The *Rpg5* NBS-LRR-STPK Gene and a Second NBS-LRR Gene are Required Together for *rpg4* Mediated Wheat Stem Rust Resistance in Barley

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Preliminary data indicated that the recessive and temperature sensitive *rpg4* gene and the dominant *Rpg5* gene are required together for resistance to *Puccinia graminis* f. sp. *tritici* races MCCF, QCCJ and TTKSK. We have cloned the *Rpg5* gene but validation of the *rpg4* gene is hindered by the complex nature of the locus. Recombinant analysis of the Q21861 (resistant) X Steptoe (susceptible) high resolution mapping population determined that *rpg4* was distinct from but tightly linked to *Rpg5*. The *rpg4* gene is required for full resistance to the wheat stem rust races but resistance is only expressed in the presence of *Rpg5*; however, *Rpg5* alone confers full resistance against the rye stem rust isolate 92-MN-90. Using virus-induced gene silencing (VIGS), we post-transcriptionally silenced each gene within the *Rpg5* genetic interval (*Rpg5*, *Hv*Rga1, *Hv*Adf2 and *Hv*Adf3) followed by inoculation with *Pgt* race QCCJ. *Rpg5* and *Hv*Adf2 were also silenced and tested with race TTKSK. Preliminary data has determined that *Rpg5* is required for *Pgt* race QCCJ and TTKSK resistance, but a second NBS-LRR gene, *Hv*Rga1, is also required for resistance against QCCJ. Thus, the *Rpg5* resistance mechanism may follow the emerging theme that pairs of unrelated genetically linked NBS-LRR genes are required for pathogen recognition and resistance. We will report on the post-transcriptional gene silencing and recombinant analysis data indicating that the complex stem rust resistance locus contains three genes required for or contributing to the expression of wheat stem rust resistance against several races including TTKSK.

Towards the Identification of Two Recessive Net Form Net Blotch Resistance Genes, *rpt.r* and *rpt.k,* in Barley

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Previous research identified two recessive net form net blotch (NFNB) resistance genes, *rpt.r* and *rpt.k,* from the barley lines Rika and Kombar, respectively. The two genes were co-localized to an ~25 cM region of barley chromosome 6H in a Rika x Kombar double haploid population consisting of 118 individuals. Here we report on the generation of a Rika x Kombar high-resolution mapping population consisting of 2,976 recombinant F2 gametes. Recombinants within the *rpt.r*/*rpt.k* region were identified using the flanking SSR markers Bmag0173 and Rbah21g15. Utilizing genome synteny between barley Ch. 6H and *Brachypodium* Ch.3 an ~ 1mb *Brachypodium* sequence was identified spanning the *rpt.r*/*rpt.k* region. Predicted *Brachypodium* genes were used to identify homologous barley ESTs. The EST sequences were utilized to develop PCR based molecular markers specific to the Rika x Kombar population allowing for the marker saturation of the *rpt.r*/*rpt.k* region. Currently we have delimited *rpt.r* and *rpt.k* to an ~0.5 cM region representing ~150 kbp of *Brachypodium* sequence. BAC clones have been identified from the cv. Morex BAC library using barley probes from the *rpt.r*/*rpt.k* region and a barley physical map across the locus is under construction. The delimited *Brachypodium* sequence contains two LRR-receptor-like gene families. Two orthologous barley genes with high homology to one of the *Brachypodium* LRR-receptor-like gene families were identified and shown to cosegregate with *rpt.r* and *rpt.k* in the high-resolution map. The candidate genes are being evaluated by allele analysis and BSMV-VIGS mediated gene silencing followed by infection type assays with NFNB isolates.

Identifying disease resistance QTLs in barley germplasm from Latin America using genome-wide association mapping

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Diseases are the main problem for barley in Latin America. Spot blotch (caused by *Cochliobolus sativus*), stripe rust (caused by *Puccinia striiformis* f.sp. *hordei*) and leaf rust (caused by *Puccinia hordei*) are three of the most important diseases that attack the crop in the region. Chemical control of those diseases is both economically and environmentally inappropriate, making the development of durable resistant varieties a priority for breeding programs. However, the availability of new resistance sources is a limiting factor. In order to identify genomic regions associated with quantitative resistance to these diseases we determined the associations between disease severities measured in several environments across the Americas and 1536 SNPs (belonging to the Barley OPA1), using a population of 360 genotypes from ICARDA and national breeding programs. We used association mapping with mixed models incorporating structure and kinship matrixes (Q+K). This model considers the structure of the population (Q) though PCA analysis and identity by descent through coancestry information (K). Preliminary results show significant marker-trait associations for spot blotch in chromosomes 1H (at 50 cM), 2H (at 39.1 and 129.3 cM), 3H (at 55 and 69.6), 4H (at 23.1 and 69.5 cM), 5H (at 50, 69.5 and 151.4 cM), 6H (at 55.6 cM), and 7H (at 21.1 and 79.6 cM). For leaf rust we detected significant marker-trait associations in chromosomes 1H (at 49.3 and 92.0 cM), 2H (at 50.6, 63.5, 149.6 and 156.7 cM), 3H (at 56.4 and 22.4), 5H (at 51-59, 130.1, and 159.7 cM), 6H (at 44.7, 55.6 and 123.8 cM), and 7H (at 1 and 98.5 cM)..Results related with stripe rust are being processed.

Individual QTLs Operative At Different Periods Within The Crop Growth Cycle Explain Anthesis Date QTLs Additivity

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We used a doubled haploid (DH) mapping population derived from the cross BCD47 x Baronesse as a tool to analyze the genetic factors controlling flowering date under Uruguayan conditions. Both parents have similar heading dates but the population shows transgressive segregation. Two QTLs - on chromosomes 2H and 3H - explained most of the phenotypic variation for anthesis date, with Baronesse contributing late alleles in 2H and BCD47 in 3H. We measured in the population the lengths of three plant developmental periods: plant emergence to tillering (E-Z20), tillering (Z20-Z30) and end of tillering to anthesis (Z30-Z65) at the field in three contrasting planting dates, and also E-Z20 at semi-controlled conditions. We performed QTL analyses on the data and detected period-specific QTL effects coincident with the main anthesis date QTL. The QTL effects on 2H (in the *EBmac684-Bmac093* interval) were coincident with QTLs for the E-Z20 and Z30-Z65 periods, whereas the QTL effects on 3H (in the *EBmac684-Bmac093* interval) were coincident with QTL effects for the length of the Z20-Z30 period. Each of the two QTLs for the end-point phenotype – anthesis date – was a determinant of flowering at a different developmental stage. No QTL by planting date interactions were detected.

Genome-wide association mapping of agronomic traits in relevant barley germplasm in Uruguay

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Information regarding genomic regions associated with key agronomic traits is essential for efficient barley breeding, but is very limited in the germplasm used in South America. The aim of this study was to determine by mapping by linkage disequilibrium (LD) the key genetic basis of agronomic traits in a population of 76 different genotypes consisting of historical varieties, commercial cultivars and advanced lines representative of barley breeding in Uruguay... The population was characterized in five contrasting environments where yield, yield components, biomass production and phenological traits were measured, and genotyped with 1033 polymorphic SNPs. We detected marker-trait associations through linkage disequilibrium mapping using a mixed linear model (MLM) Q+K containing a structure matrix (Q) and a coancestry matrix (K) obtained through pedigree data. QTL effects were detected for all traits, with some genomic regions showing a high concentration of significant associations, e.g. chromosomes 2H (mainly in two LD blocks significant for several traits), 4H and 7H. A coincidence between effects for phenology and grain size traits was observed in most of those regions. The largest number of QTL was found on environments of high yield potential.The results allow an advance in the understanding of the genetic complexity of the main agronomic goals for barley breeding in Uruguay and provide some of the first data regarding genetic basis of relevant traits in the germplasm used in the region.

QTL Identification for Seed Dormancy in the Midwest Spring Barley CAP Lines

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Seed dormancy is a mode of developmental arrest characterized by the inability of viable seed to germinate under favorable conditions. Dormant genotypes need a prolonged storage time before malting, which increases the probability of seed decay if problems during storage occur. In contrast, low dormant genotypes are more prone to have pre-harvest sprouting (PHS), which affects seed viability and makes the grains worthless for malting. Seed dormancy was determined on 3,072 spring growth habit barley lines coming from eight breeding programs participating in the USDA-NIFA Barley Coordinated Agricultural Project (CAP). Seeds were harvested at physiological maturity (Zadocks 8.9) and stored at -20°C until dormancy tests were done. Genotype data on 3,072 SNPs were gathered on the CAP lines using the Illumina GoldenGate Assay. Both phenotype and genotype data of the Midwest CAP entries were subjected to association mapping (AM) analyses across breeding all programs and years, for each of the three years across breeding programs, and each breeding program within a year using the computer software TASSEL and a mixed linear model with population structure estimated by principal component analysis (PCA) and kinship. QTL regions for seed dormancy were found on all seven chromosomes, but the most conspicuous were ones in the telomeric region on the long arm of chromosme 5H (5HL), the centromeric region on chromosome 5H, and the short arm of chromosme 4H. Additional analyses on marker-trait association between dormancy and alpha-amylase levels are currently ongoing and will be discussed.

The road to quality hulless malting barley - Where to now?

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Hulless barley malt has always intrigued brewers with significantly higher levels of malt extract, reduced amounts of spent grain and lower transportation costs. The advent of mash filters, with reduced need for hulls in the lautering process, further increased interest in hulless barley malt. Original testing of malting potential in Canadian hulless barley varieties confirmed significant improvements in extract but identified problems with adhering hulls, friability and wort ß-glucan. However, industry players and researchers indicated grain protein, malt extract and wort ß-glucan were the quality parameters to address in breeding programs of hulless malting barley. Friability was not considered a concern as malts are generally hammer milled when using mash filters. Concerted efforts to breed hulless barley specifically for malting subsequently led to several registered varieties (CDC ExPlus, Taylor) with low grain protein levels, exceptional malt extract levels, low levels of wort ß-glucan and no problems with adhering hulls. Despite availability of suitable hulless barley varieties for malting commercial demand has yet to develop. Large commercial maltsters and brewers remain skeptical due to potential processing concerns, the need for separate storage bins and general conservatism. Small micro brewers have shown some interest in this unique product and commercial quantities of hulless barley malt have recently been produced in the US. Hulless barley malt, however, will only become a commercial success if the malting and brewing industries pursue the full processing potential of the high quality, hulless malt barley varieties now available.

New algorithm improves fine structure of the barley consensus SNP map

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The need to integrate information from multiple linkage maps is a long-standing problem in genetics. One way to visualize the complex ordinal relationships is with a directed graph, where each vertex in the graph is a bin of markers. When there are no ordering conflicts between the linkage maps, the result is a directed acyclic graph, or DAG, which can then be linearized to produce a consensus map. New algorithms for the simplification and linearization of consensus graphs have been implemented as a package for the R computing environment called DAGGER. The simplified consensus graphs produced by DAGGER exactly capture the ordinal relationships present in a series of linkage maps. Using linear programming, DAGGER generates a consensus map with minimum error relative to the linkage maps while remaining ordinally consistent with them. When applied to four barley linkage maps genotyped at nearly 3000 SNP markers, in less than one minute DAGGER produced a consensus map with improved fine structure compared to the existing barley consensus SNP map. The mean error between the linkage maps and the DAGGER map was 0.65 cM per marker interval compared to 1.89 cM for the existing consensus map. Examination of the barley hardness locus at the 5HS telomere, for which there is a physical map, confirmed that the DAGGER output is more accurate for fine structure analysis. DAGGER is an effective, freely available resource for the construction of dense consensus maps.

BREEDING FOR LODGING RESISTANCE

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Lodging defined as “the state of permanent displacement of the stems from their upright positions”, is a serious problem in cereals worldwide. Lodging results in yield losses, lower grain quality and reduced efficiency of harvest. Stem lodging, which includes bending or breaking of the straw is the primary cause of field lodging in Ontario grown barley. Lodging can occur at various stages of maturity. The problem of lodging is a complex one due to the various interactions between environmental factors and the plant characteristics related to lodging. The complex interactions make selection for field lodging ineffective. While field lodging has very low heritability, several straw characteristics have been shown to have fairly high heritability thus making it feasible to use indirect selection to breed for lodging resistance through selection of straw characteristics correlated to field lodging.

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Dissection of a signaling pathway controlling barley development, senescence and grain protein concentration

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Grain protein concentration (GPC) is an important cereal quality trait. Map-based cloning of the gene responsible for a major wheat GPC QTL on chromosome 6BS has identified a NAC transcription factor, *TtNAM-B1*. Comparative analysis of a ~0.8 cM segment on barley chromosome 6HS has identified an orthologous gene, *HvNAM-1*, and found allelic differences within its coding sequence between low- and high-GPC barley germplasm. To characterize the processes controlled by *Hv*NAM-1, we have performed a transcriptomic analysis of flag leaves of near-isogenic barley germplasm varying in the allelic state of this transcription factor. Upregulation of several senescence-associated genes in high- as compared to low-GPC germplasm indicated that high GPC is functionally associated with earlier leaf senescence. The most strongly upregulated genes (at both 14 and 21 days past anthesis) included two leucine-rich repeat receptor protein kinases and a gene coding for a glycine-rich RNA-binding protein (GRP), which is highly similar to an *Arabidopsis* gene (*AtGRP7*) involved in output events from the circadian clock, in plant anthesis timing and in pathogen defense. In both barley and *Arabidopsis*, high levels of *GRP* expression are associated with earlier anthesis. While floral transition at the shoot apical meristem occurred simultaneously in low-GPC (with low *GRP* expression) and high-GPC barley grown in long days, subsequent development was faster in the high-GPC line, and sequential (pre-anthesis) leaf senescence was accelerated as well. Detailed analysis of this system will allow the dissection of a signaling pathway controlling barley development, senescence and grain protein concentration.

Development of barley with heat stress tolerance for northeastern Australia

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Merging of two germplasm pools adapted to substantially different production environments offers significant opportunities and imposes considerable challenges. In this paper we describe large scale intermating of two-rowed barley (*Hordeum vulgare*) introductions from North Dakota (ND) with genetic resources from Australia. The target production area is the northern cropping region of Australia, on the western side of the Great Dividing Range in southern Queensland and northern New South Wales, where about 20% of Australia's barley crop is grown. Spring barley is grown as a winter crop with productivity limited by variable rainfall, barley pathogens, lodging and post-anthesis heat stress. Based on yield trials conducted in the northern region, certain morphological traits were more frequent in high yielding selections having good grain quality. These traits were from ND lines and included: 1) the *sdw4* gene on chromosome 7H, a semi-dwarf gene from Chinese cultivars; 2) the *lin1* gene (2H) for a reduced number of fertile rachis nodes; and 3) the *trp1* gene (4H) for awn branching and occasionally triple awned lemmas. The latter two traits were associated with tolerance to heat stress. Since the phenotypic expression of these genes is variable and segregation for favorable traits previously considered fixed occurs in the combined germplasm pool, tracing desirable genes is based in part on molecular technology. The presentence of critical genes in elite lines was determined by using whole genome profiles based on DArT and SNP molecular markers and the concept of identity by descent for specific haplotype patterns.

CBF Transcription Factors Improveding the Frost and Drought and Frost Tolerance of of Wheat and Barley by Transgenic Plantsformation with CBF Transcription Factors

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Key words: barley, *CBFbf*, freezing, drought, transformation, wheat, barley.

Two main loci have been identified on chromosome 5 in wheat and barley that influence the capacity to overwinter in temperate climates on chromosome 5. The first major locus, was designated *FROST RESISTANCE-1 (FR-1)* co-segregates with *VRN-1*gene, which affects the vernalization requirement. The second locus, designated *FROST RESISTANCE-2* (*FR-2*), is approximately 30 cM proximal to *VRN-1* and includes a cluster of 11 (or more) *C-repeat Binding Factor* (*CBF*) genes. Transcription profiling and high-density mapping strategies have been used to identify possible candidate genes for frost tolerance within the *FR2-CBF* cluster.

Currently our aim is to directly prove the role of these genes in abiotic stress acclimation processes. To doFor this, the wheat *CbfBF14* and *CbfBF15* genes were cloned into BRACT vectors especially developed for over-expression or silencing purposes. The spring barley 'Golden Promise' and the spring wheat 'Cadenza' varieties were transformed using an *Agrobacterium*-mediated and a bombardment methods. The transgene copy number was estimated by Real Time PCR. Phenotypic characterization of the transgenic lines is in progress. The frost tolerance of barley and wheat *CBF* over-expressing lines has been tested, and it was characterised by the injury of the photosystem, by examining the level of cell damage, and also by studying the level of plant survival. Osmotic stress was induced by gradually increased PEG solution in hydroponics.

Several lines showed an improved level of frost tolerance and better osmotic adjustment than compared to the control plants, indicating the involvement of these two genes in improved stress adaptation. The best performing lines were selected for further studies in the next generations.

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Genetic variation in nitrogen efficiency of spring barley

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Genetic improvement in nitrogen efficiency (NE) is important to reduce input costs and the negative impact of excessive N on the environment. Twenty-five spring barley genotypes, assembled from different sources, were evaluated for NE under low (120 kg ha^{-1}) and normal (170 kg ha^{-1}) N regimes at Lacombe, AB during the 2010 field season. The objective of the study was to determine genetic variation and heritability for NE. Nitrogen efficiency was computed as grain N yield divided by N supply, where grain N yield was the product of grain yield and grain N concentration. Analysis of variance revealed significant differences among the genotypes in grain yield, grain N content, grain N yield, and NE under both low and normal N regimes. The NE ranged from 0.41 to 0.70 under low N and from 0.35 to 0.61 under normal N. Averaged over N regimes, the best genotypes, including I09501, I09502, I09505, I09507, and I08128, had NE of over 0.6 while the inefficient check, F09438, had a NE of less than 0.4. The lines with higher NE recovered, in the grain, over 60% of the N supplied as compared to less than 40% recovery for F09438. The top NE lines also out-yielded F09438 by 60% or more. Broad sense heritability for NE was 0.56. These results indicate that genetic progress could be made in NE through selection and some of the efficient genotypes identified are being used in the FCDC crossing program to develop high yielding and NE lines.

Pathological and genotypic characterization of *Puccinia striiformis* f. sp. *hordei* isolates from central Alberta. K. Kumar[1], K. Xi[1,] M. Holtz[1], L. Langford[1,] [1]Field Crop Development Centre, Alberta Agriculture and Rural Development, 6000 C and E Trial, Lacombe, AB. Canada T4L 1W1.

Stripe rust of barley, caused by *Puccinia striiformis* Westend. f. sp. *hordei* Eriks (Psh), has recently become increasingly common in breeding nurseries and commercial fields in Alberta. Twenty three *Puccinia striiformis* Westend. isolates collected primarily from central Alberta during 2007- 2008 were identified to be *P. striiformis* f.sp. *hordei* (Psh) based on virulence tested under the artificial epiphytotic conditions on 12 barley lines each carrying zero to a few resistance genes. Out of twenty three Psh isolates tested, more than 70 precent isolates each showed different virulence pattern and was identified as individual Psh races with virulence on 3 to 10 barley differentials. Consequently, the majority of races consisted of a single isolate and the remaining few races each consisted of two isolates. The narrow virulence spectra of Psh races identified in the present study may reflect the similar virulence spectra of the races from the Pacific North West. The narrow spectrum of local Psh races is also considered to be the result of fewer resistance genes deployed in barley in Alberta. Psh was easily distinguished from *Puccinia striiformis* Westend. f. sp. *tritici* Eriks (Pst) genetically with most Psh isolates from 2007-2008 sharing identical single sequence repeats (SSR) genotypes. In 2010 a shift in the population occurred with previously rare or undetected SSR genotypes becoming dominant in the population. The two formae speciales (Pst and Psh) had overlapping virulence on highly susceptible barley and wheat, suggesting that integrated disease management should be practiced on both crops in central Alberta.

Effect of a major quantitative trait locus for pre-harvest sprouting resistance from the Australian barley cultivar 'Baudin' on malting quality.

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Pre-harvest sprouting (PHS) is a problem for many malting barley cultivars when wet conditions occur prior to harvest, but too much seed dormancy (or PHS resistance) can be detrimental in the malt house. Australian cultivars 'Chebec' and 'Stirling' possess a major quantitative trait locus (QTL) that controls PHS resistance on chromosome 5HL, while the Canadian cultivar 'Harrington' has an allele at that locus that favours susceptibility to PHS and increases malt extract, diastatic power, alpha amylase and free-amino nitrogen (FAN). This study was undertaken to determine the effect of the major QTL for PHS resistance from the Australian cultivar 'Baudin' on PHS and malting quality in a cross with the Canadian breeding line TR253. An experiment was conducted over 6 site-years with 26 recombinant inbred lines (RIL's) carrying Baudin's PHS resistance allele, 26 RIL's carrying the TR253 allele for susceptibility and high malting quality, 2 parents, and 6 other cultivars differing in PHS resistance and malting quality. As a group, the RIL's with the Baudin allele had higher PHS resistance as indicated by higher Rapid Visco Analyzer (RVA) stirring numbers, but poorer malting quality for nearly all traits including barley protein content, germination energy at 8 ml, malt extract, malt protein, soluble protein, ratio of soluble to total protein, diastatic power, alpha-amylase, beta glucan, viscosity, wort colour and FAN. No differences were found for germination energy at 4 ml or friability. Individual RIL's carrying the Baudin allele but with more acceptable malting quality will be examined in another study.

Mapping QTL Controlling Fermentable Sugars in a Narrow Cross

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The requirements for brewing beer from barley (*Hordeum vulgare* L.) malt are specific and unique for each brewer. Two major brewers that utilize six-rowed barley malt in the United States, Anheuser-Busch InBev (ABI) and MillerCoors Brewing Company (MillerCoors), require malt with different quality parameters. Robust and Stander are two closely related cultivars that differ greatly in agronomic performance and malt quality. Robust fits the requirements of MillerCoors and Stander has many of the parameters desired by ABI. A total of 54 doubled-haploid (DH) lines and the parents from the Robust x Stander cross were evaluated in 10 locations in North Dakota the past five years. Agronomic data were collected at all locations and cleaned samples from six of the locations were micro-malted at NDSU. A linkage map using 168 DArT and SSR markers was constructed and used for identification of QTL controlling agronomic and malt quality traits. QTL for the different fermentable sugars including glucose, maltose, and maltotriose mapped to chromosomes 4H, 5H, and 6H. An additional 139 Robust x Stander DH lines were generated in 2009, and will be evaluated with the current DH population in the summer of 2011 at three locations. A map utilizing all 193 DH lines is being developed using existing DArT and SSR markers, and 200 new SNP markers. The new map will be used to better localize QTL controlling agronomic and malt quality traits in the larger population.

A gene for basal host and nonhost resistance in barley to *Puccinia* rust fungi

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In barley (*Hordeum vulgare* L.) two types of resistance occur to the leaf rust fungus *Puccinia hordei* Otth, viz. (1) the major genic resistance based on *Rph* genes that cause a hypersensitive resistance, and (2) partial or basal resistance that inherits polygenically and is not based on hypersensitivity, but associated with longer latency period and lower infection frequency despite a compatible infection type. *Rph* gene based resistance is notoriously isolate specific and not durable, but basal resistance is effective to all isolates and believed to be durably effective. Although the nature of the genes underlying basal host resistance is unknown, there is evidence that basal host resistance is a quantitative form of PAMP triggered immunity, and hence a mild form of nonhost resistance.

One of the most consistent quantitative trait loci (QTL) uncovered in the L94 × 'Vada' mapping population was *Rphq2* located on chromosome 2HL. This gene was introgressed by marker-assisted backcrossing into two susceptible lines to obtain near-isogenic lines (QTL-NILs). One of these lines, SusPtrit, is an experimental line that was developed to be susceptible to several heterologous rust fungi, and is a tool to study the genetic basis of nonhost resistance.

We developed two BAC libraries to achieve map-based cloning of the gene. We also phenotyped the QTL-NILs with several rust isolates and species. We report on the fine map position of the *Rphq2* gene and on its effect on infection by *P. hordei* and several rust fungi to which barley normally is a nonhost.

Response of Some Canadian Barley Genotypes Grown in a Stem Rust (Ug99) Environment

Joseph M. Nyachiro[1]*, James H. Helm[1], Kequan Xi[1], Patricia E. Juskiw[1], Laura L. Hoge[1] and Ruth Wanyera[2]

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Stem rust caused by *Puccinia graminis*: Pers. f. sp. *tritici* and f. *secalis* Eriks and E. Henn is a destructive disease of barley and wheat in warm, moist environments. The reactions of a range of Canadian barley genotypes, comprising varieties and advanced breeding lines from the Field Crop Development Centre (FCDC), to stem rust including the new race *Ug99* were screened at the National Plant Breeding Research Centre, Njoro, Kenya in 2009 and 2010. Each year 140 genotypes were planted on 1-m plots in two replicates arranged in a randomized block design with spreader rows comprised of a mixture of very susceptible barley varieties and wheat including 'Morocco'. Infection was natural with some artificial inoculation using a hand sprayer and syringe. Stem rust scoring was done on two dates taking into account rust infection types (R=resistant, MR=moderate resistant, MS=moderate susceptible and S=susceptible), and disease infection severity of 0 to 100%. Spreader rows had 70-80S infection. There was a general increase of disease infection type and percent severity from the first to the second score date. The check varieties showed variable responses to stem rust. 'Falcon', a six-rowed hulless barley, appeared to be very susceptible, whereas 'AC Metcalfe' and 'Seebe', both two-rowed hulled barley, showed good resistance to stem rust. The advanced breeding lines had stem rust resistance ratings ranging from 5R/5MR to susceptible. Generally, the two-year results indicate there are some promising stem rust (Ug99) R-genes in barley genotypes from the FCDC breeding program.

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Crop Development Centre, University of Saskatchewan Hulless Food Barley Varieties – from CDC Candle & CDC Alamo to CDC McGwire, CDC Rattan, CDC Fibar & CDC Hilose.

B.G. Rossnagel, A.D. Beattie, T. Zatorski, and G.J. Scoles. Crop Development Centre, University of Saskatchewan, Saskatoon, SK, S7N 5A8, Canada.

Hulless food barley development has been an ongoing objective of the Crop Development Centre, University of Saskatchewan barley breeding program for more than 25 years. While emphasis has been on the development and release of high beta-glucan waxy starch and high beta-glucan, high amylose starch types, several normal starch CDC hulless varieties have also contributed in the food barley arena. The first 95% amylopectin starch waxy variety released was CDC Candle in 1995 followed by the first 100% amylopectin starch waxy variety CDC Alamo in 1999. More recent releases in 2003 include the 95% amylopectin starch variety CDC Rattan which demonstrates significant agronomic improvement, being shorter and more lodging tolerant with higher yield potential and improved threshability, and the very high beta-glucan (10%), 100% amylopectin starch variety CDC Fibar. The most recent development is the high beta-glucan, high amylose (40%) starch variety CDC Hilose released in 2010. Along with these high beta-glucan specialty starch varieties, the highly productive, agronomically superior normal starch hulless variety CDC McGwire (released in 1999) has become the standard for hulless barley performance in western Canada and has also found a role in food barley development in the region.

CDC Meredith, CDC Reserve and CDC Kindersley - Newest Crop Development Centre, University of Saskatchewan Two Row Malting Barley Varieties.

B.G. Rossnagel, A.D. Beattie, T. Zatorski, B.L. Harvey and G.J. Scoles. Crop Development Centre, University of Saskatchewan, Saskatoon, SK, S7N 5A8, Canada.

After the development and release of the world leading two row malting barley variety Harrington in 1981, the University of Saskatchewan malting barley breeding program followed a two pronged approach for subsequent variety development. One breeding stream targeted malting and brewing end-users interested in a similar high enzyme "Harrington-type" variety resulting in the release of CDC Kendall in 1996, while the second approach was aimed at end-users desiring a somewhat lower malting enzyme package resulting in the release of CDC Copeland in 1999. Both varieties represented significant agronomic improvements and have been widely grown across western Canada. From an end-user perspective both have found success in domestic and international markets. To complement these two breeding streams, the CDC breeding program has recently placed increased emphasis on selection for lower grain protein to assist farmers in achieving malting grade and to increase malt extract levels for end-users, as well as selection for lower malt beta-glucan and more uniform/balanced modification to make our product more attractive to malting/brewing end-users. These efforts have resulted in the development and release of the moderate enzyme low protein variety CDC Meredith in 2008 and the higher enzyme, low malt beta-glucan CDC Kindersley in 2010. In collaboration with Sapporo Breweries, the program also released the relatively high enzyme, low protein, sprouting tolerant variety CDC Reserve in 2008. In addition to malting/brewing quality improvement, these varieties again represent significant advantage in agronomic performance for western Canadian barley growers. Commercialization of these varieties is underway with CDC Meredith most advanced in the marketplace.

The Road to Hulless Malting Barley Varieties at the Crop Development Centre, University of Saskatchewan – from CDC McGwire to CDC ExPlus and HB08304.

B.G. Rossnagel, A.D. Beattie, T. Zatorski, and G.J. Scoles. Crop Development Centre, University of Saskatchewan, Saskatoon, SK, S7N 5A8, Canada.

For the past 35 years hulless barley development and breeding have been a significant segment of the Crop Development Centre, University of Saskatchewan barley research and breeding program. Since the release of the first hulless feed variety Scout in 1982 program emphasis has shifted to hulless barley for food and malting/brewing. The high performing agronomically superior variety CDC McGwire released in 1999 set the stage for and provided the baseline for further hulless malting barley variety development. CDC ExPlus, with improved malting quality, was released in 2009. It has been followed by HB08304 (to be released in 2011) which demonstrates agronomic improvement versus the standard CDC McGwire and further malting/brewing quality advantage versus CDC ExPlus, having lower grain protein, lower malt beta-glucan and increased malt enzyme activity.

Collection and uses of genetic diversity

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There are clear evidences of early cultivation as well as signs of initial domestication of barley from *Hordeum vulgare* ssp. *spontaneum* ca. 10,000 years ago at The Fertile Crescent. Rather soon after the domestication six-rowed (9,500 years ago) and naked (8,000 years ago) types appeared. Barley reached India 8,000 years ago already. About 3,000 years ago cultivation of barley reached China and later, six-rowed hulled and naked barley became essential crops for feed and food supply in the ancient agriculture in Japan. During these distribution processes, significant diversity was formed and localized in each area. Collection of diversity is an important objective for genebanks. Recent activities of barley collection missions by the author in the areas eastwards of center of origin will be introduced.

One of the efficient ways of using diversity is to develop backcross introgression populations (also called recombinant chromosome substitution lines; RCSLs). This provides the opportunity to assess unadapted alleles in an adapted genetic background. This system is also useful to separate quantitative trait loci (QTL) to simplify genetic analyses to individual loci. By using Illumina OPA system, several sets of RCSLs with the genetic background of Japanese malting barley Haruna Nijo have been developed and used for the genetic analyses. The same genotyping format is also applied for the diversity estimation of barley core collection subset and used to develop further materials for genetic analyses.

Teaball Redux, or, The Upside to Downsizing. Mark R. Schmitt and Allen D. Budde. USDA ARS Cereal Crops Research Unit, 502 Walnut St., Madison WI 53726. 608-262-4480 (voice), 608-890-0302 (fax), mark.schmitt@ars.usda.gov.

Recently, we have described a number of modifications to current mashing protocols and subsequent wort and malt analysis procedures that substantially reduce the quantities of barley and malt needed for characterization of many of the primary malting quality attributes commonly examined. Results from these reduced-scale mash and QA procedures are very highly correlated with results from standard-scale procedures. In this presentation, we describe a modification of common micromalting protocols that requires only 2 g of barley lines to be malted, but provides sufficient quantities of malt for the primary malting quality analyses. In addition, the sample multiplexing used in this method can increase sample throughput 2x – 3x over current micromalting capacity with minimal staffing requirements, Briefly, we contain the small amounts (2 g) of barley inside stainless steel mesh teaball (TB) infusion containers. A number of individual TBs are then embedded within a larger volume of carrier barley that is steeped, germinated, and kilned in existing malting equipment. A few modifications to the standard malting protocols optimize grain hydration and result in malt with primary malt quality parameters similar to standard micromalted grain. The greater throughput of these combined methods raises the numbers of samples that can be analyzed annually. Importantly, this approach can substantially improve data turnaround time in the critical window between crop harvest and the preparation for the subsequent field programs. The small scale malting and analysis procedures may also be useful for research or other programs using populations that have limited seed availability.

Genetic analyses of *CBF* gene copy numbers at *FROST RESISTANCE-2*

Taniya Dhillon and Eric J. Stockinger, Department of Horticulture and Crop Science, The Ohio State University/OARDC, Wooster, OH 44691, USA

The C-Repeat Binding Factors (CBFs) are transcriptional activator proteins that play a key role effecting freezing tolerance in plants. They were originally identified from *Arabidopsis thaliana* using a functional screen for proteins capable of binding to the CRT/DRE, a cis-acting low temperature DNA regulatory element. Approximately 4% of the Arabidopsis protein-encoding genes are directly or indirectly regulated by the CBFs. Activation of this set of genes leads to increased freezing tolerance.

"Translational research" studies in which we asked, what is the role of the *CBF*s in the agronomically-important barley and wheat crops is revealing a complex story in which variation in *CBF* gene copy numbers is a central theme. On the long arm of group 5 chromosome homoeologs is a cluster of 11 – and possible more – distinct *CBF* gene orthologs. Approximately half of these orthologs are duplicated, existing as identical or nearly identical paralogs in individual genomes. More interestingly, subsets of these orthologs appear to form a haplotype relationship with *VRN-1*, a locus ~30 cM distal to the *CBF* genes and which affects regulatory control over *CBF* expression. Winter barleys carry two to eight copies of a 22 kb genomic segment encompassing *CBF2A* and *CBF4B*, and a *vrn-H1* allele that allows expression of the *CBF* genes. Spring barleys in contrast carry single copies of *CBF2* and *CBF4*, and a *Vrn-H1* allele that restricts *CBF* gene expression. In the allohexaploid wheat genome multiple copies of a different ortholog, *CBF14*, occurs in winter wheats, while single copies occurs in spring wheats – and these distinctions occur on all three homoeologs.

Genetic analyses now reveal association between copy numbers of the 22 kb *CBF2A–CBF4B* barley genomic segment and expression levels of other *CBF* orthologs in the cluster, despite an absence of detectable copy number differences of these other *CBF*s. We are working to better understand this relationship and how it in turn affects freezing tolerance.

This work was supported by grants from the Ohio Plant Biotechnology Consortium (2010-011), and USDA-CSREES subaward CO396A-F.

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Detecting and mapping breeding progress in UK elite barley cultivars.

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We have utilised the high throughput SNP genotyping platform Barley Oligo Pooled Array 1 (BOPA1) to genotype 1000 elite barley lines with 1536 genic markers to characterise the variation that exists amongst UK elite barley varieties and associate sequence variants with differences in performance and morphological characters. Over 500 of these lines had been evaluated in spring and winter barley National and Recommended List trials between 1988 and 2006 and thus an extensive body of performance data (yield, height, disease resistance, quality etc.) and morphological data used in assessing Distinctness, Uniformity and Stability (DUS) already existed for these lines. Additionally we grew a subset of lines representing market successes and failures over our survey period to both provide an unambiguous estimate of breeding progress and additional data to improve the prediction of means of varieties that generally were not grown in the same trials. We have combined the genotypic and phenotypic data in Genome Wide Association Scans to find that not only are DUS characters controlled by genes on each of barley's seven chromosomes but also that they are largely independent of genomic regions controlling performance characters, although we did detect "hot-spots" for the latter class, suggesting pleiotropy, possibly due to developmental genes.

Pyramiding genes for resistance to Scald by marker assisted selection. J. Zantinge*, P. Juskiw, K. Xi, S. Xue, and K. Steenbergen. Field Crop Development Centre, Alberta Agriculture and Rural Development, Lacombe, Alberta Canada T4L 1W8 (e-mail: jennifer.zantinge@gov.ab.ca).

Scald of barley (*Hordeum vulgare L.)* caused by the fungus *Rhynchosporium secalis* (Oudem) J.J. Davis, is prevalent in central Alberta, Canada and causes considerable yield and quality losses. Combining several scald resistance genes in a single cultivar may give rise to more long-term control of a fungi that can rapidly change in pathotype composition and frequency. Selection for disease resistance in breeding programs can be hampered by year to year environmental variability. Molecular markers for resistance to scald have been identified at the Field Crop Development Centre (FCDC) to mark the resistance genes inherited from 'Seebe', a two-row general purpose barley released in 1992, with good resistance to the scald pathogen in Alberta. Barley breeders at FCDC use a modified bulk program for the majority of their breeding efforts. In 2009, 700 F_7 lines from six populations were evaluated for scald resistance using the SSR marker Ebmac635 linked to scald susceptibility. Of these entries, 285 had the susceptibility marker. After agronomic and quality selection, 22 lines were advanced to first year yield tests in 2010 and screened in the Lacombe scald nursery. Fifteen lines rated as R or MR, six rated as I and only one line rated as S. Additional scald resistance markers were identified from the 2009 populations. These were included into MAS in 2010 on 1,300 F_7 lines from seven populations. In 2011, we will again evaluate the efficiency of the markers by comparison with reactions in the Lacombe scald nursery and continue MAS on an additional 3,100 F_7 lines from 16 populations.