at the main stages of malting using 'Morex' as a model and then compared the gene expression in these different malting stages to dry seed as control. The malting stages included steeping, Day-2 and Day-4 germination, and after kilning.

To evaluate technical and biological variability, we analyzed replication clusters for both the control and the different malting stages. The scatter plots showed that the same genes clustered in similar orders indicating that the replications gave highly reproducible results.

Gene expression at each of the four malting stages examined was compared against dry seed expression profiles. Four hundred eighty seven genes were identified which showed 5000-fold greater signal intensity than dry seed at a significance level of $P \leq 0.0005$. Majority of the genes were up-regulated during steeping or imbibition compared to dry seed. Less than half of the genes were down-regulated. However, almost all of the genes increased in expression level from steeping to Day-2 and Day-4 germination.

Genes for enzymes known to be important in malting, including α-amylase, β-amylase, and isoamylase-like proteins, are among the highly expressed genes (Table 1). The highly expressed genes include genes that function in starch degradation, sucrose metabolism/energy production, cell wall degradation, hydrolytic enzyme inhibition, senescence, cell division and growth, lipid metabolism, amino acid metabolism, and protein synthesis. Furthermore, genes that are involved in stress/defense response as well as temperature response were also included in this list. Interestingly, 26% of highly expressed genes were of unknown function. This suggests that there are other genes involved in malting which have not been identified. Similar results were recently reported in a study to identify genes that are highly expressed during malting among 1400 ESTs (*4*).

Expression of genes that were co-regulated with α-amylase were investigated further by RT-PCR for two reasons. First, is to validate the gene expression profiles observed in the GeneChip array and second, to investigate expression at additional time points (i.e., 24 h steeping, 48 h steeping, days 1, 2, 3, and 4 malting). The results of RT-PCR were consistent with the results of the microrray (not shown). Sixteen genes that were co-regulated with genes coding for α-amylase and limit dextrinase and are involved in starch and cell wall degradation are being analyzed further by RT-PCR.

Table 1. Partial list of genes significantly expressed in Morex after 24 h (steeping) and 96 h (day 4) of micromalting compared to dry seed (P < 0.0001) grouped according to function

Starch degradation
alpha-amylase [*Hordeum vulgare* subsp. *vulgare*]; Alpha-amylase type a isozyme precursor (1,4-alpha-d-glucan glucanohydrolase) (amy1) (low pi alpha amylase); Beta-amylase (1,4-alpha-D-glucan maltohydrolase); Iso-amylase-like protein

Sucrose metabolism/energy production
2-oxoglutarate/malate translocator (clones OMT134 and OMT106), mitochondrial membrane - proso millet; Phosphoglycerate kinase, cytosolic pir||TVWTGY; phosphoglycerate kinase (EC 2.7.2.3), cytosolic – wheat; Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)

Inhibitors of hydrolytic enzymes
Alpha-amylase/subtilisin inhibitor precursor (BASI) pir||S04860 alpha-amylase/subtilisin inhibitor precursor – barley; bowman-birk type trypsin inhibitor pir||TIBHB trypsin inhibitor (Bowman-Birk) - two-rowed barley

Temperature stress response
cold acclimation protein WCOR413 - wheat gb|AAB18207.1| cold acclimation protein WCOR413 [*Triticum aestivum*]; heat shock protein HSC70-1, cytosolic [imported] - spinach gb|AAA62445.1| heat shock protein

Stress response/defense
23 kd jasmonate-induced protein pir||S22514 jasmonate-induced protein 1 – barley; chitinase (EC 3.2.1.14) CH11, acidic - maize (fragment) gb|AAA62420.1| (L16798) class I acidic chitinase [*Zea mays*]; (1->3,1->4)-beta-glucanase isoenzyme II (EC 3.2.1.73) [*Hordeum vulgare*]

Senescence
Ethylene-inducible protein [*Oryza sativa*] Putative pyridoxine/pyridoxal 5-phosphate
S-adenosylmethionine synthetase 1 (Methionine adenosyltransferase 1) (AdoMet synthetase 1

Cell division and growth
Tubulin beta-2 chain (Beta-2 tubulin) gb|AAD20179.1| beta-tubulin 2 [Eleusine indica]; ubiquitin / ribosomal protein CEP52 - rice dbj|BAA02154.1| ubiquitin/ribosomal polyprotein [*Oryza sativa*]

Cell division and growth
Tubulin beta-2 chain (Beta-2 tubulin) gb|AAD20179.1| beta-tubulin 2 [Eleusine indica]
ubiquitin / ribosomal protein CEP52 - rice dbj|BAA02154.1| ubiquitin/ribosomal polyprotein [*Oryza sativa*]

Lipid metabolism
glyoxalase I [*Oryza sativa* (japonica cultivar-group)]**;** lipid transfer protein precursor 1 - barley (fragment) emb|CAA42832.1| LTP 1 [*Hordeum vulgare*]; omega-6 fatty acid desaturase [*Sesamum indicum*]

Oxygen reactive enzymes
CAD11966.1 2e-34 glutathione-S-transferase, I subunit [*Hordeum vulgare*subsp. *vulgare*]
ascorbate peroxidase [*Hordeum vulgare*subsp. V*ulgare*

Amino acid metabolism
phosphoethanolamine methyltransferase [*Triticum aestivum*]; serine acetyltransferase [*Oryza sativa* (japonica cultivar-group)]

Protein destination
Adenosylhomocysteinase (S-adenosyl-L-homocysteine hydrolase) (AdoHcyase); cathepsin B-like cysteine proteinase (EC 3.4.22.-) - wheat (fragment); Cysteine proteinase EP-B 1 precursor pir||JQ1111; cysteine proteinase (EC 3.4.22.-) EP-B 1 precursor –barley

protein synthesis
40s ribosomal protein s11 gb|aac14469.1| ribosomal protein s11 [*glycine max*]; ribosomal protein s30 homolog; protein id: at4g29390.1 [*arabidopsis thaliana*]; 60s acidic ribosomal protein p0 pir||t04309 acidic ribosomal protein p0 – rice; ef-1 alpha [*oryza sativa*] dbj|baa23659.1| ef-1 alpha [*oryza sativa*

Cell wall degradation
(1->3,1->4)-beta-glucanase isoenzyme II (EC 3.2.1.73) [*Hordeum vulgare*]; arabinoxylan arabinofuranohydrolase isoenzyme AXAH-I [*Hordeum vulgare*]

Signal transduction
adenosine kinase [*Zea mays*]; small Ran-related GTP-binding protein [*Triticum aestivum*]

Unknown or unclear

Comparative expression profiles among four malting barley cultivars

The expression profiles of three other cultivars at Day-2 and Day-4 malting were investigated and compared with the same stages in 'Morex'. Expression patterns of the subset of genes (identified in the study above) that appeared to be important in malting were analyzed in these cultivars. Other genes showing significant levels of expression but were not highly expressed in 'Morex' were identified in the three cultivars. Among the highly expressed genes, 8.4% had at least a two-fold greater level of expression in 'Morex' and 'Legacy' than in the 2-row cutivars 'Harrington' and 'Merit'. Fructokinase and peptidylprolyl isomerase are examples of genes in this category. Conversely, 11.9% of the genes had at least 2-fold greater level of expression in the 2-row cultivars than in the 6-row cultivars. Acid phosphatase, and defensin are examples of genes in the latter group. There were also some genes that were significantly expressed in one cultivar only. These genes may be involved in determining malting quality differences between 2-row and 6-row cultivars or among the cultivars.

In summary, candidate genes that appear to be important in malting or malting quality differences between cultivars were identified using the Barley 1 GeneChip probe array. Validation of these candidate genes will be important. Association with malting quality phenotypes is one approach. Genetic mapping and co-localization of candidate genes with QTLs for malting quality phenotypes will provide further evidence for their possible roles in malting.

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Molecular marker assisted introgression of loose and covered smut resistance into CDC McGwire hulless barley

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True loose smut (*Ustilago nuda* (Jens.) Rostr.) and covered smut (*U. hordei* (Pers.) Lagerh.) of barley result in yield reductions from 0.2 to 0.8 % in western Canada (Thomas and Menzies 1997). The smuts can be controlled by seed treatment, sowing disease-free seed or growing resistant cultivars. Seed treatment with fungicides is effective but adds cost and the pathogens may become resistant (Ben-yephet et al. 1975, Leroux and Berthier 1988). Furthermore, seed treatment is not an option for organic production. Resistant cultivars are generally recognized as the most economical and preferred method of control. However, breeding for smut resistance is expensive as screening is time, labour and space consuming and frequent escapes makes it necessary to screen putative resistant lines several times to confirm resistance. As both diseases infect the inflorescence, simultaneously screening is not possible. Molecular Marker Assisted Selection is a good alternative to combine resistance to both diseases at once.

CDC McGwire is a high yielding hulless barley cultivar but susceptible to true loose and covered smut. Loose smut resistant lines in a CDC McGwire background with resistance from TR251 (*Run8*) were developed. *Run8* confers resistance to most known races of *U. nuda* in western Canada (Thomas and Menzies 1997). Similarly, covered smut resistant lines (having the *Ruhq* gene) in a CDC McGwire background were available with resistance from Q21861. *Ruhq* shows resistance to western Canadian isolates of *U. hordei* (Grewal et al. 2004). Each of these lines had 50% of their background from CDC McGwire.

A sequence characterized amplified region (SCAR) marker linked to the loose smut resistance gene *Run8* has been developed (Eckstein et al. 2002). Similarly, a SCAR marker linked to covered smut resistance in Q21861 has been developed (Ardiel et al. 2002). This project was initiated to introgress the *Run8* and *Ruhq* into CDC McGwire using molecular markers.

Materials and Methods

Breeding line SH00752 (CDC McGwire/TR251) was crossed with breeding line SH01470 (CDC McGwire/Q21861). Two strategies were used to introgress covered and loose smut into CDC McGwire i.e. doubled haploidy and marker-assisted backcrossing.

Doubled haploidy

SH00752 X SH01470 F_1 seeds were used to produce doubled haploids. Thirty five DH plants were produced using microspore culture and tested with UhR450 and $Un8_{700}R$ SCAR markers as described by Ardiel et al. (2002) and Eckstein et al. (2002), respectively. The 35 DH lines were tested for covered and loose smut reactions.

Covered smut screening: For inoculation, disease screening and evaluation, the techniques used were as reported earlier (Ardiel et al. 2002, Grewal et al. 2004). The 35 DH lines (population

MC0181) were inoculated with a mixture of *U. hordei* isolates along with the original parents (Q21861, CDC McGwire, TR251) and susceptible check (CDC Candle). All lines were screened in the field in summer 2003 at the Preston Plots, U of S, Saskatoon. Covered smut infection was evaluated as percent infected heads. In fall 2003, 21 putative resistant lines (showing <3% infected heads) were re-screened in the greenhouse where the infection level was evaluated as percent infected plants. A plant showing one or more smutted heads was considered infected. Putative resistant lines were re-tested in the field in summer 2004.

Loose Smut screening: All DH lines, parents and the check were inoculated in the field in summer 2003 using the syringe inoculation technique described by Eckstein et al. (2002). Five spikes from each line were inoculated with *U. nuda* teliospores. Inoculated seeds were planted in the greenhouse in fall 2003. A line with any smutted head in any replication was rated susceptible. Twenty one lines showing zero infection were re-inoculated with loose smut and inoculated seeds were grown in the field in summer 2004. All lines were inoculated again in the field in summer 2004 and inoculated seeds grown in the greenhouse in fall 2004 for evaluation.

Marker-assisted backcrossing

Ten F_1 SH00752 X SH01470 plants were tested with both SCAR markers using a quick, simple and effective method reported by Eckstein et al. 2004. Nine of 10 plants were positive for both markers and five were backcrossed to CDC McGwire. In summer 2002, BC_1F_1 plants were tested with both markers. Fourteen BC_1F_1 plants positive for both markers were backcrossed to CDC McGwire. In fall 2002, BC_2F_1 plants were screened with both markers and plants positive to both identified (Table 2). A few plants positive for both markers were backcrossed to CDC McGwire. Similarly, BC_3F_1 plants were screened and those positive for both markers were selfed in the greenhouse. In the BC_3F_2 generation, 186 plants were screened with the markers and plants positive for both were also screened with SCAR marker $Un8_{700}S$ (linked to susceptible allele of *Run8* gene - Eckstein et al. 2002) and RAPD marker $OPJ10_{450}$ (linked to susceptible allele of *Ruhq* gene - Ardiel et al. 2002) to identify the plants homozygous for the markers. Sixty-two BC_3F_2 plants positive for both markers were selfed. Ten BC_3F_3 lines were evaluated for covered smut reaction in the field in 2004 along with the parents and check. These lines were re-tested again in the greenhouse in fall 2004 and spring 2005. All lines were inoculated with loose smut in the field in summer 2004 and grown out in the greenhouse in fall 2004 for evaluation. These lines were again inoculated with loose smut and evaluated for resistance in spring 2005. All lines were again inoculated in the greenhouse and are being evaluated in the field during summer 2005.

Results and Discussion

Doubled haploidy

Fourteen of 35 DH lines, were positive for both markers and 10 were negative. The UhR450 marker was positive in 18 lines and the Un8 was positive in 21 (Table 1). Field screening of all lines in summer 2003 against covered smut showed that Q21861 was resistant whereas CDC McGwire and TR251 were susceptible. Twenty DH lines showed resistance. For 32 of 35 lines, the phenotype defined by the covered smut reaction and genotype defined by the covered smut marker UhR450 agreed. Lines showing putative resistance (<3% infected heads) were screened in the greenhouse and field to confirm reactions.

Table 1. Phenotype and Genotype Data of 35 Doubled-Haploid Lines

Barley lines	Test	Covered smut reaction*			UhR450	Un8	Loose smut reaction**		
		Field 2003 % infected heads	GH 2003 % infected plants	Field 2004 % infected heads	covered smut marker	Loose smut marker	GH 2003	Field 2004	GH 2004
CDC Candle	check	48.5	75.0	65.1	No	No	S	S	S
Q21861	parent	0.4	0.0	0.1	Yes	No	S	S	S
TR251	parent	8.1	17.6	2.5	No	Yes	R	R	R
CDC McGwire	parent	10.5	16.7	4.4	No	No	S	S	S
MC0181-01	SH00752/SH01470	**1.1**	**8.3**	**0.2**	**Yes**	**Yes**	**R**	**R**	**R**
MC0181-02		0.6	0.0	0.1	Yes	Yes	S		
MC0181-03		15.8			No	No	S		
MC0181-04		4.1			No	Yes	R	R	R
MC0181-05		0.0	0.0	0.0	Yes	No	S		
MC0181-07		6.5			No	No	S		
MC0181-08		**0.0**	**0.0**	**0.3**	**Yes**	**Yes**	**R**	**R**	**R**
MC0181-09		0.0	0.0	0.0	No	No	R	R	R
MC0181-10		3.6			No	Yes	R	R	R
MC0181-11		4.7			No	Yes	R	R	R
MC0181-14		0.0	0.0	0.0	Yes	Yes	R	S	R
MC0181-15		**0.9**	**0.0**	**0.0**	**Yes**	**Yes**	**R**	**R**	**R**
MC0181-18		**0.0**	**0.0**	**0.0**	**Yes**	**Yes**	**R**	**R**	**R**
MC0181-21		19.9			No	Yes	R	R	R
MC0181-22		18.7			No	Yes	R	R	R
MC0181-23		3.2			No	No	S		
MC0181-24		**0.0**	**0.0**	**0.0**	**Yes**	**Yes**	**R**	**R**	**R**
MC0181-25		11.7			No	No	S		
MC0181-26		8.8			No	No	S		
MC0181-27		13.7			No	Yes	R	R	R
MC0181-28		**0.0**	**7.1**	**0.0**	**Yes**	**Yes**	**R**	**R**	**R**
MC0181-29		**0.0**	**0.0**	**0.0**	**Yes**	**Yes**	**R**	**R**	**R**
MC0181-30		**0.0**	**0.0**	**0.0**	**Yes**	**Yes**	**R**	**R**	**R**
MC0181-31		**0.0**	**0.0**	**0.0**	**Yes**	**Yes**	**R**	**R**	**R**
MC0181-32		**0.0**	**0.0**	**0.0**	**Yes**	**Yes**	**R**	**R**	**R**
MC0181-33		**0.0**	**0.0**	**0.0**	**Yes**	**Yes**	**R**	**R**	**R**
MC0181-34		3.3			No	No	S		
MC0181-37		**0.0**	**0.0**	**0.0**	**Yes**	**Yes**	**R**	**R**	**R**
MC0181-40		3.0			No	No	S		
MC0181-45		1.3	0.0	0.2	No	No	S		
MC0181-46		0.6	0.0	0.1	No	No	S		
MC0181-47		14.0			No	Yes	R	R	R
MC0181-48		2.6	5.5	0.0	Yes	No	S		
MC0181-49		0.0	0.0	0.0	Yes	No	S		
MC0181-50		0.0	0.0	0.0	Yes	No	S		

*In field, covered smut evaluated as % infected heads; in greenhouse, evaluated as % infected plants.
**R - no infected head; S - any infected head. Loose smut inoculations were performed in the field and inoculated seeds were grown in the greenhouse for disease development and vice versa.

Loose smut screening showed TR251 resistant (no infected head) and Q21861 and CDC McGwire susceptible. Twenty-one DH lines showed resistance and for 33/35 lines the phenotype and genotype data agreed. Resistant lines were screened twice to confirm their resistance. All but one were resistant in the two subsequent tests.

Testing of putative resistant DH lines three times against covered smut and loose smut, showed 12 lines resistant to both the diseases and positive for both markers, proving indirect selection using molecular markers is feasible. All 12 lines are being tested for agronomic and quality traits during 2005.

Marker-assisted Backcrossing

Plants were genotyped in each generation and plants positive to both markers were backcrossed to CDC McGwire. The number of BC_1F_1, BC_2F_1 and BC_3F_1 plants genotyped are shown in Table 2 and plants segregated in a 1:2:2:1 ratio for the markers as expected for two independent loci. In the BC_3F_2 generation, a high number of plants were positive to either Un8 and/or UhR450 markers because these are dominant, thus we were unable to distinguish between homozygous and heterozygous plants. These plants were screened with SCAR marker $Un8_{700}S$ and RAPD marker $OPJ10_{450}$ to identify the plants homozygous for the markers.

Table 2. Genotyping of Backcrossed Plants with Un8 and UhR450 Markers

Generation	Total plants screened	Positive to both markers	Run8	UhR450	No marker
BC_1F_1	166	27	79	68	46
BC_2F_1	240	61	119	115	67
BC_3F_1	103	22	51	52	21
BC_3F_2	186	99	136	131	18

Evaluation of 10 lines against covered smut in the field in 2004 and twice in the greenhouse indicated all were resistant (Table 3). These lines, along with the parents and the check, were tested twice against loose smut. All lines were resistant. These lines are being tested again in the field for loose and covered smut to exclude the possibility of escapes.

Blind selection based on the markers was conducted until the BC_3F_2. In every generation, plants for backcrossing were selected based only on genotype. We were fortunate to have markers linked to susceptible alleles, thus were able to identify homozygous plants for resistance to both diseases in the BC_3F_2. The resistance of BC_3F_3, BC_3F_4 and BC_3F_5 lines to both covered and loose smut proves MAS is practical. These lines are more than 93% similar to CDC McGwire as we started with 50% CDC McGwire in each parent. Phenotypically, they are very similar to CDC McGwire. These lines are being tested in BC_3F_6 generation against loose and covered smut to confirm reactions. Lines showing resistance to both the diseases are being evaluated in 2005 yield trials. As these lines are very similar to CDC McGwire limited testing should be required to detail overall performance. This material may be released as a new cultivar - fully smut resistant hulless barley! Release of these MAS-improved cultivars will demonstrate the power of this technology. These results confirm that molecular markers can assist in rapid introgression of disease resistance genes into elite lines with considerable savings in time and cost.

Table 3. Screening of Backcrossed Lines against Loose Smut and Covered Smut

Barley lines	Test	Loose smut*		Covered smut reaction**		
				Field 2004	Gh winter 2004	Gh Spring 2004
		Fall 2004	Spring 2005	% infected heads	% infected plants	% infected plants
CDC Candle	check	S	S	65.1	87.5	71.4
Q21861	parent	S	S	0.1	0.0	0.0
TR251	parent	R	R	2.5	35.7	37.5
CDC McGwire	parent	S	S	4.4	50.0	25.0
SH041241		R	R	0.0	6.7	0.0
SH041242		R	R	0.0	0.0	0.0
SH041243		R	R	0.0	5.9	0.0
SH041244		R	R	0.0	0.0	0.0
SH041245		R	R	0.0	0.0	0.0
SH041246		R	R	0.0	0.0	0.0
SH041247		R	R	0.0	0.0	0.0
SH041248		R	R	0.4	0.0	0.0
SH041249		R	R	0.0	0.0	7.1
SH041250		R	R	0.0	0.0	5.6

*R - Resistant, no infected head; S - Susceptible, one or more infected heads.

**In field, covered smut was scored as % infected heads; in greenhouse, scored as % infected plants.

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Coupling expressed sequences and bacterial artificial chromosome resources to access the barley genome

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Abstract

Barley is an important cereal crop with a size of approx. 5300 Mb per haploid genome. This is too large to be considered for whole-genome sequencing. But barley genome resources including the Morex BAC library, abundant ESTs, and 22K microarray enable researchers to access the barley genome. We aim to couple these resources to accelerate a transition to comprehensive physical mapping and sequencing of the barley "gene-space". We utilized unigene sequences to design more than 12,600 36-mer "overgo" probes to identify Morex barley BAC clones that carry expressed genes. These BAC clones will be fingerprinted to create BAC contigs, and a minimal set will be identified. In Phase I of this project, 21,161 BACs identified in our own work and that of A Kleinhofs, G Muehlbauer, R Wise, P Hayes, K Gill, N Stein, MA Saghai Maroof and co-workers were fingerprinted, with 13,067 BACs assembled into 2262 contigs comprising ca 9.4% (470 Mb) of the genome. These results are available through the "The Barley Genome" website http://phymap.ucdavis.edu:8080/barley/. In Phase II, more than 7700 abiotic stress related genes (drought, salinity, low temperature or ABA treatment) were identified using the Affymetrix Barley1 GeneChip. In total ~7000 overgos have been used as of June 2005. Of these, about 2149 overgo probes were related to an objective to genetically map 1000 genes associated to abiotic stress. For the purpose of anchoring these abiotic stress related regions on the genetic map, we investigated single feature polymorphisms (SFPs) using the Barley1 GeneChip data using Morex, Steptoe, Oregon Wolfe Barley (OWB) dominant and OWB recessive. We also developed a single nucleotide polymorphisms (SNPs) database from HarvEST:Barley EST sequences. A high throughput method for SNP mapping with R Waugh and N Rostoks (Scottish Crop Research Institute; SCRI) and N Stein, R Varshney and A Graner (Institute of Plant Genetics and Crop Plant Research; IPK) is in progress. Polymorphisms, and genetic and physical map data, will be added to HarvEST:Barley (http://harvest.ucr.edu). Phase III has a goal of probing the BAC library with the remaining ~5500 overgos to identify around 60,000 gene-bearing BACs in all, and to fingerprint and align them into contigs to derive a physical map of the overall minimal set.

Introduction

Triticeae genomes contain at least 80% of repetitive DNA (Bennet and Leitch, 1995), which has so far prevented the Triticeae from becoming the focus of large-scale genomic sequencing projects. In recent years, however, a number of barley genomic resources such as ~400,000 ESTs representing about 70% of all genes, the 6.4X Morex BAC library (Yu et al. 2000), cDNA libraries, several widely used mapping populations, a 22K microarray and its diploid nature have made barley a model Triticeae crop to access its genome.

The most commonly used probes for screening arrayed libraries have been sub-cloned DNA fragments, PCR amplified products (Xu et al. 1998) or DNA oligonucleotides (Klein et al. 2000). A novel approach for making probes, developed by Ross et al. (1999), has the advantage of oligonucleotides and also yields slightly larger probes with better hybridization kinetics and higher specific activity of labeling. These probes, termed overgos are made by annealing two 24-bp oligonucleotides with an 8-bp overlapping region at the 3' end and filling in the overhanging bases with Klenow enzyme and radiolabeled nucleotides. Multiplexing of overgos enables the hybridization of large numbers of probes in a single experiment. For example, 10,642 overgos designed from ESTs were applied to 165,888 maize BACs in a 24×24×24 experimental design with an 88% success rate (Gardiner et al. 2004).

In our effort to isolate a large number of BAC clones from gene rich loci in the barley genome, we have developed a novel strategy that integrates the technical advantages of currently available library screening methods. We modified the labeling protocol and developed stringent criteria for the selection of sequences used for overgo probes. We developed software that can extract overgos from unique as well as popular sequences from the HarvEST:Barley database.

Following this approach, we designed a total of 12,661 "overgos". The "OligoSpawn" website http://oligospawn.ucr.edu provides access to elements of our oligo design algorithms. We have been able to pool >200 overgo probes per hybridization for highly parallel hybridization-based screening of the Morex barley BAC library.

Materials and Methods

Barley BAC library. The library was derived from DNA of cultivar Morex using restriction endonuclease *Hind*III. This library consists of 313,344 individual clones stored in 816 384-well microtiter plates. This library provides about 6.3 haploid genome equivalents with an average insert size is 106 kb. The library is arrayed on 17 high-density DNA filters for screening by hybridization (http://www.genome.clemson.edu).

Oligonucleotide probe design. A computer program "OligoSpawn" was used to design the overgo probes used in this study (Zheng et al. 2004, http://www.oligospawn.ucr.edu). A total of 18,766 overgos were designed from the probesets used in 22K barley GeneChip (Close et al. 2004), and of these 9500 were selected for the present studies. The rest of the overgos (around 2600 for this project) were designed from unigenes that were not covered as probesets on the barley gene chip. These latter probes were chosen on the basis of functional categories of the unigenes from which they were derived.

Oligonucleotide probes and probe pairs. All barley oligonucleotide primers were purchased from Illumina Inc. (San Diego, CA). Each oligonucleotide was synthesized as a 22-mer at 25 nmol scale, dissolved in 250 μl of TE buffer, and diluted 50-fold in the final probe pair mix (final concentration 1 μM).

Oligonucleotide probe labeling. Ten microliters of each probe pair mix was labeled in a separate well of a 96-well PCR microplate with 10 μl of freshly prepared master mix composed of 4.0 μl of 2.5X Overgo Labeling Buffer, 1.0 μl 2 mg/ml acetylated bovine serum albumin (BSA)

(Promega), 0.125 µl of all the four radioactive nucleotides ([α-^{32}P] dATP, [α-^{32}P] dCTP, [α-^{32}P] dGTP and [α-^{32}P] dTTP) each at a concentration of 10 µCi/µl (~ 3000 Ci/mmol) (Perkin Elmer) and 1 unit of Klenow enzyme (New England Biolabs). A dNTP solution composed of 10 mM each of four non-radioactive dNTPs was used for cold chase. An oligonucleotide pair with sequences 5'-AACGGGCGAGTGATGTAAAATA-3' and 5'-TGATGGGATCGGGCTATTTTAC-3' was used as background overgo to light up all of the bacterial clones. Labeling reactions were carried out at room temperature for 1 h followed by addition of 5 µl of the cold chase solution to each of the reaction tubes. Later, all the reactions were pooled and probes were denatured at 95°C for 5 min and immediately transferred to the hybridization tubes containing prehybridized high density BAC membranes.

High-density filter hybridization. Hybridization was performed in 40 ml of Church's buffer at 60°C for two nights in a hybridization oven. After hybridization, membranes were extensively washed in solutions with increasing stringency starting with 2 liters of 4X SSC with 0.1% SDS followed by 2 liters of 1.5X SSC with 0.1% SDS and finally with 2 liters of 0.75X SSC with 0.1% SDS at 50°C. Membranes were then sealed in plastic wrap and exposed to Kodak X-ray films (Kodak BIOMAX MS Double Emulsion, 24 x 30 cm) at -80°C for 5-6 days.

Results

Phase I

As the initial step to compile all the barley resources, all of the available BAC addresses from major barley genomic researchers were collected. A total of 21,616 BAC addresses were compiled from seven sources including those identified from our own work and that of A Kleinhofs, G Muehlbauer, R Wise, P Hayes, K Gill, N Stein, MA Saghai Maroof and co-workers. The majority of these BAC clones were identified using mapped cDNA probes, while most of the others were recognized using EST-derived overgo probes. In Phase I of this project, an attempt was made to fingerprint all of these BACs, with 15,513 clones ultimately used for FPC assembly. Of these, 13,067 BACs assembled into 2262 contigs, while 2446 were singletons. These 2262 contigs account for 470 Mb which is about 9.4% of the barley genome. All data is publicly available at the "Barley Genome" website http://phymap.ucdavis.edu:8080/barley/ providing access to BAC contig data.

Phase II

The strategy that we have developed consists of identifiying gene-containing BAC clones through hybridization to pools of overgos designed from EST-derived unigene sequences in the HarvEST:Barley database. We design overgos using algorithms available through the OligoSpawn website (http://oligospawn.ucr.edu). OligoSpawn provides efficient selection of two types of oligos, namely unique and popular, from large unigene datasets. In the context of BAC library screening, unique oligos serve to unambiguously link one gene to BAC clones, while the purpose of popular oligos is to identify the largest possible list of gene-bearing BAC clones using the smallest number of probes. In order to obtain oligos for many genes of interest, and to probe selectively by functional category, we created a local information management software called oSearch. The majority of the overgos were derived from probesets on the Affymetrix barley GeneChip that were up- or down-regulated during abiotic stress including salinity, drought, low temperature or ABA treatment. This software recommends a 36-mer for each unigene or probeset and lists all unigenes represented by a given 36-mer.

Using oSearch we have generated a total of 12,661 overgos to be used in this work. Through early June 2005, a total of about 7000 overgos had been used, generally in pools of 96 to 300 simultaneous probes, most often 192 probes per pool. The reading of positive BAC addresses from all these hybridizations using Incogen's High Density Filter Reader software (http://www.incogen.com) is in progress. Interestingly, results using 96 popular overgos detected about 4000 positive BAC clones, which is about 40 per overgo, about 6-7 times the expected frequency of probes representing unique genes. This result seems to validate our hypothesis that popular overgos provide economical screening of genomic libraries for gene-bearing clones that carry sequences found in numerous genes. Another result from screening the BAC library with 576 overgos, a mixture of unique and popular, identified more than 5000 positive BAC clones with an average of 9.2 clones per probe (Figure 1).

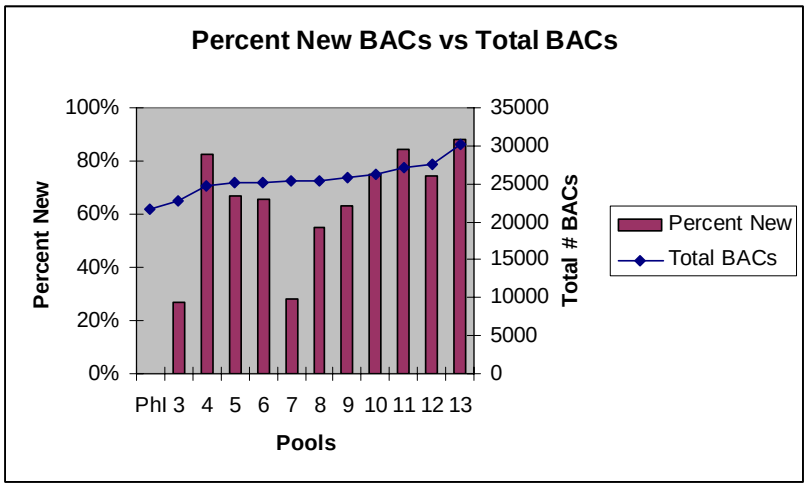

Figure 1: This chart shows the percent new BACs in each pool for this first 13 pools that we used, as well as the total number of BACs that were identified up to that point.

In order to tie the physical map of BAC clones to the genetic linkage map, around 1000 genes with unambiguous BAC address and contigs will be mapped. To identify and map these unigenes we developed a two-pronged approach based on single nucleotide polymorphisms (SNPs) and single feature polymorphisms (SFPs). SNP discovery was done "*in silico*" using a relaxed assembly (#32) from HarvEST:Barley and 36 pairwise comparisons between eight barley genotypes. This resulted in 12,615 eSNPs in 3509 unigenes, of which 29 of 32 (91%) randomly chosen eSNPs were validated by direct sequencing. Of these 3509 unigenes, only the subset in the abiotic stress list has been further considered for our mapping purposes. We combined our list of SNPs with others provided by collaborators N Rostocks and R Waugh at SCRI and N Stein, R Varshney and Andreas Graner at IPK. SNPs from 565 and 217 unigenes were provided by SCRI and IPK, respectively, the former list being mainly a subset of the abiotic stress unigene list that we previously shared with our SCRI colleagues. The collective list of SNPs was used to design of an Illumina Oligo Pool Assay (OPA). The OPA is a high throughput genotyping

platform designed to genotype 1536 loci simultaneously. Among these, we hope to map as many as 1000 related to abiotic stress in order to accomplish our abiotic stress gene mapping objective. We plan to genotype 96 maplines each from Steptoe x Morex, Barke x Morex, and Oregon Wolfe Barley (OWB) dominant and recessive parents. Also 96 different cultivars, landraces and elite lines will be examined. Designs have been finalized for the Illumina OPA chip. To identify the BAC clones corresponding to these ~1000 abiotic stress genes, about 2149 corresponding overgo probes have been used to screen the BAC library filters.

For our second approach we investigated single feature polymorphisms (SFPs) using the Affymetrix Barley1 GeneChip hybridized with labeled cRNA from the parents of each of three barley mapping populations: OWB dominant x OWB recessive, Steptoe x Morex, Barke x Morex. We developed a detection method using the robustified projection pursuit (RPP) method in order to evaluate the overall differentiations of signal intensities of probe sets comparing two genotypes and to measure the individual contribution of each probe, from which the probes covering polymorphisms (SNPs or INDELs) can be identified (Cui et al., submitted). We randomly selected SFP-containing unigenes for sequence validation and found that 59 of 72 were validated (82%). A total of 2090 SFPs were detected of which 844 (722 probe sets) were abiotic stress responsive as defined by our expression data. A 12,000 probe Nimblegen array was designed to further test the performance of SFPs and optimize SFP representation. The results from the Nimblegen chip indicate that a 25-mer with the polymorphic nucleotide(s) positions within a central region of 6-18 nucleotides is best suited for obtaining higher signal intensity differences between the polymorphic parents.

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Analysis of barley necrotic mutants in relation to disease resistance/susceptibility

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Plants respond to pathogen attack with complex signaling and defense mechanisms including hypersensitive response, which results in rapid cell death. Numerous mutants, resulting in unregulated cell death, have been identified in many species. In barley, such mutants are called "necrotic" while in Arabidopsis they are labeled "lesion mimic" to suggest their involvement in mimicking response to pathogen attack. Lesion mimic or necrotic mutants have been extensively characterized in maize (reviewed in Johal et al., Bioessays 17:685, 1995) and Arabidopsis (reviewed in Lorrain et al., Trends Plant Sci. 8:263, 2003). In barley, the most famous necrotic mutant is *mlo* (Wolter et al., Mol. Gen. Genet. 239:122, 1993), but otherwise such mutants have received only limited attention. The wild-type *Mlo* gene encodes a unique membrane anchored protein with six membrane-spanning helices and a postulated dual negative control function in leaf cell death and onset of pathogen defense (Buschges et al., Cell 88:695, 1997). The recessive *mlo* allele confers durable broad-spectrum resistance to almost all known isolates of the biotrophic fungal pathogen *Erysiphe graminis* f. sp. *hordei* (powdery mildew) (Jorgensen, Euphytica 63:141, 1992). However, all *mlo* lines are hyper-susceptible to the hemibiotrophic fungi *Bipolaris sorokiniana* (teleomorph *Cochliobolus sativus*) and *Magnaporthe grisea* (Kumar et al., Phytopath. 91:127, 2001; Jarosch et al., Mol. Plant-Microbe Interact. 12:508, 1999). Since the survival of hemibiotrophic pathogens in their necrotrophic phase depends on host cell death, the lack of *Mlo* gene function may antagonize plant defenses to these organisms. The simplest interpretation of these observations may be that increased susceptibility to cell death, as in necrotic mutants, may be antagonistic to biotrophic organisms and favor necrotrophic organisms. Here we report the identification of fast neutron induced mutants FN085 and FN338 as allelic to the barley *nec1* locus. By homology to the Arabidopsis *Hlm1* gene, the *Nec1* gene encodes the cyclic nucleotide-gated ion channel (CNGC) 4 protein (Rostoks et al., submitted). This protein belongs to a family of proteins that are weakly selective cation channels, permeable to K^+, Na^+ and/or Ca^{++} and regulated by cyclic nucleotides and calmodulin. In Arabidopsis the *hlm1* allele confers increased resistance to *Pseudomonas syringae* pv. tomato (Balague et al., Plant Cell 12:365, 2003). We tested these mutants and several other necrotic mutants for their reaction to stem rust *Puccinia graminis* f. sp. *tritici* (B. Steffenson, unpublished). The CNGC4 mutants FN085, in susceptible cv. Steptoe background, and FN338, in resistant cv. Morex background, did not differ from the wild-type in their reaction to the stem rust pathotype MCC, thus this mutation did not affect susceptibility or resistance to the pathogen. Surprisingly four other fast neutron-induced necrotic mutants, all in susceptible cv. Steptoe background, showed remarkable resistance to the stem rust pathotype MCC, while several others showed no change in their response when compared to the wild-type. In order to identify the genes involved, the four resistant mutants were subjected to analysis on the Barley 1 Affymetrix microarray. Preliminary data indicate that several genes are deleted in each mutant. These could be multiple deletions at several loci or due to one large deletion. To simplify the analysis, the mutants were backcrossed to wild-type and reselected for new analysis on the microarray. The mutants are also being tested for response to different pathogens.