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Poster Abstracts

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Table of contents

Poster Abstracts

Theme 4: Molecular Breeding for Rust Resistance

55. Characterization of the durable leaf rust resistance gene *Lr34* in European winter wheats

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Many European winter wheats (EWWs) have been reported to possess high levels of adult plant resistance (APR) and/or slow rusting to the leaf rust pathogen *Puccinia triticina*. However, the identities of the genes conferring APR in these wheats are largely unknown. Currently, there are seven genetically characterized APR genes (*Lr12*, *Lr22a*, *Lr22b*, *Lr34*, *Lr35*, *Lr46* and *Lr67*). Of these, *Lr34* is considered to be the most durable and widely utilized in wheat. To assess the potential presence of *Lr34* in 135 EWWs, the molecular marker csLV34, closely linked to *Lr34*, was applied. The marker confirmed the presence of *Lr34* in 7 (5%) wheats (Forno, Lona, MV Palma, Panda, Pegaso, Sarka and Viginita). All of these cultivars showed high levels of APR to leaf rust in the field when tested against prevalent Australian *P. triticina* pathotypes, to which they were susceptible as seedlings. Two of the seven resistant cultivars (Sarka and Viginita) were from the Czech Republic, two (Forno and Lona) from Switzerland, two (Pandas and Pegaso) from Italy and one (MV Palma) from Hungary. Pedigree analyses identified the cultivar Frontana (derived from Mentana) in the pedigree of Lona; and Bezostaya (traced back to Ardito) in the pedigrees of Pandas, Pegaso and Sarka. A set of wheat cultivars (including Beaver, Estica, Georg and Mec) was identified with high levels of APR despite lacking *Lr34*, indicating the presence of APR genes different from *Lr34*. Genetic studies have been initiated on these cultivars to determine if they carry new genes conferring APR.

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56. Dissecting adult plant stripe rust resistance in the wheat cultivar Cappelle Desprez

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Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most important diseases of bread wheat in South Africa. The deployment of disease resistant cultivars is the most cost-effective approach to minimise crop losses. Although the full complement of stripe rust resistance in Cappelle Desprez remains unclear, its resistance has been incorporated into many European varieties and has proven to be effective in South Africa. The objective of this study was to validate *Yr16* and identify undetected adult plant resistance in Cappelle Desprez using a QTL mapping approach. Yr16DH70, a Cappelle Desprez derivative, was crossed with the spring wheat cultivar Palmiet to develop a recombinant inbred line (RIL) mapping population, devoid of resistance genes expressed in seedlings. SSR and DArT markers were typed in the population. The presence of the *Yr16* gene on chromosome 2D was confirmed as well as major QTL on chromosomes 2A and 5B. Markers are being implemented in tracking Cappelle Desprez's resistance in South African wheat breeding programs.

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57. Quantitative resistance conferring durable leaf rust resistance in wheat cultivar Toropi

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Leaf rust appears almost everywhere wheat is grown, making resistance to the causal agent, *Puccinia triticina* Eriks., an important issue in breeding programs. The deployment of durable resistance is the best strategy worldwide to protect wheat against leaf rust; however, only genes *Lr34*, *Lr46* and *Lr67* are described as capable of conferring durable resistance. Toropi, a Brazilian variety, has maintained its resistance for more than 40 years, indicating the presence of durable resistance. To characterize Toropi resistance and to fine map the gene involved, double haploid and backcross populations were developed by crossing Toropi and Thatcher. These populations were tested in the greenhouse and under field conditions in Canada and New Zealand. Toropi had good leaf rust resistance in the range of 0 to 10MS during three years in Canada and 0 in New Zealand. Segregation data indicated the presence of one seedling resistance gene, a dominant race-specific adult plant resistance and two complementary race non-specific APR genes in Toropi. The seedling and race-specific APR genes do not confer good resistance in the field in Canada, but seem to be effective in New Zealand. Resistance conferred by the complementary race non-specific APR was effective in both locations. The combination of all genes conditioned an almost immune phenotype, typical of Toropi, which was also resistant to both stem rust and stripe rust. Further experiments are being conducted to confirm the rust resistance in Toropi, and to fine map the genes involved.

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58. Discovery, mapping, and validation of QTL conferring partial resistance to broadly-virulent post-2000 North American races of the stripe rust pathogen

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A hexaploid wheat mapping population of 186 recombinant inbred lines developed from a cross between UC1110, an adapted Californian spring wheat, and PI 610750, a synthetic derivative from CIMMYT's wide-crossing program, was evaluated for response to current Californian races of *Puccinia striiformis* f. sp. *tritici* in replicated field trials over four seasons (2007-2010) in the northern Sacramento Valley. Using 1,493 polymorphic probes (SSRs, DArTs, and ESTs) mapped to 559 unique loci, QTL analysis revealed four stripe rust resistance QTL segregating in this population; two from UC1110 (on chromosomes 3BS and 2BS) and two from PI 610750 (5AL and 2AS). The two QTL with largest effects (on 3BS and 5AL) were validated in independent populations and their intervals narrowed to 2.5 cM and 4.7 cM, respectively. The 3BS QTL (*QYr-ucw.3BS*) was shown, by a test of allelism and genotype, to carry a gene different from the *Yr30*/*Sr2* complex. The mapped position also suggests that *QYr.ucw-3BS* is associated with a gene different from either *Yrns-B1* or *YrRub*, two stripe rust resistance genes mapped to this region in other studies. The 5AL QTL carries a previously unreported partial stripe rust resistance gene, designated here as *Yr48*. This poster presents an initial characterization of these four QTL and reports the availability of donor lines and markers to interested breeding programs.

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59. QTL mapping for adult-plant resistance to stripe rust in Italian common wheat cultivars Libellula and Strampelli

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Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is a devastating disease of common wheat (*Triticum aestivum* L.) worldwide. Deployment of wheat cultivars with adult-plant resistance (APR) is the most environmentally friendly and economical way for controlling this disease. In the present study, 1,136 SSR markers were used to map QTLs for APR to stripe rust in two F_3 populations with 255 and 252 lines derived from the crosses Libellula/Huixianhong and Strampelli/Huixianhong, respectively. Composite interval mapping detected five QTLs for APR in Libellula, designated *QYr.caas-2DS*, *QYr.caas-4BL*, *QYr.caas-5BL.1*, *QYr.caas-5BL.2* and *QYr.caas-7DS*, respectively, explaining from 2.6–35.0% of the phenotypic variance. The QTLs *QYr.caas-4BL*, *QYr.caas-5BL.1* and *QYr.caas-7DS* were also detected in Strampelli. Three interactions between different pairs of QTLs accounted for 6.1-35.0% of the phenotypic variance. The QTL *QYr.caas-7DS* flanked by markers *csLV34* and *Xgwm295* showed the largest effect for resistance to stripe rust. Sequence analyses confirmed that lines with the *QYr.caas-7DS* allele for resistance carried the resistance allele of *Lr34/Yr18/Pm38*. SSR markers *Xgwm165* and *Xgwm149*, *Xwmc415* and *Xwmc537*, and *csLV34*, were closely linked to *QYr.caas-4BL*, *QYr.caas-5BL* and *QYr.caas-7DS*, respectively. These markers could be used for marker-assisted selection in wheat breeding.

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60. The presence of *SrCad* and *Sr2* influences reaction to stripe rust and *Fusarium* head blight

R. M. DePauw[1], A. Singh[1], A. K. Singh[1], R. E. Knox[1], D. Singh[2], S. Bhavani[3] and T. Fetch[4]

AC Cadillac has stem rust resistance genes *Sr2* and *SrCad* that confer resistance to races TTKSK and TTKST. Carberry has *Fhb1* providing some resistance to Fusarium head blight (FHB), but linked in repulsion to *Sr2* on chromosome 3BS. Both lines have the *Lr34* gene linked to *Yr18*. This study was conducted to determine the effect of *SrCad* and *Sr2* loci on Fusarium head blight and to see if these loci also affect stripe rust response in a Canadian wheat background. Stripe rust and FHB were assessed in the Carberry/AC Cadillac doubled haploid (DH) population of 219 DH lines. Parents and DH lines were evaluated with molecular markers linked to *Sr2* (*Xgwm533* and *X3B028F08*) and *SrCad* (*Xcfd49*). FHB incidence and severity were evaluated in 2010 near Portage La Prairie, Canada, whereas stripe rust was rated for severity and pustule type in the 2009 rust nursery near Njoro, Kenya. AC Cadillac was resistant to stripe rust while Carberry had higher severity and pustule type ratings than AC Cadillac. The AC Cadillac *Sr2* marker molecular variants were significantly associated with reduced stripe rust severity and pustule type. Association of *Sr2* and stripe rust response could be due to *Yr30* known to be in the *Sr2* region. No significant *Sr2* by *SrCad* interaction was observed for stripe rust response. The Carberry molecular variants for both the *Sr2* and *SrCad* loci were associated with reduced severity and incidence of FHB. In addition to *Fhb1*, Carberry appeared to have another factor for resistance on chromosome 6D. Lines possessing resistance to stem rust, stripe rust and FHB were identified; lines with *Fhb1*, *Sr2* and *Yr30* in coupling will be useful for further genetic enhancement.

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61. Multi-environment quantitative trait loci analysis for resistance to stripe rust and Cephalosporium stripe in two recombinant inbred line populations.

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Stripe rust (*Puccinia striiformis*) and Cephalosporium stripe (*Cephalosporium gramineum*) can cause severe yield and grain quality losses in wheat (*Triticum aestivum L.*) in the Pacific Northwest, USA. Favorable weather conditions and new races are important factors for annual development of stripe rust in the region. Cephalosporium stripe can be a limiting factor in the adoption of conservation tillage practices and little is known about the inheritance of resistance. Two recombinant inbred line (RIL) populations (Einstein x Tubbs and Tubbs x NSA 95-0995) were assessed for quantitative trait loci (QTL) analysis of responses to stripe rust and Cephalosporium stripe. A linkage map was created for each population based on diversity array technology (DArT) and simple sequence repeat (SSR) markers. Field assessments of stripe rust response were done in six environments under natural inoculation. Field screening of response to Cephalosporium stripe under artificially inoculated conditions and based on whitehead frequencies (sterile heads caused by pathogen infection) was done in three environments for the Einstein x Tubbs population and in two environments for the Tubbs x NSA 95-0995 population. Results from the QTL analysis identified common regions associated with resistance to stripe rust and Cephalosporium stripe. More thorough assessments with additional phenotypic data and production of a denser genetic map are ongoing.

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62. Investigating the role of *SrCad* and *Sr2* on stem rust race TTKST in wheat

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Stem rust race TTKST with virulence to *Sr24* and *Sr31* is a threat to wheat production globally. AC Cadillac expresses resistance to TTKST. AC Cadillac has *Sr2* and *SrCad*, whereas Carberry has neither. Both lines are thought to have other unidentified *Sr* genes, and both have *Lr34* which reportedly works synergistically with *SrCad*, where *SrCad* stem rust resistance is better in the presence of *Lr34*. The objective of this study was to use a doubled haploid (DH) population derived from Carberry/AC Cadillac to study the effect of *Sr2* and *SrCad* in controlling TTKST. The parents and 219 DH lines were evaluated to TTKST in a stem rust nursery near Njoro, Kenya, for adult plant stage resistance while seedling reactions against TTKSK were evaluated in a bio-containment facility (Morden, Canada). Stem rust severity and pustule type were recorded. Parents and DH lines were evaluated with molecular markers linked to *Sr2* (*Xgwm533* and *X3B028F08*) and *SrCad* (*Xcfd49*). In the DH population, *Xgwm533* and *X3B028F08* mapped 3 cM apart. Single factor analysis of variance indicated that *Xcfd49* was significantly associated with seedling resistance as well as adult plant resistance in the 2009 and 2010 Kenya nurseries. The *Sr2*-linked markers were significantly associated with resistance associated with pustule type in 2010. No significant *SrCad* by *Sr2* interaction was observed. Results suggested that *SrCad* provided good resistance to race TTKST in an *Lr34* background and simultaneous presence of *Sr2* with *SrCad* did not confer significantly better resistance than *SrCad* alone.

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63. Using molecular markers to detect favorable linkages between *Sr2* and *Fhb1* in SRWW germplasm

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Since the 1930s, the gene *Sr2* has served as a source of durable adult plant resistance to stem rust of wheat, caused by *Puccinia gramini* f. sp. *tritici* Pers.. The *T. turgidum* L. ssp. *dicoccum*- derived introgression carrying *Sr2* is located on the short arm of chromosome 3B in the same region as the *Fhb1* locus conferring resistance to Fusarium head blight, caused by *Fusarium graminearum* Schwabe. Isolation of *Sr2* and *Fhb1* in the same cultivar requires selection for recombination between the resistance genes. This favorable recombination event has been difficult to select for using traditional disease screening given the large population sizes needed and the quantitative nature of resistance conferred by *Fhb1* and *Sr2*. We used simple sequence repeat and single nucleotide polymorphism markers to detect recombination on 3BS in a population of 384 F_2 plants generated from crosses between soft red winter wheat germplasm carrying the introgressed genes. The observed linkage distance between the marker UMN10 (closely linked to *Fhb1)* and csSr2 (closely linked to *Sr2)*, was approximately 10 cM. Fourteen recombinant plants having marker alleles associated with resistance in coupling were identified. Test-crosses with susceptible lines were made that will be used to confirm the presence of *Sr2* and *Fhb1* in coupling by disease screening. In addition, crosses have been made to pyramid these linked genes with other rust resistance genes in SRWW backgrounds. Pyramiding of FHB and stem rust resistance genes should generate useful germplasm for wheat breeding programs.

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64. Quantitative trait loci for adult plant resistance to wheat stem rust in cultivar K-Nyangumi

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The challenge posed by Ug99 and other *Puccinia graminis* f. sp *tritici* races has necessitated faster discovery of effective sources of resistance to stem rust and their efficient use through marker assisted selection. The Kenyan bread wheat cultivar K-Nyangumi exhibits adult plant resistance in the field, but is susceptible at the seedling stage. To dissect this resistance, the cross K-Nyangumi/PBW343 was made and 223 F_6 RILs generated by single seed descent were inoculated and evaluated for adult plant stem rust resistance for two seasons at Njoro-Kenya in 2010. RILs were phenotyped for leaf rust resistance at Cd. Obregon, Mexico during 2009-2010 season. Results indicated continuous variation implicating quantitative resistance. Inclusive composite interval mapping (ICIM) (http://www.isbreeding.net) software was used to analyze the QTL maps with 599 polymorphic DArT markers. Preliminary analysis revealed three chromosomes - 1B, 3B and 6A - significantly associated with stem rust resistance and explaining 5%, 23% and 5% of the phenotypic variation, respectively. These results paved the way for current fine mapping of the significant genomic regions. The identified markers are candidates for MAS.

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65. Mapping resistance to race Ug99 stem rust in Norin 40 (*Sr42*)

***H. Ghazvini, C. Hiebert, T. Zegeye and T. Fetch*[1]**

Stem rust of wheat can be controlled by deploying cultivars carrying effective resistance genes. Ug99-type stem rust races are virulent to many designated stem rust resistance (Sr) genes, but resistance is available. Unfortunately, much of the resistance to Ug99 was derived from beyond the primary gene pool of wheat, which decreases the desirability of those sources of resistance due to decreased end-use quality or agronomic performance often associated with alien transfers. Thus, Sr genes conferring resistance to Ug99 that are from the primary gene pool represent valuable genetic resources. *Sr42*, previously discovered in Norin 40, is one such gene. The purpose of this study was to map *Sr42* with DNA markers. A doubled haploid population consisting of 252 lines from the cross LMPG x Norin 40 was tested with TTKST, a variant of Ug99 that is virulent to *Sr24*. There were 123 resistant and 129 susceptible lines which fitted a single gene ratio (p = 0.71). Simple sequence repeat (SSR) markers specific to chromosome 6D, the previously described location of *Sr42*, were tested for linkage. *Sr42* mapped to the short arm of chromosome 6D and the nearest SSR marker was *gpw5182* with a genetic distance of 1.2 cM. *Sr42* maps to the same region as *SrCad*, another Sr gene that confers resistance to Ug99. While *SrCad* confers resistance to races virulent to *Sr42*, further research is needed to determine if these two resistances represent unique loci.

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66. The effect of *Lr34* on wheat stem rust responses

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The gene *Lr34* conditions adult plant leaf rust resistance, which has been durable in Canada and throughout the world for over 50 years. This gene also conditions resistance to stripe rust (*Yr18*), powdery mildew (*Pm38*), and barley yellow dwarf virus (*Bdv1*). Resistance to stem rust, conditioned by *Lr34,* was reported by Dr. Peter Dyck in the 1980s. Recent evidence has further demonstrated that *Lr34* provides stem rust resistance independently, and enhances the expression of other stem rust resistance genes. To investigate the effect of *Lr34* on stem rust response, a backcross population was created in the Sumai 3 background. Sumai 3 has *Lr34*, but carries no seedling or additional adult plant resistance to stem rust. Pairs of sister lines were selected in 25 Sumai 3*6/Thatcher families, with one sister line homozygous resistant *Lr34Lr34* and the other homozygous susceptible *lr34lr34*. These pairs of sister lines were grown in a stem rust-inoculated nursery at Winnipeg in 2010. In all pairs of lines, the sister line with *Lr34* had an intermediate stem rust response and average severity of 40%, whereas the sister line without *Lr34* had a susceptible stem rust response and average severity of 80% and was badly lodged. This study demonstrates that *Lr34* conditions resistance to stem rust.

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67. Identification of QTLs associated with adult plant resistance to stem rust race Ug99 in the 'Avocet' x 'Pavon76' recombinant inbred line population

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Stem rust, caused by *Puccinia graminis* f. sp. *tritici* (Pgt), has historically caused severe losses to wheat production worldwide, but was effectively controlled by resistance for almost 30 years. However, stem rust re-emerged in 1998 in eastern Africa in the form of race Ug99. Furthermore, it has u ndergone changes to produce separate variants with added virulence to resistance genes *Sr24* and *Sr36*. The lineage is once again threatening the global wheat crop. The objective of our study was to identify genomic regions contributing to APR to stem rust in 'Pavon 76'. A recombinant inbred line (RIL) population of 298 lines was previously developed at CIMMYT from a cross between 'Avocet S' and 'Pavon 76. The RILs segregated for APR to Ug99 when evaluated in Kenya for three years. Single year and combined year analyses by inclusive composite interval mapping using 450 DArT markers identified six quantitative trait loci (QTLs) contributing to resistance . avon 76' contributed three QTLs, *Sr2*-linked *QSr.cim-3B-1*, *Lr46/Yr29/Pm39*-linked *QSr.cim-1B* and *QSr.cim-3D* (possibly an ortholog of *Sr2*), which explained 32%, 24% and 20% of the phenotypic variance, respectively. The remaining three QTLs, *QSr.cim-3B-2*, *QSr.cim-4B* and *QSr.cim-5A*, were contributed by 'Avocet' and explained 24%, 8% and 6% of the phenotypic variance, respectively. Our results indicate that it is possible to accumulate several minor genes (each with a small-to-intermediate effect) to produce a variety that exhibits negligible disease levels even under high stem rust pressure.

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68. The presence *Sr2* resistance reinforced *Sr24* against the virulent race TTKST

P. N. Njau[1], S. Bhavani[2], B. Keller[3], J. Huerta-Espino[4] and R. P. Singh[2]

Utilization of stem rust resistance genes in breeding programs resulted in effective control of stem rust in most countries during the second half of the 20th century. However, the detection of stem rust race Ug99 in East Africa in 1998, a *Pgt* race with broad virulence, and adaptive capacity, and frequent stepwise mutations to new strains, indicated that continued efforts were necessary to control the disease. This study was aimed at evaluating the effectiveness of quantitative resistance in reducing the susceptibility of wheat in case a major gene breaks down during the onset of a new virulent race. In 2005 a trial was set to evaluate the effectiveness of *Sr24* in controlling Ug99. The trial consisted of wheat lines with *Sr24±Sr2*, and was carried out for four seasons; viz. 2005, 2006 and 2 seasons in 2007. During the first two seasons, *Sr24* gene was very effective conferring a near immune response. In the last season of 2007 a new race identified as TTKST and virulent to *Sr24* appeared in Kenya. The results indicated that lines combining *Sr24* and *Sr2* remained resistant whereas those lacking *Sr2* were susceptible scoring up to 90S. These results imply that deployment of quantitative disease resistant genes in a major gene resistance background may safeguard wheat from an epidemic in case of evolution of a virulent race like TTKST. Breeding to incorporate both major and minor genes may increase durability.

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69. Gene-gene interaction reveals complexity of resistance to race Ug99 in wheat

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The recent emergence of wheat stem rust Ug99 and evolution of new races within the lineage threatens global wheat production because they overcome widely deployed genes that had been effective for many years. To identify loci conferring resistance to stem rust in wheat, we previously employed an association mapping approach for 276 spring wheat breeding lines, from the International Maize and Wheat Improvement Center (CIMMYT), phenotyped for stem rust resistance at the adult plant stage in two stem rust resistance screening nurseries in Njoro, Kenya in seasons 2008, 2009 and 2010 where races of the Ug99 lineage predominate. We identified 15 loci associated with stem rust resistance. Using the same data and a Q matrix in the present study, we investigated the interactions among marker loci using linear regression models to calculate p-values for pairwise marker interactions. Resistance marker loci including the *Sr2* locus on 3BS and the *wPt-1859* locus on 7DL had significant interaction effects with other loci on the same chromosome arm and with markers on chromosome 6B. Similarly, five markers on chromosome arm 1DL interacted with both the resistance loci, *wPt-7750* on 2BL and *wPt-9822* on 7DL. Other resistance marker loci had significant pairwise interactions with markers on different chromosomes. Based on these results, we propose that a complex network of gene-gene interactions is in part responsible for resistance to Ug99. Further investigation may provide insight for understanding mechanisms that contribute to this resistance gene network.

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70. Genome-wide markers can predict adult plant resistance to wheat stem rust

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Genomic selection (GS) prediction models for stem rust adult plant resistance (APR) were created and validated using a training population consisting of lines from the 2nd SRRSN (Stem Rust Resistance Screening Nursery), selected candidates of the 5th and 6th SRRSN, and 3 biparental APR mapping populations. The lines were phenotyped for adult plant resistance to stem rust race Ug99 and/derivatives at KARI, Njoro, Kenya. Lines with low reactions characteristic of major gene resistance were removed from the training population. Diversity Arrays Technology (DArT) markers were used for genome-wide genotyping of the training population. Marker imputation, implemented in MACH 1.0, a Markov Chain based haplotyper, was used to unite datasets genotyped with different sets of markers. Four different statistical models were applied and compared for prediction accuracy. Accuracies were calculated using 5-fold cross validation, where accuracy was measured as the Pearson's correlation between the genomic estimated breeding values (GEBVs) and best linear unbiased predictors (BLUPs) of the phenotypes. Removal of 10 empirically identified outlying individuals led to gains of 0.1 in accuracy. Prediction accuracies ranged from 0.2 to 0.8 with a mean of 0.6, indicating that GS would lead to an increase in gain from selection per unit time compared to phenotypic selection.

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71. Association mapping to identify stem rust resistance loci in durum wheat germplasm

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A panel of 183 elite durum accessions suitable for association mapping was characterized for stem rust response in four artificially inoculated field trials in Ethiopia using race Ug99 and a bulk of other highly virulent Ethiopian *Pgt* races. The seedling responses of the accessions were determined using several *Pgt* races at CDL, St. Paul MN. The phenotypic distributions indicated that the overall genetics of resistance is complex. Twenty-five resistant accessions were identified (DSS below 20%). The molecular genotypes of the accessions using 300 SSR and 900 DArT markers were subjected to association mapping analysis. Several chromosome regions putatively involved in stem rust response, both under field conditions and at the seedling stage, were identified. Genotypes at the *Sr13* locus (chromosome 6AL) were involved in both field and seedling tests and a resistant haplotype tagged by a series of SSRs was often present (*wmc580*, *gwm427* and *barc104*). Significant associations were also detected on chromosomes 1B (*gwm11*, *wmc128* and *gpw3013*) and 2B (*wmc257*, *gwm1300* and *wmc356*) as well as other regions. These results suggest that durum has several genes for resistance to Ethiopian races, including Ug99, and that there is potential to exploit some of them in breeding.

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72. First report of slow rusting gene *Lr46* in durum wheat

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Leaf rust (LR) (caused by *Puccinia triticina*) poses a major threat to durum wheat (DW) (*Triticum turgidum* ssp. *durum*) production in developing countries. The most effective way to control LR is through the deployment of resistant cultivars. Even though new sources of resistance have been identified in CIMMYT germplasm, several genes have lost their effectiveness due to the appearance of new virulent races in Mexico. The CIMMYT DW line 'Quetru' is moderately resistant to LR in the field displaying severities of 15MR-50MR depending on the environment. An F_5 population was developed from the cross of Quetru with highly susceptible 'Atil*2/Local Red' for investigating the genetic basis of resistance. A total of 113 F_5 lines were evaluated under high disease pressure in Obregon in 2009-10 and in El Batan in 2010 . At least two genes that interact in an additive manner governed resistance in the field. One of the resistance genes in Quetru seemed to be a race-specific kind since Quetru displayed IT 'X' on adult plants based on greenhouse tests, but susceptible infection type on seedlings. The second gene in Quetru was found to be *Lr46* based on molecular analysis using the associated marker developed by CSIRO, Australia. Phenotypic reductions of 50-58% in Obregon for lines in the population carrying the *Lr46* positive marker allele, and of 14-29% in El Batan were observed. This is the first report of *Lr46* in DW. This gene plays an important role in enhancing resistance in gene combinations and further studies are needed to investigate its frequency in DW.

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73. Highly expressed RPG1 protein in a five-copy *Rpg1*-transgenic barley line results in susceptibility to stem rust

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The stem rust resistance gene *Rpg1* has provided durable resistance in barley for over 60 years in the Upper Midwest region of the United States. In previous research, a susceptible barley cultivar Golden Promise was transformed into a resistant cultivar and a single copy of *Rpg1* was sufficient for resistance. However, other transgenic lines with different copy numbers exhibited different infection phenotypes. Thus, to investigate the relationship between *Rpg1* transgene copy number and stem rust resistance level, we selected five transgenic lines containing different copy numbers of *Rpg1* and tested them for stability of transgene inheritance, level of *Rpg1* transcript and protein expression, and degradation of RPG1 protein upon stem rust infection. Southern blot transgene copy number estimation revealed unstable transgene inheritance over several generations in a five copy T_0 line. All transgenic lines exhibited higher transcription and protein levels than cultivar Morex (the cultivar from which *Rpg1* was cloned), but the relationship between these factors and copy number was not linear. Western blot assays of RPG1 protein degradation after challenge by avirulent stem rust pathotype MCCF revealed rapid degradation between 20-28 hours post-inoculation in all transgenic lines, except the susceptible five-copy line G04-288.

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74. 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 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.

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75. Genetics of resistance to stem rust race TTKSK in barley landraces from Switzerland

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Stem rust race TTKSK poses a serious threat to both wheat and barley worldwide. Several barley landraces from Switzerland were found to possess a high level of resistance to this race at both the seedling and adult plant stages. To determine the inheritance of resistance in two of these landraces (Hv545 and Hv612), crosses were made to the susceptible cv. Steptoe. F_3 families from the Steptoe/Hv545 and Steptoe/Hv612 populations were evaluated for reaction to race TTKSK at the seedling stage. The numbers of homozygous resistant : segregating : homozygous susceptible families in the Steptoe/Hv545 (χ^2=0.50, P=0.47) and Steptoe/Hv612 (χ^2=2.6, P=0.28) populations approximated a 1:2:1 ratio. These data indicate that a single gene controls resistance in both populations. Reactions of F_1 plants and proportions of resistant plants in segregating F_3 families suggested recessive gene action in both populations. Molecular mapping is currently underway to determine if the respective resistance genes map to the same chromosome 5H region containing the *rpg4/Rpg5* complex. This gene complex was derived from line Q21861 and was previously implicated in race TTKSK resistance. *Polymerase chain reactions* and sequencing indicated that Hv545 and Hv612 contain a functional *Rpg5* gene. Assays for *rpg4* and other possible genes in the complex are under development.

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76. Vulnerability of *Hordeum* germplasm to wheat stem rust race TTKSK

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The threat of stem rust (caused by *Puccinia graminis* f. sp. *tritici*) race TTKSK (aka Ug99) on wheat is widely known and is being addressed by many research groups. Comparatively little research has been advanced on barley, due to its smaller worldwide area and lower rank as a food crop. Yet in the very countries where TTKSK now exists (Ethiopia and Yemen), barley is one of the most important food crops, especially for people living at high elevation. To assess the vulnerability of *Hordeum* germplasm worldwide, over 2,600 cultivated (i.e. cultivars, breeding lines, and landraces) and wild barley (*Hordeum vulgare* subsp. *spontaneum*) accessions were evaluated at the seedling stage in the Biosafety Level 3 containment facility at St. Paul. More than 90% of the cultivated germplasm was susceptible to race TTKSK. A similar frequency of susceptibility was also observed in *H. vulgare* subsp. *spontaneum* accessions. These data clearly demonstrate the extreme vulnerability of *Hordeum* germplasm to African stem rust. Wheat and barley are often cultivated in the same areas. To effectively control stem rust, it is essential that both crops be bred for resistance to race TTKSK, otherwise inoculum from one crop could initiate epidemics on the other.

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77. Mapping and haplotype analysis of adult plant resistance to stem rust race TTKS in barley breeding germplasm from the USA

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Race TTKS (synonym Ug99) of the wheat stem rust pathogen (*Puccinia graminis* f. sp. *tritici*) is highly virulent and capable of attacking more than 70% of wheat and barley cultivars worldwide. To identify genes conferring resistance to isolates in the Ug99 lineage in barley, we employed an association mapping approach with breeding germplasm (3,072 lines) from the U.S. Barley Coordinated Agricultural Project (BCAP). Breeding lines were genotyped with 3,072 SNP markers. Half of the germplasm (1,536 lines) was phenotyped for adult plant response (0-100% severity scale) at the Kenya Agricultural Research Institute, Njoro, Kenya, in 2009 and the other half (1,536 lines) in 2010. Disease pressure was high in 2009 with a mean rust severity of 37.2%. Rust infection was lower in 2010 with a mean severity of 5.4%. Association mapping analyses were performed separately for the two groups of germplasm. One single nucleotide polymorphic (SNP) marker on the long arm of chromosome 5H was significantly associated with adult plant resistance in 2009. The same marker was again found significantly associated with resistance in spite of the lower disease severity in 2010. Resistant and susceptible haplotypes were flanked on the left by two distal SNPs and on the right by three proximal SNPs--a region spanning 10 cM on chromosome 5H. Mean disease severities were significantly lower in both years for lines with the resistance haplotype. The haplotype for resistance found in this study will be useful for marker-assisted development of stem rust resistant barley cultivars.

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78. Molecular tagging of an Ug99-effective stem rust resistance gene *Sr28*

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Stem rust of wheat, caused by *Puccinia graminis* f. sp. *triticii* (Pgt), is a major threat to global wheat production. Detection of Pgt pathotype Ug99 (TTKSK) in Kenya rendered *Sr31* and many other stem rust resistance genes ineffective. An Indian wheat cultivar VL404 produced high infection type (IT3+) against the prevalent Pgt pathotypes of Australia. It showed a low seedling infection type (IT; to 2-) when tested against TTKSK and TTKST in the USA. A recombinant inbred line population (RIL) from the cross VL404/WL711 was phenotyped using the Pgt pathotype TTKST. Chi-squared analysis conformed to segregation at a single locus and the locus was temporarily named as *SrVL*. Molecular mapping using DArT and SSR markers placed it in the long arm of chromosome 2B flanked by markers *gwm120* and *wmc175* at 3.3cM each proximally and distally. Comparison of infection type and relative position of markers in chromosome 2BL led us to conclude that *SrVL* was *Sr28*. Virulence for *Sr28* has been reported in all wheat growing areas of the world. The identification of flanking markers in this study would enable pyramiding of *Sr28* with molecularly tagged stem rust resistance genes *Sr22*, *Sr26*, *Sr33*, *Sr39*, *Sr40* and *Sr50* that are effective against a wide range of Pgt pathotypes. The Australian wheat genotypes Janz and CSP44 also carry *Sr28*. The presence of *Sr28* in Janz and CSP44 was traced back to LV-Rus parent of Kota.

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