



**Borlaug Global Rust Initiative**

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# Proceedings Poster Abstracts

*Edited by Robert McIntosh*

# Poster Abstracts

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# Theme 2: New Sources of Rust Resistance for Wheat

## 13. A Survey of Genetic Variation for Adult Plant Stem Rust Resistance Among the A.E. Watkins Collection of Hexaploid and Tetraploid Wheat Genotypes

*HS Bariana, UK Bansal, H Miah, AK Toor, F Hussain, RF Park*

The pathotype 'Ug99' of the wheat stem rust pathogen was first detected in Uganda in 1999. Since its first detection, it has produced variants with added virulence for *Sr24* and *Sr36*. A strategic global effort was undertaken to tackle this menace through deployment of genetic resistance in new wheat cultivars. The identification and characterisation of diverse sources of resistance is essential to combat the threat posed by new variants of pathogens. We studied genetic variation for stem rust resistance among the AE Watkins collection of hexaploid and tetraploid wheat genotypes. A specific attempt was made to identify new sources of durable minor gene controlled adult plant stem rust resistance. Tests on these genotypes under field conditions, followed by seedling tests with the same pathotype (s) of the stem rust pathogen, indicated the presence of minor (non- hypersensitive) genes for resistance in both hexaploid and tetraploid genotypes. Genotyping using *Sr2*-linked molecular markers enabled identification of genotypes that lacked *Sr2* and carried as yet uncharacterised adult plant resistance gene(s). These putative new sources of resistance were crossed with susceptible cultivars to develop mapping populations for genetic characterisation of the resistance. Bulked segregant analysis will be performed to identify genomic regions that control adult plant resistance in some selected genotypes.

## 14. Sources of Resistance to Stem Rust Race Ug99 in Wild Tetraploid Wheat Accessions

*E Alwan<sup>1,2</sup>, FC Ogbonnaya<sup>2</sup>, B Ayele<sup>3</sup>, K Nazari<sup>2</sup>, D Worku<sup>3</sup>, O Abdalla<sup>2</sup>, SH Hakim<sup>1</sup>, G Bedada<sup>3</sup>*

Stem rust caused by *Puccinia graminis* f. sp. *tritici* race TTKS commonly known as "Ug 99" is becoming a serious threat to wheat production worldwide. To cope up with the rapidly changing stem rust pathogen, new sources of seedling and adult plant resistances might be sought from the wild relatives of cultivated tetraploid wheat. A total of 1,524 wild tetraploid wheat accessions were evaluated against the prevailing Syrian stem rust population under field conditions at the International Center for Agricultural Research in the Dry Areas (ICARDA), Tel Hadya, Aleppo, Syria.

Two hundred and thirty eight accessions with adult plant resistance were selected for further seedling and adult plant assessments at the Debre Zeit Research Center, Ethiopia; a reputed 'hotspot' site for stem rust epidemics on tetraploids. The accessions were exposed to a mixture of isolates comprising Ug99 and a local bulk of urediniospores collected from hexaploid and tetraploid wheats. About 37% and 36% of the accessions showed resistance to stem rust at the seedling and adult growth stages, respectively. About 15% exhibited resistance at both the seedling and adult plant stages, leaving 21% with adult plant resistance only. This preliminary result indicated that wild tetraploid wheats could be potentially important sources of resistance to the prevailing stem rust races including Ug99. Some accessions have been selected for repeat testing to confirm the results. Crosses between these and elite bread wheat and durum varieties have also been initiated. Further ongoing genetic and genomic studies using these accessions should identify and characterize the resistance genes and reveal potentially new stem rust resistance genes for deployment in both durum and bread wheat breeding.

The University of Sydney Plant Breeding Institute-Cobbitty, PMB11, Camden, NSW2570, Australia

<sup>1</sup>Aleppo University, Faculty of Agriculture, Field Crops Department, Aleppo, Syria; <sup>2</sup>International Centre for Agricultural Research in the Dry Areas, PO Box 5466, Aleppo, Syria; <sup>3</sup>Ethiopian Institute of Agricultural Research (EIAR), Box 2003, Addis Ababa, Ethiopia

## 15. SSR-Genotyping Of *Triticum aestivum* x *T. timopheevii* Introgression Lines and Mapping of Genes for Leaf Rust Resistance

IN Leonova<sup>1</sup>, EB Budashkina<sup>1</sup>, MS Röder<sup>2</sup>, EA Salina<sup>1</sup>

Twenty-four leaf rust resistant *T. aestivum* x *T. timopheevii* hybrid lines were developed using five common wheat cultivars. The resistances were analyzed using microsatellite markers specific for *T. aestivum* and *T. timopheevii*. Microsatellite analysis revealed two major areas of introgression of the *T. timopheevii* genome: chromosomes of homoeologous groups 2 and 5. Translocations were detected in the 2A and 2B chromosomes in 11 lines. The length of the translocated fragment in the 2B chromosome was identical in all hybrid lines and did not depend on the parental wheat variety.

The hybrid line 842-2 was used for detailed characterization of introgression and mapping of loci determining resistance to leaf rust. Molecular analysis using 350 specific short sequence repeat (SSR) markers identified genes from the *T. timopheevii* genome in chromosomes 1A, 2A, 2B, 5A, 5B, and 6B. An F<sub>2</sub> mapping population of line 842-2 crossed with common wheat cultivar Skala was used for analysis of association of phenotypic and genotypic data. Adult plant leaf rust resistance was determined by loci in chromosomes 5B and 2A. The major locus transferred from *T. timopheevii* chromosome 5G mapped to the microsatellite interval *Xgwm408* – *Xgwm1257* and controlled 72% of the phenotypic diversity in leaf rust response. The other, less effective gene was located on chromosome 2A at a distance of 10 cM from *Xgwm312*, and accounted for 7% of the trait expression. Microsatellite markers located near these loci may be used for the transfer of these valuable genes to new lines and cultivars.

## 16. Association Mapping of Loci Conferring Resistance to Race TTKSK in Cultivated and Wild Barley Germplasm

BJ Steffenson<sup>1</sup>, J Roy<sup>1</sup>, H Zhao<sup>1</sup>, Y Jin<sup>2</sup>

The threat that race TTKSK (Ug99) poses to wheat worldwide is well known and documented. However, this race also threatens barley throughout the world, including those cultivars carrying the durable rust resistance gene *Rpg1*. To identify and map loci conferring resistance to race TTKSK, we are using an association mapping approach in both cultivated (Barley Coordinated Agricultural Project or BCAP) and wild (Wild Barley Diversity Collection or WBDC) *Hordeum* germplasm. BCAP accessions were genotyped with 1,536 SNP markers and WBDC with 3,072 SNP and 558 DArT markers. Marked variation in the germplasm was observed in response to race TTKSK at the seedling stage, with some accessions exhibiting a high level of resistance. Association mapping analyses of BCAP germplasm identified resistance QTL on chromosomes 1H, 2H, 3H, 5H and 7H ( $p=2.01E-07$  to  $8.00E-04$ ,  $r^2=1.4$  to 2.4%). The QTL on chromosome 5H was coincident with the previously identified resistance gene complex *rpg4/Rpg5*. In the WBDC germplasm, QTL for resistance were identified on all seven chromosomes ( $p=0.000$  to 0.002,  $r^2=2.9$  to 7.4%). Several identified QTL on chromosomes 5H and 7H were coincident with those found in the same region of the BCAP germplasm. Additionally, QTL were found coincident with both *Rpg1* on chromosome 7H and *rpg4/Rpg5* on chromosome 5H. This work documents the power of association mapping for identifying and mapping stem rust resistance loci in cultivated and wild *Hordeum* germplasm.

<sup>1</sup>Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences, Novosibirsk, 630090 Russia; <sup>2</sup>Leibniz Institute of Plant Genetics and Crop Research, Gatersleben, D-06466 Germany

<sup>1</sup>Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, USA; <sup>2</sup>USDA-ARS Cereal Disease Laboratory, St. Paul, MN 55108, USA

## 17. R-Genes *Rpg4* and *Rpg5* are Required for Resistance to Stem Rust Race TTKSK in Barley

R Brueggeman<sup>1</sup>, B Steffenson<sup>2</sup>, Y Jin<sup>3</sup>, A Kleinhofs<sup>1</sup>

We characterized a 70 kbp genomic region from barley containing two stem rust resistance genes, *rpg4* and *Rpg5*, conferring resistance to *Puccinia graminis* f. sp. *tritici* (*Pgt*) pathotypes QCCJ, MCCF and TTKSK (Ug99), and *P. g. f. sp. secalis* (*Pgs*) isolate 92-MN-90. *Rpg5* is a novel R-gene containing a nucleotide binding site (NBS)-leucine-rich repeat (LRR) domain in combination with a serine threonine protein kinase (STPK) domain. The predicted RPG5 protein has two putative transmembrane sites, possibly involved in membrane localization and potentially presenting the LRR domain outside the cell, while the NBS and STPK domains remain intracellular. High-resolution mapping, allele and recombinant sequencing identified *rpg4* as encoding an actin depolymerizing factor-like protein (ADF2). Both *Adf2* and *Rpg5* appear to be essential for resistance against the *Pgt* pathotypes, but not the *Pgs* isolate. A possible hypothesis for *Adf2* gene function is that it might be modified by fungal invasion, activating *Rpg5* to initiate signal transduction pathways resulting in resistance. An alternative hypothesis is that *Adf2* controls actin networks that may be redirected by the fungus to obtain nutrients from the plant via a haustorial-plant interface. If the *adf2* gene is inactive or inappropriately active, the actin network required to feed the fungus might fail leading to resistance. The recessive nature of *rpg4* makes the alternative hypothesis appealing. Study of the *rpg4/Rpg5* locus may provide insight into how stem rust maintains its biotrophic life style on its host to possibly be utilized in disease management strategies.

<sup>1</sup> Department of Crop and Soil Science, Washington State University, Pullman, WA 99164, USA<sup>2</sup>. Department of Plant Pathology, and <sup>3</sup>USDA-ARS Cereal Disease Laboratory, St. Paul, MN 55108, USA

## 18. Slow Rusting Resistance to Stripe Rust and Leaf Rust in Indian Wheat Genotypes Under Artificially Inoculated Conditions

MS Saharan, AK Sharma

Stripe (yellow) rust and leaf (brown) rust of wheat are important wheat diseases worldwide, including India. Slow-rusting resistance is a useful type of rust resistance. Sets of wheat genotypes in advanced breeding trials numbering 220 (150 advanced entries and 70 checks), 247 (180 advanced lines and 67 checks) and 228 (154 advanced lines and 74 checks) were inoculated with bulked prevalent pathotypes of *Puccinia striiformis tritici* (Indian designations 67S8, 47S102, 46S103, 70S69, 46S119, 78S84) and *P. triticina* (Indian designations 12-2, 77-2, 77-5, 104-2) at Karnal during the 2005-06, 2006-07 and 2007-08 crop seasons, respectively. The rust intensities recorded at equal intervals were computed to relative Area Under the Disease Progress Curve (AUDPC) values, and the genotypes were categorized into four groups. Group I included genotypes exhibiting AUDPC values <1% of the susceptible checks Bijaga Yellow (AUDPC 2,000) for stripe rust and Agra Local (AUDPC 2,000) for leaf rust. Genotypes exhibiting AUDPC values for stripe rust in the range of 1-100, 101-200 and 201-500 were allocated to: Group II (2 genotypes in 2005-06, 15 in 2006-07, 16 in 2007-08); Group III (12 genotypes in 2005-06, 27 in 2006-07, 14 in 2007-08); and Group IV (48 genotypes in 2005-06, 57 in 2006-07, 27 in 2007-08). Similarly, of 220 genotypes evaluated during 2005-06 for leaf rust, 39, 5 and 9 genotypes were placed in Groups II, III and IV, respectively. During 2006-07, of 99 genotypes evaluated for leaf rust, 5, 27 and 22 were placed in Groups II, III and IV, respectively. During 2007-08, 13, 9 and 17 genotypes were in the Groups II, III and IV, respectively. Thirteen (WH 542, HD 2932, K 9107, HS 295, PBW 373, PBW 502, MACS 3313, GW 1189, NIDW 295, VL 882, VL 804, RAJ 3765 and HD 2833) and eight genotypes (HS 490, VL 829, HD 4717, PBW 175, PBW 373, WH 542, HUW 234 and MP 1203) generated AUDPC values of 101-500 (groups III and IV) for both rusts during 2006-07 and 2007-08, respectively. Group III and IV genotypes were characterized as partially resistant as these genotypes exhibited AUDPC values less than 50% of the checks.

Crop Protection, Directorate of Wheat Research, Karnal (Haryana)-132001, India

## 19. Identification of Chromosomal Regions Determining Leaf Rust, Yellow Rust and Stem Rust Resistances in CIMMYT Germplasm Through Association Mapping

SA Herrera-Foessel<sup>1</sup>, RP Singh<sup>1</sup>, J Crossa<sup>1</sup>, J Burgeno<sup>1</sup>, S Bhavani<sup>1</sup>, J Huerta-Espino<sup>2</sup>, S Dreisigacker<sup>1</sup>, PK Singh<sup>1</sup> and D Singh<sup>1</sup>

A historical set of 170 bread wheat (*Triticum aestivum*) lines originating from the CIMMYT 1<sup>st</sup>, 6<sup>th</sup>, 10<sup>th</sup>, 20<sup>th</sup> and 24<sup>th</sup> elite spring wheat yield trials (ESWYT) were evaluated for resistance to leaf rust (LR) (caused by *Puccinia triticina*) and stripe rust (YR) (*P. striiformis* f. sp. *tritici*) in field trials established in Mexico in 2007, and to stem rust (SR) (*P. graminis* f. sp. *tritici*) in Kenya in the off- and main seasons in 2008 under high disease pressure using prevalent races. In addition, leaf rust resistance genes present in these wheat lines were postulated from seedling reaction data obtained in the greenhouse using 13 Mexican *P. triticina* races. The ESWYT set was used previously for identifying genomic regions associated with resistance to the three rusts and other traits utilizing phenotypic data collected between 1979 and 2004, and genotypic data generated through Diversity Array Technology markers (DARs) (813 in total) together with 831 other markers. In this study, association analyses were conducted using new rust data and the previously available genotypic data. We used the mixed model for association analyses incorporating the relationship matrix comprising the coefficients of parentage among lines and the population structures. This increases the power of detecting more reliable marker-trait associations. Results reveal that markers identified to be associated with resistance to all three rusts were located on chromosome arms 1AS, 1AL, 3BS, 3BL, 4AL, 4BL, 5BS, 5BL, 6BS, 7AS, 7BL, and 7DS. Additional markers associated with rust resistance were located on the short arms of 1B (LR, YR), 2A (YR), 2B (SR), 2D (LR), 4B (LR, SR), 4D (LR), 5A (YR), 6A (SR), 7B (SR), and on the long arms of 1B (YR, SR), 1D (YR, SR), 2A (SR), 2B (LR, SR), 3A (LR, SR), 5A (LR), 6A (LR, SR), and 7A (LR, SR). The identified genomic regions carrying resistance genes are being verified through further genetic analyses.

<sup>1</sup>International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, 06600 México, D.F., México; <sup>2</sup>Campo Experimental Valle de México INIFAP, Apdo. Postal 10, 56230 Chapingo, Edo de México, México

## 20. Resistance to Wheat Stem Rust in Triticale (*X Triticosecale*)

PD Olivera<sup>1</sup>, S Gale<sup>2</sup>, L Wanschura<sup>2</sup>, M Rouse<sup>1</sup>, Y Jin<sup>1,2</sup>

Triticale (*X Triticosecale*) is an amphiploid between wheat (*Triticum aestivum*) and rye (*Secale cereale*) developed as a crop in the late 20<sup>th</sup> century, and it is grown commercially mostly in Europe, China, Australia and South Africa. Triticale is an excellent source of resistance to wheat stem rust as several stem rust resistance genes have been described. A collection of 567 triticale accessions from 21 countries was evaluated at the seedling stage for resistance to several races of *Puccinia graminis* f. sp. *tritici* with broad virulence ranges, including TTKSK, TRTTF, and TTTTF. A high frequency of resistance to race TTKSK was observed; 417 (73.5%) exhibited low infection types ranging from 0; to 2. Based on infection types, we postulated genes *Sr27*, *SrSatu* and other known or predicted *Sr* genes of rye origin. Accessions exhibiting resistance to races TTKSK, TRTTF, and TTTTF were further characterized for reaction to other races in the TTKS lineage and additional US races. Several resistant accessions from diverse geographic origins and exhibiting different infection types were selected as parents to develop crosses in an attempt to determine the genetic control of resistance to race TTKSK.

<sup>1</sup> Department of Plant Pathology, University of Minnesota, and <sup>2</sup>USDA-ARS, Cereal Disease Laboratory, St. Paul, MN 51108, USA

## 21. Characterisation of a Leaf Rust Resistance Gene Transferred into Wheat from *Aegilops speltoides*

CW Hiebert, BD McCallum, GF Marais, DG Humphreys

Wheat leaf rust can be controlled by host resistance. Relatives and progenitors of wheat have been abundant sources of leaf rust resistance (*Lr*) genes. Effective *Lr* genes were transferred from *Aegilops speltoides* to wheat by J. Dvorak and D. Knott. Subsequently, P. Dyck produced a near-isogenic line (RL6161) carrying this gene in a Thatcher background. To further characterise the resistance in RL6161, agronomic, quality and genetic tests were undertaken. Compared to the recurrent parent (Thatcher), RL6161 showed no penalty in yield or quality that sometimes accompanies alien transfers. Monosomic analysis placed the *Lr* gene on chromosome 1B. A doubled-haploid population from the cross Thatcher / RL6161 was tested with microsatellite markers specific to chromosome 1B and the results showed that the *Ae. speltoides* DNA carrying the *Lr* gene was linked to markers on the long arm. Preliminary mapping data showed that recombination occurred between the *Ae. speltoides* and wheat DNA. Therefore, lines with reduced introgression size can be identified and used as sources of resistance in breeding populations. Whereas the uniqueness of the resistance in RL6161 is not known, it is possible that the resistance gene is *Lr51*, or an allele, since *Lr51* was also transferred from *Ae. speltoides* to wheat chromosome 1BL. Experiments to demonstrate the relationship between the two resistance sources are in progress.

## 22. Mapping of New Sources of Resistance to *Puccinia graminis* f. sp. *tritici* Race Ug99

S Bhavani<sup>1</sup>, RP Singh<sup>1</sup>, J Huerta-Espino<sup>2</sup>, D Singh<sup>1</sup>, Y Jin<sup>3</sup>

One of the best approaches to alleviate the threat from *Puccinia graminis tritici* race Ug99 (TTKSK) is to identify and characterize sources of resistance within the available wheat (*Triticum aestivum*) breeding materials and commercial cultivars. Genes identified can then be deployed in combinations. Identification of molecular markers tightly linked to resistance genes can aid their pyramiding, and allow selection of plants without the need for disease screening. This is especially important with Ug99 and its derivatives, which are absent in many countries. F<sub>3</sub> and F<sub>4</sub> populations derived from the crosses of susceptible PBW343 with three resistant parents with race-specific resistance genes were developed and characterized for reaction to TTKSK in the greenhouse at USDA-ARS CDL, St. Paul, MN; and, during 2008, in the field at Njoro, Kenya, where the *Sr24* virulent Ug99 variant was present. Bulk-segregant analysis was performed to identify marker trait associations and the linked markers were used for genotyping lines clearly identified in field trials as homozygous resistant and homozygous susceptible. Genomic regions with 3 putative new resistance genes, temporarily designated as *SrA*, *SrB* and *SrC* were identified. Gene *SrA* was mapped on chromosome 3DL (linked markers, *Xgwm52*, *Xgwm341*) of Milan/Sha7/3/Thb/CEP7780//Sha4/Lira/4/Sha4/Chil, *SrB* on chromosome 3BS (*Xgwm566*, *Wmc231*) of Ning9415/3/Ures/Bow//Opata/4/Ningmai 7, and *SrC* on chromosome 5DL (*Xgwm292*, *Xgwm212*) of Chen/Ae.Sq//2\*Weaver/3/Oasis/5\*Bor195. Like several other characterized stem rust resistance genes, the three new resistance genes provide moderate levels of resistance at the seedling and adult stages. Further studies to confirm the results and development of targeted mapping populations to identify closely linked markers are under progress.

## 23. Allosyndetic Recombinants of the *Ae. peregrina*-Derived *Lr59* Translocation in Common Wheat

GF Marais, L Kotze, A Eksteen

The wild relatives of wheat constitute a valuable source of rust resistance genes that can be utilized in breeding. Translocation of desirable genes from wild species inevitably results in co-transfer of un-needed alien chromatin. The *Lr59* translocation appears to involve the complete long arm of chromosome 1A. An attempt was made to replace some of the *Aegilops peregrina* chromatin with wheat chromatin through induction of homoeologous chromosome pairing by deleting *Ph1*. Resistant testcross F1 plants were characterized for the presence of three mapped wheat microsatellite loci and a newly discovered SCAR locus that maps to the *Lr59* translocation. Within the mapped region primarily single crossovers occurred, as expected with homoeologous chromosome pairing. Overall, the recombination data were reflective of comparatively regular pairing within a highly homoeologous chromosome region. Strong segregation distortion resulted in the recovery of an abnormally high frequency of recombinants. Eight of the 160 resistant recombinants had recovered wheat chromatin at each of the four marker loci and apparently retained comparatively short terminal segments of foreign chromatin. The latter plants were used in a search that identified 12 anonymous AFLP loci that could be used for continued mapping. The data obtained suggested reduced homoeology between 1AL and the *Lr59* translocation in the distal chromosome regions, most likely due to the presence of a paracentric inversion. Up to six or seven of the eight shortest recombinants may have been produced through crossing over within an inversion loop and are thus genetically imbalanced. Development and field evaluation of near-isogenic lines of five of the eight recombinants will be necessary to identify those that retained the shortest balanced translocations.

Department of Genetics, University of Stellenbosch, Private Bag X1, Matieland, 7602 Stellenbosch, South Africa

## 24. Stem Rust Resistance in *Triticum monococcum* Germplasm

M Rouse<sup>1</sup>, B Steffenson<sup>1</sup>, Y Jin<sup>1,2</sup>

Wheat stem rust, caused by *Puccinia graminis* f. sp. *tritici*, has been effectively controlled through the use of genetic resistance. The recently identified race TTKSK (Ug99) possesses virulence to many resistance genes that have been used in wheat breeding worldwide. One strategy to aid breeders in developing resistant varieties is to provide resistance genes transferred from wild relatives to wheat. Stem rust resistance genes *Sr22* and *Sr35*, derived from *Triticum monococcum* are effective against race TTKSK. In order to identify additional genes from this relative of wheat, we screened 1,062 accessions of *T. monococcum* deposited in the National Small Grains Collection against TTKSK and two additional races with broad virulence. We identified 625 accessions (58.85%) with resistance to TTKSK with infection types ranging from 0 to 2+. Among these resistant accessions, 90 accessions (8.47% of the total) were also resistant to TTTTF and TRTTF. Results from the preliminary screening suggested that new resistance genes are likely to be present in *T. monococcum*. These resistant accessions are being characterized further by testing with additional stem rust races. Crosses among selected resistant *T. monococcum* accessions have been initiated to determine the number and allelic relationships of stem rust resistance genes.

<sup>1</sup>Department of Plant Pathology, University of Minnesota, and <sup>2</sup>USDA-ARS Cereal Disease Laboratory, St. Paul, MN 51108, USA

## 25. Toropi, a Source of Leaf Rust Resistance Genes in Wheat

SB Rosa<sup>1,2</sup>, B McCallum<sup>1</sup>, A Brule-Babel<sup>2</sup>

Leaf rust is one of the most prevalent diseases in wheat and is found almost everywhere wheat is grown. The most cost effective method to control leaf rust is resistance. Sixty one leaf rust resistance loci have been formally designated. Most leaf rust resistance genes are race-specific. Some adult plant sources and resistance genes have provided more durable protection than many genes expressed at the seedling stage. Toropi, a Brazilian cultivar released in 1965, and grown extensively for 15 years, has maintained its resistance for over 40 years. Two complementary recessive genes on chromosomes 1AS and 4DS were identified when *P. triticina* virulence phenotype LCG-RS was used as the test culture. The objective of our study was to identify, characterize, and fine map the leaf rust resistance genes in Toropi. Resistant lines derived from crosses between Toropi and susceptible parent IAC 13-Lorena, and both parents, were inoculated at the seedling and adult stages with isolates of six *P. triticina* pathotypes (BBBD, TDBG, TBJ, MGBJ, MBDS, MBRJ) and with a mixture of pathotypes. The results achieved to date demonstrated that Toropi has at least one race-specific seedling resistance gene and three adult plant resistance genes, two of which are non-race specific. Crosses between Toropi and Thatcher are being made to develop a new mapping population to better characterize the source of resistance present in Toropi and to map the resistance genes.

<sup>1</sup>Cereal Research Center, Agriculture and Agri-Food Canada, Winnipeg, MB R3T 2M9, Canada; <sup>2</sup>Department of Plant Science, University of Manitoba, Winnipeg, MB R3T 2N2, Canada

## 26. Sources of Resistance to the Ecuadorian Yellow Rust Population in Bread Wheat Germplasm of CIMMYT

J Ochoa, E Falconí

Resistance to yellow (stripe) rust (caused by *Puccinia striiformis* f. sp. *tritici*) (YR), the major threat to bread wheat (*Triticum aestivum*) in commercial varieties in Ecuador, has been ineffective so far. New sources of resistance available in CIMMYT germplasm, the basis of the Ecuadorian breeding program, should be efficiently exploited. With this consideration and taking the advantage of the rapid evolution of YR in Ecuador, systematic selection and characterization of the resistance sources in CIMMYT germplasm were carried out at INIAP, near Quito, Ecuador. In 1995, 104 of 2,812 CIMMYT lines were selected with disease severity equal to, or lower than, 30%. These lines, together with modern Ecuadorian cultivars were evaluated at the seedling and adult stages to the races 110 E207, 198 E10 and 7 E8 in 1996, 1997 and 2000, respectively. As a group these races carry the individual virulences identified so far in Ecuador. Most of the lines, initially selected, were susceptible to at least one of the races, whereas 32 lines carried effective levels of resistance to all three races. In the following years, the responses of some of the promising lines or derivatives were monitored in the field, and in year 2008, also at the seedling stage. Effective seedling resistances were identified in Milan, Catbird, Corrydon, SW89.3243, SW89-1862, Chuanha 118 and Child, among which at least the resistances in Milan and Catbird have remained effective over many years. Similarly, the adult plant resistances in Chum 18 and Tinamou were confirmed to be effective, and results indicated that PF74354, IAN8/FINK'S', ALUCAN/YMI#6, ALDAN/IAS58 might also have adequate resistance. Residual resistance levels in Burrion, NANJING8331/3/SUZ10//ALD/PVN and GZ156/NAC//PSN/URES as well as of the commercial cultivars INIAP-Altar and INIAP-Quilindaña are comparatively high. These studies identified new and different types of resistance which will help in improving the management of YR in Ecuador.

Instituto Nacional Autónomo de Investigaciones Agropecuarias (INIAP), Quito-Ecuador, Pan, Sur Km1, Ecuador

## 27. Attempts to Remove Gametocidal Genes Co-Transferred With Rust Resistance from *Aegilops speltoides*

GF Marais<sup>1,2</sup>, TE Bekker<sup>1</sup>, A Eksteen<sup>1</sup>, B McCallum<sup>2</sup>, T Fetch<sup>2</sup>, AS Marais<sup>1</sup>

Transfers of rust resistance genes from *Aegilops speltoides* are often accompanied by completely linked gametocidal (*Gc*) genes that preclude their commercial utilization. Two such introgressions, S13 (with resistance genes *LrS13*; *YrS13* and *SrS13*) and S24 (with *SrS24*), were studied with the aim to separate the *Gc* and resistance genes. Evaluation with western Canadian pathotypes of *Puccinia triticina* and *P. graminis* f. sp. *tritici* races showed that the S13 genes are worth exploring, whereas the *SrS24* source is susceptible to one Canadian pathotype. Attempts to remove *Gc* genes were nonetheless continued with both introgressions as it also provided for a better understanding of *Gc* mechanisms. An attempt to rid S24 of *Gc* genes through homologous chromosome pairing and rigorous selection for increased fertility was unsuccessful and the fertilities of the better selections could not be maintained in subsequent generations. The S13 introgression was mapped to chromosome 3A with the use of wheat marker loci following which allosyndetic pairing induction was attempted. This produced seven putative primary recombinants. Following microsatellite mapping, the best recombinant (04M127-3) was identified. Resistance in this recombinant had exchanged a small region of intercalary donor chromatin for wheat chromatin, but was still associated with somewhat reduced *Gc* effects. Selection 04M127-3 was crossed with wheat and then testcrossed. The progeny yielded a total of 35 resistant progeny, all of which were secondary recombinants. Microsatellite and DArT markers showed that the recombinants were similar, and that in each, a major portion of the

*Ae. speltoides* chromatin was replaced with wheat chromatin. Both *YrS13* and *SrS13* were lost together with the exchanged chromatin. Preliminary indications are that the *Gc* system had largely broken down in some of the secondary recombinants; however, these need to be characterized further to find the most useful recombinant for continued exploitation. The nature of the recombinants produced in the two S13 experiments suggests that a complex multigenic interaction governs the gametocidal response, explaining why it is so difficult to dismantle. However, it appears possible to completely separate the gametocidal genes from *LrS13*.

## 28. New Sources of TTKSK Resistance Derived from *Thinopyrum* and *Aegilops* Species

SS Xu<sup>1</sup>, Y Jin<sup>2</sup>

Several stem rust resistance genes derived from *Thinopyrum* and *Aegilops* sources are highly effective against race TTKSK (Ug99) of *Puccinia graminis* f. sp. *tritici*. We evaluated and characterized the seedling resistances to TTKSK of 62 wheat lines derived from crosses of common wheat or durum with the grass species *Th. junceum*, *Th. intermedium*, *Th. bessarabicum*, *Th. elongatum*, *Th. ponticum*, *Ae. caudata*, and *Ae. speltoides*. Thirty four wheat-alien species derivatives had resistance to TTKSK. Comparisons of the wheat-alien species derivatives and their parental lines for reactions to different stem rust races suggested that several lines, including seven wheat-*Th. intermedium* amphiploids, one wheat-*Th. ponticum* amphiploid, six durum-*Ae. speltoides* amphiploids, one wheat-*Th. junceum* disomic addition line, two wheat-*Ae. caudata* disomic addition lines, and a wheat-*Th. bessarabicum* 7J disomic addition line, may carry novel genes for TTKSK resistance. These lines will be useful for introducing the resistance genes into wheat. Research efforts are currently underway to introduce the resistance genes into wheat genomes through *ph1b*-induced homoeologous recombination.

<sup>1</sup>Department of Genetics, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa; <sup>2</sup>Cereal Research Centre, Agriculture and Agri-food Canada, 195 Dafoe Road, Winnipeg, MT R3T 2M9, Canada

<sup>1</sup>USDA-ARS, Northern Crop Science Laboratory, Fargo, ND 58105 USA; <sup>2</sup>USDA-ARS, Cereal Disease Laboratory, University of Minnesota, St. Paul, MN 55108, USA

## 29. Haplotyping New Sources for Stem Rust Resistance in Wheat Using Available Markers

LX Yu, ME Sorrells

Stem rust is one of the most serious diseases of wheat. The recent emergence of wheat stem rust race Ug99 threatens global wheat production. The development of durable and effective disease resistant wheat varieties is our primary goal. To develop and optimize markers for stem rust resistance, a survey of available stem rust resistance genes including those conferring resistance to Ug99 has been completed in our group. All mapped major stem rust resistance genes were characterized for source, markers available, current research activities, and prioritized for this project (<http://rustopedia.get-traction.com/traction>). We screened 58 markers for 23 stem rust resistance genes among 24 randomly selected wheat lines. About 80% of the markers showed PCR products. Of those amplified, 75% showed polymorphism. We then performed haplotyping analysis with selected polymorphic markers among 248 wheat lines. To date, 15 markers associated with major resistance genes, including Sr1A1R, Sr2, Sr9a, Sr13, Sr17, Sr22, Sr24, Sr32, Sr36, Sr40 and Sr44 were analyzed. Preliminary analysis of haplotyping data from 15 markers, using PCR amplicons, generated a group of haplotypes among the diverse wheat lines. Phylogenetic analysis using the same data showed 3 major and 12 minor clusters. More markers will be used for haplotyping stem rust resistance among those wheat lines, and statistical tools such as association and regression may provide a way for Sr genotypic prediction.

Department of Plant Breeding and Genetics, Cornell University, Ithaca, NY 14853, USA

## 30. Molecular and Pathological Characterization of Slow Rusting Against Leaf Rust in Common Wheat

S Kumar<sup>1</sup>, P Sareen<sup>2</sup>, L Prasad<sup>3</sup>, Uttam Kumar<sup>4</sup>, RP Singh<sup>5</sup>, AK Joshi<sup>6</sup>

Rust diseases, especially leaf rust caused by *Puccinia triticina*, are globally important fungal pathogens of wheat and may cause significant yield losses of up to 40% or more, worldwide. Due to rapid changes in pathogen races, single gene resistances are generally short lived when deployed in wheat cultivars. A more durable form of resistance, known as slow leaf rusting, has been identified and characterized in some genotypes.

Genetic studies indicate that slow rusting resistance is under polygenic control with moderate to high heritability. Such resistance, also known as adult plant resistance (APR), is controlled by minor genes. Although 10-12 slow rusting genes are present in CIMMYT spring wheats, only two such genes, *Lr34* and *Lr46*, have been characterized. Fifteen wheat genotypes, including twelve CIMMYT lines, two elite Indian wheat cultivars, HUW 234 and HUW 468, and one known leaf rust susceptible cultivar, Agra Local, were included in the present study. These lines were firstly evaluated under field conditions for disease severity, latent period and incubation period. They were subsequently evaluated under controlled laboratory conditions using a detached leaf technique with three pathotypes, designated 29R45 (12-5), 121R63-1 (77-5) and 21R55 (104-2). They were also tested in the field. Genotypes, G-5, G-11, G-12 and G-13 showed the lowest disease severities, very close to immunity, against all three pathotypes. In addition, 10 tightly linked microsatellite markers were also used to characterize the 15 lines for presence or absence of the known slow rusting leaf rust resistance genes.

<sup>1</sup>Department of Molecular Biology & Genetic Engineering, <sup>2</sup>Department of Biotechnology, <sup>3</sup>Department of Plant Pathology, Sardar Vallabh Bhai Patel University of Agriculture & Technology, Modipuram, Meerut 250110, India; <sup>4</sup>Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, 06466 Gatersleben, Germany; <sup>5</sup>CIMMYT, Apdo. Postal 6-64106600 Mexico, D.F., Mexico; <sup>6</sup>CIMMYT, South Asia Regional Office, PO Box 5186, Singha Durbar Plaza, Marg Bhadrakali, Kathmandu, Nepal

## 31. Quantitative Trait Loci for High-Temperature Adult-Plant Resistance to Stripe Rust and Molecular Mechanisms of Durable Resistance

X Chen<sup>1,2</sup>, T Coram<sup>1,2</sup>, X Huang<sup>2,4</sup>, F Lin<sup>2</sup>, J Zhao<sup>2,4</sup>, D Santra<sup>3</sup>, A Carter<sup>3</sup>, K Kidwell<sup>3</sup>, K Campbell<sup>1,3</sup>, Z Kang<sup>4</sup>

High-temperature, adult-plant (HTAP) resistance, which expresses when the weather becomes warm and as plants grow older, has been used successfully to control stripe rust of wheat caused by *Puccinia striiformis* f. sp. *tritici* in the Pacific Northwest and other regions of the U.S. since the 1960s when Dr. Vogel developed the semi-dwarf wheat cultivars 'Gaines' and 'Nugaines' with partial resistance. Leading cultivars with adequate levels of HTAP resistance were developed over later years. Recently, we identified and mapped several genes, or quantitative trait loci (QTL), for HTAP resistance in commercial wheat cultivars and genotypes. A major QTL (gene) in 'Alpowa' spring wheat, named *Yr39*, was mapped to the long arm of chromosome 7B. A major QTL (*Qyrlo.wgp-2BS*) in 'Louise' spring wheat was mapped on chromosome 2BS. A major QTL (*Qyr8.wgp-2DS*), tightly linked to the race-specific all-stage resistance gene *Yr8*, was mapped on chromosome 2DS in the 'AVS/6\*Yr8' NIL and its donor genotype, 'Compair'. Three QTL (*Qyrex.wgp-6AS*, *Qyrex.wgp-3BL*, and *Qyrex.wgp-1BL*) were mapped on chromosomes 6AS, 3BL, and 1BL, respectively in 'Express' spring wheat. Two QTL (*Qyrst.wgp-6BS.1* and *Qyrst.wgp-6BS.2*) were mapped in 'Stephens' winter wheat. Wheat lines completely free of stripe rust were developed through molecular marker-assisted pyramiding of HTAP resistance QTL from Alpowa and Express. HTAP resistance is durable because it is non-race-specific. Transcript profiling studies using microarrays revealed that more genes are involved in non-race-specific HTAP resistance than those involved in race-specific all-stage resistance. Different HTAP resistance genes share relatively few regulated genes compared to genes controlling all-stage resistance. Broader spectra of defense genes contribute to the molecular basis of non-race-specific, and therefore, durable types of HTAP resistance.

<sup>1</sup>USDA-ARS Wheat Genetics, Quality, Physiology and Disease Research Unit, <sup>2</sup>Department of Plant Pathology, <sup>3</sup>Department of Crop and Soil Sciences, Washington State University, Pullman, WA 99164-6430, USA, <sup>4</sup>College of Plant Protection, Northwest A&F University, Yangling, Shaanxi, China

## 32. Quantitative Trait Loci for Adult-Plant Resistance to Stripe Rust in a Recombinant Inbred Line Population Derived from a Stephens x Platte Cross

M Dolores Vazquez<sup>1</sup>, A Heesacker<sup>1</sup>, C James Peterson<sup>1</sup>, X Chen<sup>3</sup>, K Ammar<sup>4</sup>, C Mundt<sup>2</sup>, JM Leonard<sup>1</sup>, O Riera-Lizarazu<sup>1</sup>

Stephens wheat (*Triticum aestivum* L.) has been grown commercially in the U.S. Pacific Northwest for 30 years, in part due to its durable resistance to stripe rust (caused by *Puccinia striiformis* f. sp. *tritici*). This resistance is believed to be due to a combination of genes or quantitative trait loci (QTL). The location and role of most of these loci are unknown. To better understand the genetic basis of stripe rust resistance, diversity arrays technology (DArT) and simple sequence repeat (SSR) markers were used to construct a linkage map. This map was based on 160 recombinant inbred lines (RILs) from a cross between Stephens and Platte, a stripe rust susceptible hard white winter wheat from the U.S. Great Plains. This population was also assessed for stripe rust response at four U.S. locations (Corvallis, OR; Pendleton, OR; Mt. Vernon, WA; and Whitlow, WA) and at one location in Mexico (Toluca, MX). Quantitative trait analysis revealed loci that were significant across environments on chromosomes 2A, 4B, and 7A, explaining 15, 20, and 16% of the phenotypic variance, respectively. The QTL on chromosome 4B was contributed by Platte and was significant in the Oregon locations, Toluca, Mexico, and Whitlow, WA. QTL on chromosomes 1A and 5A gave resistance only at the two Washington locations. These results indicated that QTL of moderate effect contribute to resistance in this population and that there were QTL x environment interactions. Because our linkage map is based mostly on DArT markers, additional genotyping is ongoing to identify breeder-friendly markers for marker-assisted selection. A more thorough assessment with additional phenotypic data is ongoing.

<sup>1</sup>Department of Crop and Soil Science, <sup>2</sup>Department of Botany & Plant Pathology, Oregon State University, Corvallis, OR 97331, USA; <sup>3</sup>USDA-ARS, Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430 USA; <sup>4</sup>International Maize and Wheat Improvement Center (CIMMYT), Apartado, Postal 6641, Mexico, 06600 DF, Mexico

### 33. Genetic Analysis of Wheat Leaf Rust Resistance Associated with the Solid Stem Trait

BD McCallum<sup>1</sup>, FR Clarke<sup>2</sup>, RE Knox<sup>2</sup>, RM De Pauw<sup>2</sup>

The solid stem trait conditions resistance to wheat stem sawfly (*Cephus cinctus* Nort.) which causes major losses in some areas. Improved resistance to wheat leaf rust (*Puccinia triticina*) was observed when the solid stem character was transferred into wheat from a synthetic hexaploid, *Triticum turgidum* L. var. durum cv. Golden Ball/*Triticum tauschii*. The solid stem trait was backcrossed into the cultivar AC Elsa and lines with solid stem were more leaf rust resistant than the recurrent parent. To investigate the genetic basis of this resistance both AC Elsa and an AC Elsa backcross line with solid stem were crossed to the leaf rust susceptible cultivar Thatcher. The F<sub>1</sub>s were backcrossed to Thatcher. Preliminary results suggest that AC Elsa has two seedling resistance genes, whereas the AC Elsa backcross solid stem line has three. However, both backcross populations appeared to have only one main resistance gene effective in adult plant field trials. This effective gene is likely *Lr34* based on marker and phenotypic data. Additional genes in the solid stem AC Elsa backcross line enhanced the level of rust resistance in progeny with *Lr34*, but were not effective in isolation.

### 34. A Major QTL for Leaf Rust Resistance, Widely Exploited in Durum Wheat Breeding, Maps on Chromosome 7BL

M Maccaferri<sup>1</sup>, P Mantovani<sup>1</sup>, MC Sanguineti<sup>1</sup>, A Demontis<sup>2</sup>, A Massi<sup>2</sup>, K Ammar<sup>3</sup>, J Kolmer<sup>4</sup>, JH Czembor<sup>5</sup>, A Breiman<sup>6</sup>, R Tuberosa<sup>1</sup>

Linkage and association mapping were used to investigate the resistance to leaf rust (*Puccinia triticina* Eriks.) from Creso and its derivatives. Creso's leaf rust resistance in durum wheat has remained effective since 1975. A Colosseo (C; resistant cultivar derived from Creso × Mexa) × Lloyd (L; susceptible) population and a panel of 164 elite accessions suitable for association mapping were tested under both seedling (greenhouse) and adult plant (open field) conditions with isolates of diverse origin. Field experiments were conducted in northern Italy (Argelato, Bologna), using inoculum of a mixture of Italian isolates, and in Mexico (El Batán and Obregon), where plants were challenged with Mexican *P. triticina* races BBG/BN and BBG/BP. Infection type responses in seedlings were recorded for isolates from Italy, central Europe, Ethiopia, Israel and Mexico. A major QTL (*R*<sup>2</sup> up to 77%) for both the adult plant and the seedling-responses was mapped in C×L on chromosome 7BL in a 5 cM interval (Maccaferri et al (2008); TAG 117:1225-1240). This chromosome region was the only one with markers consistently associated to leaf rust resistance at *P* = 0.001 in all five field trials. Candidates for the 7BL QTL include *Lr14a* (Herrera-Foessel et al. (2008) Plant Disease 92:469-473), a major hypersensitive leaf rust resistance gene introgressed from emmer wheat. Further genetic and molecular work is underway at DiSTA and PSB to fine map and eventually clone this QTL.

<sup>1</sup>Agriculture and Agri-Food Canada, Cereal Research Center, 195 Dafoe Road, Winnipeg, MB, Canada R3T 2M9; <sup>2</sup>Agriculture and Agri-Food Canada, Semiarid Prairie Agricultural Research Center, P.O. Box 1030, Swift Current, SK Canada S9H 3X2

<sup>1</sup>Department of Agroenvironmental Science and Technology (DiSTA), University of Bologna, Viale G. Fanin 44, 40127 Bologna, Italy; <sup>2</sup>Società Produttori Sementi (PSB), Via Macero 1, 40050 Argelato (BO), Italy; <sup>3</sup>Wheat Program, International Maize and Wheat Improvement Center (CIMMYT) Apdo Postal 6-641, 06600, Mexico DF, Mexico; <sup>4</sup>USDA/ARS/Cereal Disease Laboratory, 1551 Lindig Street, Univ. Minnesota, St. Paul, MN 55108, USA; <sup>5</sup>Plant Breeding and Genetics Department, Plant Breeding and Acclimatization Institute, 05-870 Blonie, IHAR Radzikow, Poland; <sup>6</sup>Department of Plant Sciences, Tel Aviv University, 69978 Tel Aviv, Israel

## 35. Screening of International Wheat Germplasm for Multiple Disease Resistances in Morocco

<sup>1</sup>A Ramdani, <sup>1</sup>M Jlibene, <sup>2</sup>N Nsarellah, <sup>3</sup>SM Udupa

There are many biotic constraints to wheat production in Morocco. While leaf rust and *Septoria tritici* leaf blotch were known from early times, yellow (stripe) rust appeared in the area near the Atlas Mountains during the late 1980s. It recently spread to other cereal-growing areas, probably because of changes in virulence patterns (eg, *Yr9* is no longer effective). Hence, a search for multiple disease resistances in wheat cultivars is a major objective. The best lines from international nurseries were screened to widen the genetic base for wheat crop improvement. Since diseases are not regularly expressed under field conditions, testing with local pathogen populations under controlled conditions was carried out for some nurseries. The objective of this study was to identify wheat lines from international nurseries that carry simultaneously adult-plant resistances to leaf rust, yellow rust, and *Septoria tritici* leaf blotch. Severities and reaction types for leaf rust and yellow rust, and pycnidial coverage for *Septoria* under field conditions, and latent period and severity of *Septoria* under greenhouse conditions, were scored. A high frequency of multiply resistant entries was observed among these accessions, reinforcing the importance of international co-operation.

## 36. Introgression of Resistance to Wheat Stem Rust Race TTKSK from Sharon Goatgrass into Wheat

E Millet<sup>1</sup>, PD Olivera<sup>2</sup>, BJ Steffenson<sup>2</sup>

Sharon goatgrass (*Aegilops sharonensis*) is a wild cereal endemic to the coastal plains of Israel. It is a diploid species (2n=14) and possesses the S<sup>sh</sup> genome, which is closely related to the B genome of wheat. Sharon goatgrass exhibits a high frequency and level of resistance to a number of wheat diseases including leaf rust, stripe rust and stem rust. Many accessions of this species are also resistant to the widely virulent stem rust race TTKSK (Ug99). Gene transfer from Sharon goatgrass is not straightforward due mainly to a lack of homology between the alien and wheat chromosomes, and also to the presence in the wild species of gametocidal genes that prevent recovery of the pure wheat genetic background through backcrossing. We developed a method which combines the use of the *ph1* gene to promote pairing between homoeologous (partially homologous) chromosomes and an anti-gametocidal mutant gene to overcome the gametocidal effect. Production of wheat breeding material with a segment carrying the desired TTKSK resistance gene is under way. Selection of TTKSK resistant progenies during the transfer process will be aided by molecular markers linked to the gene.

<sup>1</sup>INRA – CRRA, PO Box 578, Meknès, Morocco; <sup>2</sup>INRA – CRRA, PO Box 589, Settat, Morocco; <sup>3</sup>ICARDA, Rabat Institutes, PO Box 6299, Rabat, Morocco

<sup>1</sup>Institute for Cereal Crops Improvement, Tel Aviv University, Tel Aviv, Israel; <sup>2</sup>Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, USA

## 37. Stem Rust Resistance in *Aegilops tauschii* Germplasm

M Rouse<sup>1</sup>, E Olsen<sup>2</sup>, M Pumphrey<sup>3</sup>, B Steffenson<sup>1</sup>, Y Jin<sup>1,4</sup>

*Aegilops tauschii*, the D genome donor of hexaploid wheat, has been used extensively for the transfer of agronomically important traits to wheat, including stem rust resistance genes *Sr33* and *Sr45*. In order to identify potentially new stem rust resistance genes in *Ae. tauschii* germplasm, we evaluated 530 non-duplicated accessions of *Ae. tauschii* deposited in the USDA National Small Grains Collection and Wheat Genetic and Genomic Resources Center collection, with races TTKSK (Ug99), TRTTF, TTTTF, TPMKC, QFCSC, and RKQQC of *Puccinia graminis* f. sp. *tritici*. Our preliminary results indicated that 33% of *Ae. tauschii* accessions were resistant to TTKSK with infection types ranging from ; to 2+. Based upon different compatibility phenotypes displayed to the various races by the resistant accessions, we postulated that novel resistant genes to race TTKSK are present in this species. Selected accessions are being backcrossed into wheat for the introgression of resistance to race TTKSK.

## 38. Resistance to Wheat Stem Rust in Spelt Wheat (*Triticum aestivum* ssp. *spelta*)

PD Olivera<sup>1</sup>, S Gale<sup>2</sup>, L Wanschura<sup>2</sup>, M Rouse<sup>1</sup>, Y Jin<sup>1,2</sup>

Spelt wheat (*Triticum aestivum* ssp. *spelta*) is a hexaploid hulled wheat that was extensively cultivated in Europe until the early 1900s. This species has extensive genetic diversity, and the existence of several stem rust resistance genes were postulated by previous investigators. We evaluated a collection of 495 spelt wheat accessions at the seedling stage for resistance to several races of *Puccinia graminis* f. sp. *tritici* with broad virulence, including TTKSK (Ug99), TRTTF, and TTTTF. Resistance with infection types 2 and 2+ to race TTKSK was found in 16 (3.3%) accessions. We observed a near complete association for resistance to the three races, suggesting that these spelt wheat accessions may share a common set of stem rust resistance genes. Accessions exhibiting resistance to races TTKSK, TRTTF, and TTTTF were further characterized for reaction to other races in the TTKS lineage and additional US races. Since spelt and bread wheat have the same genomic constitution (2n=6x=42; AABBDD), resistance to stem rust from spelt could be easily introgressed into bread wheat. We selected resistant accessions as parents to develop crosses in an attempt to determine the genetic basis of resistance to race TTKSK.

<sup>1</sup>Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, USA; <sup>2</sup>Department of Plant Pathology, Kansas State University, and <sup>3</sup>USDA-ARS Plant Science & Entomology Research Unit, Manhattan, KS 66506, USA; <sup>4</sup>USDA-ARS Cereal Disease Laboratory, St. Paul, MN 55108, USA

<sup>1</sup> Department of Plant Pathology, University of Minnesota, and <sup>2</sup>USDA-ARS, Cereal Disease Laboratory, St. Paul, MN 55108, USA

## 39. Progress and Prospects in Discovery and Use of Novel Sources of Stem Rust Resistance

Y Jin<sup>1,2</sup>, M Rouse<sup>2</sup>, PD Olivera<sup>2</sup>, BJ Steffenson<sup>2</sup>

A number of stem rust resistance genes derived from wild relatives of wheat appeared to be more effective against race TTKSK (Ug99) of *Puccinia graminis* f. sp. *tritici* than *Sr* genes of wheat origin. In an attempt to identify novel sources of stem rust resistance genes effective against TTKSK, we evaluated several cultivated and wild relatives of wheat for resistance to TTKSK and other stem rust races with broad virulence in seedling tests. Preliminary results indicated that TTKSK resistance was common, but the frequencies of resistant accessions varied between species. *Secale cereale* (533 accessions) and *Aegilops speltoides* (90 accessions) had the highest frequencies of resistance (nearly 100%). Other species having high frequencies of TTKSK resistance include triticale (74% of 567 accessions), *Ae. sharonensis* (69% of 107 accessions), *Triticum urartu* (97% of 186 accessions), and *T. monococcum* (61% of 1,020 accessions). Frequencies of TTKSK resistance in further species were: 18% in *Ae. tauschii* (114 accessions), 15% in *T. timopheevii* (298 accessions), and 17% in *T. dicoccoides* (153 accessions). Based on specific infection types to several races, known genes effective against TTKSK in some of these species were postulated. Accessions with putative new resistance genes were selected for crossing and introgressing resistances into wheat, and for developing mapping populations.

## 40. Wheat-Stripe Rust Interactions Involving 'Moro' Resistance

DA Gaudet<sup>1</sup>, X Wang<sup>2</sup>, B Puchalski<sup>1</sup>, F Leggett<sup>1</sup>, A Kuzyk<sup>1</sup>, A Laroche<sup>1</sup>

The wheat cultivar Moro possesses *Yr10*, which confers seedling resistance to *Puccinia striiformis* Westend. f. sp. *tritici* Eriks. race 44 E14 (European nomenclature) in Western Canadian soft white spring and winter wheats. Race CDL-29 (US nomenclature) is virulent and both races are also virulent on Fielder, but differ in aggressiveness. In a time-course study from 0 to 16 days post-inoculation (dpi), we studied compatible and incompatible stripe rust interactions in seedlings of Fielder and Moro inoculated with race 44 E14 and race CDL-29. We employed microscopy, DAB staining for detection of the oxidative burst, and qRT-PCR for expression of different PR-proteins. Penetration and early infection stages for the resistant cultivar Moro and the susceptible cultivar Fielder were similar for the first 9-10 dpi. In Moro, fungal development failed to progress beyond haustorium formation from 10-13 dpi, and the hypersensitive response occurred from 10 to 16 dpi. An oxidative burst at 6 and 14 dpi was recorded in the incompatible interaction compared to a single peak at 14 dpi in the compatible interactions. Differences in the time-course expression of the different PR-proteins were observed among treatments.

<sup>1</sup>USDA-ARS, Cereal Disease Laboratory, and <sup>2</sup>Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, USA

<sup>1</sup>Agriculture and Agri-Food Canada, Lethbridge Research Centre, PO Box 3000, 5430-1st Avenue, South, Lethbridge, Alberta T1J 4B, Canada; <sup>2</sup>College of Plant Protection and Shaanxi Key Laboratory of Molecular Biology for Agriculture, Northwestern A&F University, Yangling, Shaanxi 712100, PR China.

## 41. Effect of Silencing Gene *Yr10* for Stripe Rust Resistance in Moro Wheat

W Liu<sup>1,2</sup>, A Laroche<sup>1</sup>, Z-S Kang<sup>2</sup>, DA Gaudet<sup>1</sup>

Wheat stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is a destructive disease of wheat worldwide and the development of resistant cultivars is the most economical control method. The *Yr10* gene in Moro wheat, that encodes a cytoplasmic NB-LRR protein containing nucleotide-binding sites (NBS) and leucine-rich repeats (LRR), imparts seedling resistance to stripe rust. Virus-induced gene silencing (VIGS) is a rapid and powerful tool to analyze the function of plant genes. We employed the barley stripe mosaic virus (BSMV)-VIGS system to study the function of different domains of the *Yr10* gene, in the resistance response in Moro wheat. A series of DNA fragments based on different domains of *Yr10* were inserted into BSMV-VIGS vectors. Moro wheat infection by *P. striiformis* following transfection with vectors was examined at the morphological, cytological and molecular levels. Susceptible responses consisting of pustule formation and symptoms of compatibility were observed in Moro leaves transfected with some of the fragments. We evaluated the expression of the *Yr10* gene by probing different domains. The effects of changes in expression of *Yr10* on function of plant responses at the leaf and cellular levels will be presented.

## 42. Cloning and Characterization of the *Avr1* Gene from *Puccinia triticina*

A Pacheco, H Zhang, DB Hays

Leaf rust is the most common, and one of the most important, wheat diseases of the world. Current leaf rust control in the U.S. consists of breeding resistant cultivars by using identified *Lr* genes in the host. Cultivars with such genes usually become susceptible to infection due to the tremendous extant genetic diversity of the pathogen that allows it to overcome resistant cultivars in 2-4 years. Development of alternate methods of control is limited since little is known about *Puccinia* genomes and plant : pathogen interactions. Construction of a genome-wide physical map is important in order to fully understand the molecular basis of the infection mechanism of the pathogen and its interaction with the host. In an effort to discover more about the genetic potential of leaf rust in terms of AVR and VIR gene regulation, and to create future novel plant resistance breeding strategies, we have proposed a study of the pathogen genome by constructing a BIBAC library and a physical map of the pathogen. The BIBAC library is being constructed from the *P. triticina* type culture PRTUS 3 which has *AVR1* (avirulence gene corresponding to *Lr1*) disrupted using T-DNA mutagenesis via particle bombardment. The characterization of *AVR1* in the BIBAC library will serve as a point of reference for cloning heterologous AVR and VIR genes, and for defining their regulation and modes of inheritance and recombination.

<sup>1</sup>Agriculture and Agri-Food Canada, Lethbridge Research Centre, PO Box 3000, 5403 1st Avenue, South, Lethbridge, Alberta T1J 4B1 Canada; <sup>2</sup>College of Plant Protection and Shaanxi Key Laboratory of Molecular Biology for Agriculture, Northwestern A&F University, Yangling, Shaanxi 712100, PR China

Department of Soil and Crop Science, Texas A&M University, College Station, TX 77843, U.S.A.