



Poster Abstracts

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Table of contents

Poster Abstracts

Theme 1: Rust Surveillance and Genetics	155
1. Global distribution of aggressive wheat yellow rust strains	155
2. Status of wheat diseases in Morocco during the 2009-10 growing season: Yellow rust is becoming a more dangerous disease	155
3. The reason behind the serious outbreaks of wheat yellow rust in Morocco: Yr27 is no longer effective.....	156
4. Last decade (2000-2010) cereal pests situation in Algeria	156
5. Races of <i>Puccinia graminis</i> f. sp. <i>tritici</i> in Ethiopia and Kenya	157
6. Races of <i>Puccinia graminis</i> f. sp. <i>tritici</i> detected on wheat in South Africa during 2010	157
7. The expression of Sr21 to South African Ug99 and related races	158
8. Wheat Rusts Status in Iraq	158
9. Detection of Yr27 virulence in <i>Puccinia striiformis</i> f.sp. <i>tritici</i> populations	159
10. Role of date of sowing and meteorological parameters on stripe rust of wheat under Punjab conditions (India).....	159
11. Wheat Rusts in Bangladesh	160
12. Occurrence of wheat rusts in Turkey during the 2010 growing season.....	160
13. Virulence of the wheat stem rust pathogen in the Volga region of Russia	161
14. Distribution of wheat rusts and effectiveness of resistance genes in the Russian North Caucasus region	161
15. Virulence phenotypes and molecular genotypes in collections of <i>Puccinia triticina</i> from Italy.....	162
16. Virulence of <i>Puccinia triticina</i> on wheat in Poland from 1998 to 2009	162
17. Genetic variability in collections of <i>Puccinia graminis</i> f. sp. <i>avenae</i> and <i>P. graminis</i> f. sp. <i>tritici</i> from Sweden, Ethiopia and Tajikistan	163
18. Recent wheat disease changes in Brazil	163
19. Emerging virulences to race-specific resistance genes in Mexican populations	164
20. Reactions of western Canadian wheat and triticale varieties to stripe rust	164
21. Races and virulences of <i>Puccinia striiformis</i> in the United States in 2010	165
22. Potential summer and winter survival regions of the stripe rust pathogen in the United States	165
23. Role of barberries in stem rust epidemics and race diversity in Washington and Idaho U.S.A., 2007-2009.....	166
24. Barberry is more important as an alternate host for stem rust than for stripe rust in the U.S. Pacific Northwest.....	166
25. Identification of <i>Berberis</i> spp. as alternate hosts of <i>Puccinia striiformis</i> f. sp. <i>tritici</i> in China.....	167
26. Stem rust epidemic modeling: adapting ryegrass models to wheat	167

27. Modeling leaf rust of wheat in response to combined effects of temperature and wetness duration.....	168
28. Wheat leaf rust (<i>Puccinia triticina</i> Eriks) genome sequencing and comparative resources for rust fungi	168
29. Functional genomic approaches for the wheat leaf rust fungus	169
30. Development of SNP markers to infer evolutionary relationships of <i>Puccinia triticina</i>	169
31. A method to linearize Stakman infection type ratings for statistical analysis.....	170
Theme 2: National Efforts to Breed for Rust Resistance	171
32. Seedling and adult plant resistance to stem rust race Ug99 in spring wheat landraces.....	171
33. Resistance to stem rust in Australian barley cultivars	171
34. Resistance to stem rust race Ug99 in selected wheat germplasm	172
35. Collaborative research against ug99 in wheat-BARs-09 a success story.....	172
36. Status of Rust Resistance in Indian Wheat Cultivars.....	173
37. Breeding strategies to improve tolerance in Indian wheat genotypes against leaf and stripe rusts to enhance productivity under global climate change.....	173
38. Identification of slow rusting wheat genotypes for stripe and leaf rusts under artificially inoculated conditions.....	174
39. The national wheat breeding program for development of high yielding and rusts resistant of bread wheats for Tajikistan.....	174
40. Postulation of genes for adult plant resistance to leaf rust in wheat cultivars	175
41. Strategy of wheat breeding for resistance to leaf rust in various regions of Russia	175
42. Identification of resistance to wheat stem rust race Ug99 in Iran	176
43. Bioinformatics integration in breeding for rust resistance in wheat: prospects and challenges in Morocco.....	176
44. Effective genes for wheat stem rust resistance at New Halfa, Sudan	177
45. Plan to recover wheat production in Ecuador	177
46. Adult plant stem rust responses of a doubled haploid wheat population tested under greenhouse conditions	178
Theme 3: New Sources of Rust Resistance.....	179
47. Introgression of stem rust resistance into <i>Triticum aestivum</i> L. from <i>Aegilops tauschii</i> Coss. by direct crossing.....	179
48. Wheat-wheatgrass hybrids as new sources of resistance to African and U.S. races of stem rust.....	179
49. Using a wild wheat relative to tackle stem rust race Ug99	180
50. Mapping stem rust resistance genes in <i>Aegilops sharonensis</i> , a diploid wheat relative.....	180
51. Screening of cytogenetic stocks for resistance to race Ug99	181
52. Resistance to TTKSK in durum (<i>Triticum turgidum</i> ssp. durum) and emmer (<i>Triticum turgidum</i> ssp. dicoccum) wheat	181
53. Chromosome engineering of wheat stem rust resistance gene <i>Sr47</i> in a tetraploid wheat background	182
54. Histopathology of some non-specific resistance mechanisms expressed on wheat cultivar <i>Toropi</i>	182

Theme 4: Molecular Breeding for Rust Resistance	183
55. Characterization of the durable leaf rust resistance gene <i>Lr34</i> in European winter wheats.....	183
56. Dissecting adult plant stripe rust resistance in the wheat cultivar <i>Cappelle Desprez</i>	183
57. Quantitative resistance conferring durable leaf rust resistance in wheat cultivar <i>Toropi</i>	184
58. Discovery, mapping, and validation of QTL conferring partial resistance to broadly-virulent post-2000 North American races of the stripe rust pathogen	184
59. QTL mapping for adult-plant resistance to stripe rust in Italian common wheat cultivars <i>Libellula</i> and <i>Strampelli</i>	185
60. The presence of <i>SrCad</i> and <i>Sr2</i> influences reaction to stripe rust and <i>Fusarium</i> head blight.....	185
61. Multi-environment quantitative trait loci analysis for resistance to stripe rust and <i>Cephalosporium</i> stripe in two recombinant inbred line populations.	186
62. Investigating the role of <i>SrCad</i> and <i>Sr2</i> on stem rust race <i>TTKST</i> in wheat.....	186
63. Using molecular markers to detect favorable linkages between <i>Sr2</i> and <i>Fhb1</i> in <i>SRWW</i> germplasm	187
64. Quantitative trait loci for adult plant resistance to wheat stem rust in cultivar <i>K-Nyangumi</i>	187
65. Mapping resistance to race <i>Ug99</i> stem rust in <i>Norin 40 (Sr42)</i>	188
66. The effect of <i>Lr34</i> on wheat stem rust responses	188
67. Identification of QTLs associated with adult plant resistance to stem rust race <i>Ug99</i> in the 'Avocet' x 'Pavon76' recombinant inbred line population	189
68. The presence <i>Sr2</i> resistance reinforced <i>Sr24</i> against the virulent race <i>TTKST</i>	189
69. Gene-gene interaction reveals complexity of resistance to race <i>Ug99</i> in wheat	190
70. Genome-wide markers can predict adult plant resistance to wheat stem rust.....	190
71. Association mapping to identify stem rust resistance loci in durum wheat germplasm	191
72. First report of slow rusting gene <i>Lr46</i> in durum wheat	191
73. Highly expressed <i>RPG1</i> protein in a five-copy <i>Rpg1</i> -transgenic barley line results in susceptibility to stem rust	192
74. The <i>Rpg5</i> NBS-LRR-STPK gene and a second NBS-LRR gene are required together for <i>rpg4</i> -mediated wheat stem rust resistance in barley.....	192
75. Genetics of resistance to stem rust race <i>TTKSK</i> in barley landraces from Switzerland.....	193
76. Vulnerability of <i>Hordeum</i> germplasm to wheat stem rust race <i>TTKSK</i>	193
77. Mapping and haplotype analysis of adult plant resistance to stem rust race <i>TTKS</i> in barley breeding germplasm from the USA	194
78. Molecular tagging of an <i>Ug99</i> -effective stem rust resistance gene <i>Sr28</i>	194
Theme 5: Delivering Seed to Farmers	195
79. Farmer participation in promoting rust resistant wheat genotypes in the hills of Nepal enhances food security.....	195
80. <i>Muqawim 09</i> - a <i>Ug99</i> resistant success story in Afghanistan	195
81. Gender-responsive variety selection (GVS) for rust-resistant wheat varieties at <i>Kulumsa, Ethiopia</i>	196

Theme 3:

New Sources of Rust Resistance

47. Introgression of stem rust resistance into *Triticum aestivum* L. from *Aegilops tauschii* Coss. by direct crossing

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An evaluation of a diverse set of 456 accessions of *Aegilops tauschii* Coss. ($2n=2x=14$, DD) with six races of the stem rust pathogen *Puccinia graminis* f. sp. *tritici* Pers. identified 98 lines with seedling resistance to race TTKSK (Ug99) (Rouse et. al, Crop Science, submitted). Stem rust resistance has been transferred to *Triticum aestivum* L. ($2n=6x=42$, AABBDD) from six Ug99-resistant *Ae. tauschii* accessions: TA1615, TA1662, TA1718, TA1693, TA10171, and TA10187. Introgression was done by direct crossing of the *Ae. tauschii* accessions as males and the hexaploid wheat, KS05HW14, as female. Progeny from direct crosses ($2n=28$, ABDD) were embryo rescued between 14 and 18 days after pollination in MS media containing kinetin. Upon the development of shoots, plantlets were transferred to a modified MS medium until the full development of roots and then placed in vernalization. The sterile F_1 plants were backcrossed as females to the hexaploid KS05HW14 to generate BC_1F_1 progeny. Phenotyping of BC_1F_1 progeny with avirulent stem rust races identified stem rust-resistant progenies which were used as males to generate euploid BC_2F_1 plants. A bulked segregant analysis of BC_1F_2 or BC_2F_1 genotypes with SSR markers will identify loci linked to stem rust resistance and determine the chromosome location of the genes for subsequent linkage analysis. Crosses are being made among *Ae. tauschii* accessions to determine allelic relationships.

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48. Wheat-wheatgrass hybrids as new sources of resistance to African and U.S. races of stem rust

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Perennial wheatgrass (*Thinopyrum* spp.) is recognized as a source of genetic variation to improve annual wheat germplasm and for its potential use as a perennial grain crop. *Thinopyrum* species have provided many genes for improving resistances various diseases, including stem rust, leaf rust, eyespot, and powdery mildew. *Thinopyrum intermedium* is the source of *Sr44* and *Th. ponticum* (syn. *Th. elongatum*, *Agropyrum elongatum*, *Lophopyrum ponticum*) is the source of *Sr24*, *Sr25*, *Sr26*, and *Sr43*. Hybrid lines made by crossing *Thinopyrum* species and *Triticum aestivum* (common wheat) can be used to improve both species. Seventeen hybrid wheat-wheatgrass lines from crosses of *T. aestivum* with *Th. intermedium* (intermediate wheatgrass), *Th. Junceum*, *T. carthlicum*, and *T. turgidum*, developed at the Land Institute were tested for seedling reaction to two African stem rust races (TTKSK and TRTTF), and 14 lines were screened with 5 U.S. races (MCCFC, TPMKC, TTTTF, QTHJC, and RKQQC). Genomes of these hybrid lines ranged in numbers of chromosomes from common wheat (12-44 chromosomes) and wheatgrass (8-46 chromosomes). Thirteen of the 17 different pedigrees tested were highly to moderately resistant (infection type ≤ 2 -) to TTKSK, with highly resistant ratings (IT 0;) occurring in seven resistant lines. Of the 14 lines tested with U.S. races, 12 were resistant (IT ≤ 2 -) to RKQQC, 11 were resistant to TTTTF, and 9 of 14 were resistant to MCCFC, TPMKC, and QTHJC. These lines are currently being screened with genetic markers for known stem rust resistance genes to help determine if they contain new resistance genes.

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49. Using a wild wheat relative to tackle stem rust race Ug99

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Stem rust has been a devastating disease of wheat since ancient times. The new super-virulent stem rust strain Ug99 can overcome ~90% of commercial wheat cultivars, highlighting the urgent need for new sources of resistance. Our strategy is to isolate multiple novel *Sr* genes and deploy them in combination at a single transgene locus in wheat.

To this end we are developing a suite of genetics and molecular genomics tools in Sharon goatgrass (*Aegilops sharonensis*), a wild diploid “B genome” relative of wheat. These tools include: genetic, molecular, and phenotypic resources for a core set of wild germplasm; crossing programs and advancement of mapping populations through single seed descent; a high-density custom-made *Ae. sharonensis* DArT array; SNP discovery via 454 transcriptome sequencing; an integrated DArT and gene-based genetic map; characterization of synteny to barley and sequenced monocot genomes; a BAC library; and an *Ae. sharonensis* and wheat oligo capture array for resistance genes of the nucleotide-binding leucine-rich-repeat (NB-LRR) class. The tools and resources developed will be a significant resource to clone NB-LRR genes and to study other traits of agronomic importance in wheat.

We will give a current update on these tools and our characterization of mapping populations segregating for resistance to Ug99.

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50. Mapping stem rust resistance genes in *Aegilops sharonensis*, a diploid wheat relative

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Aegilops sharonensis (Sharon goatgrass) is known to be a rich source of genetic diversity for resistance to diseases. To clone new resistance genes functional against stem rust from this genetically uncharacterized grass, the development of molecular genomics and reverse genetics tools is important. We have developed a linkage map, based on Diversity Array Technology (DArT) markers, that allowed the localization on putative chromosome 1S^{sh} of a single dominant stem rust resistance gene (named *SrAeSh1644*) functional against race TTTTF and at least two dominant *Sr* genes (*Sr1644-A* and *Sr1644-B*) functional against Ug99. To map these genes with conventional molecular markers, we converted wheat DArT markers into CAPS (Cleaved Amplified Polymorphic Sequences) and COS (Conserved Orthologous Set) markers based on the synteny between wheat and *Brachypodium distachyon*. Currently, we are increasing the marker density in the region around these *Sr* genes by developing CAPS markers, based on micro-synteny between *Ae. sharonensis* chromosome 1S^{sh} and putative orthologs in barley and *Brachypodium*, using Single Nucleotide Polymorphisms (SNPs) identified by sequencing normalized leaf tissue cDNA from *Ae. sharonensis* accessions 1644 and 2232. As a result, by combining an integrated DArT and gene-based genetic map, and characterizing the micro-synteny to barley and sequenced monocot genomes, we are creating a platform to clone *Sr* genes and other traits of agronomic importance from the diploid wild relative, *Ae. sharonensis*. In addition, we will present the latest advances in the development of reverse genetics and effectoromic screen tools for virus-induced gene silencing and virus-mediated heterologous gene overexpression in *Ae. sharonensis*.

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51. Screening of cytogenetic stocks for resistance to race Ug99

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Screening of our cytogenetic stocks with the TTKST variant of the Ug99 lineage revealed a number of resistant accessions. Screening of wild species detected resistance in eight lines of *T. monococcum* (A genome) and one accession of *T. miguschovae* (AGD genome). Two Brazilian rye landraces, Boller and Vacaria, were resistant as were primary triticales produced from them. Amphiploids or partial amphiploids originating from hybrids of wheat with *Th. elongatum* (E), *Th. intermedium* (EEst), *L. ponticum* (EsEsEEE) and *Haynaldia villosa* (V) also included resistant lines. Fifteen of 21 translocation lines derived from wheat x *L. ponticum* hybrids were resistant. Resistance was also detected in stable lines selected from populations derived from crosses of wheat to *T. miguschovae*, *Ae. cylindricai* (CD) and *Elymus repens* (StStH). Mapping populations are being developed with these lines.

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52. Resistance to TTKSK in durum (*Triticum turgidum* ssp. durum) and emmer (*Triticum turgidum* ssp. dicoccum) wheat

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Stem rust, caused by *Puccinia graminis* f. sp. *tritici* is one of the most destructive diseases of durum and bread wheat. Races that recently emerged in Eastern Africa (TTKSK [Ug99] and its derivatives) possess broad virulence to wheat cultivars worldwide, and only a few genes in the adapted cultivars have resistance to these races. The objective of this study was to identify additional effective resistance genes in durum (*T. turgidum* ssp. durum) and emmer (*T. turgidum* ssp. dicoccum) wheat that could be utilized in wheat breeding. We evaluated 1236 accessions of durum and emmer for stem rust resistance in the field screening nurseries at EIAR (Debre Zeit, Ethiopia) and Saint Paul, MN. Three hundred ninety nine and 526 accessions exhibited resistant to moderately resistant responses to stem rust in Debre Zeit and St. Paul, respectively. The highest frequencies of resistance in emmer were from Ethiopia (49%) and Middle East (14%), and in durum were Africa (26%) and North America (18%). Four hundred eighty accessions exhibiting resistant to moderately susceptible responses (up to 30MS) were characterized for their reaction to races of TTKSK and TRTTF at the seedling stage. Seventy-nine durum and 118 emmer accessions were resistant to the two races, and 83 accessions of durum and 96 accessions of emmer were susceptible to these two races. These accessions may possess adult plant resistance. Race specific resistance was also observed as 26 and 70 accessions were resistant to race TTKSK and susceptible to race TRTTF and vice versa. Accessions exhibiting resistance to races TTKSK and TRTTF were further characterized for their reaction to seven US races in an attempt to postulate the presence of known genes. Genetics of TTKSK resistance in selected lines are being investigated.

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53. Chromosome engineering of wheat stem rust resistance gene Sr47 in a tetraploid wheat background

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Durum wheat (*Triticum turgidum* L. ssp. *durum*) line DAS15 carries *Sr47*, a gene conferring resistance to stem rust (caused by *Puccinia graminis* f. sp. *tritici*), including race TTKSK (Ug99). The *Ae. speltooides* segment harboring *Sr47* in line DAS15 accounts for most of the T2BL-2SL-2SS chromosome. Our objective was to use high-throughput DNA marker-assisted chromosome engineering to shorten the alien segment present in resistant plants. Following a previously described crossing procedure, we tested 1,086 BC₂F₁ plants (Rusty/3/47-1 5D(5B)//Rusty 5D(5B)/DAS15) for resistance to race TMLK and for dissociation from SSR markers *Xgwm55*, *Xgwm319*, *Xcfa2278*, *Xwmc474*, and *Xbarc55*. There were 893 resistant and 193 susceptible plants, indicating strong segregation distortion. Two infection types (IT) were observed among resistant plants: 856 plants had IT 0; and 37 plants had IT 2. Seven IT 0; and three IT 2 plants with small *Ae. speltooides* segments were identified based on marker analysis and genomic *in situ* hybridization. We concluded that the *Ae. speltooides* segment in DAS15 carried two stem rust resistance genes. The IT 2 gene was located on chromosome arm 2BS/2SS, and close to marker *Sr39#22r*, indicating that it may be allelic, or possibly identical, to *Sr39*. Stem rust tests of the dissociation lines with several races also suggested that the IT 2 gene was similar or identical to *Sr39*. The gene conferring IT 0; was located on 2BL/2SL near dominant markers *Xgwm47*, *Xwmc332*, and *Xwmc627*. The proximity of the gene to these three markers suggested that the gene may be homoeoallelic to *Sr9* or *Sr28*.

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54. Histopathology of some non-specific resistance mechanisms expressed on wheat cultivar Toropi

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Leaf rust, caused by *Puccinia triticina*, can cause significant damage in all regions where wheat (*Triticum aestivum*) is cultivated. The primary means of controlling leaf rust has been through resistance, although in most cases this has quickly been overcome by the pathogen. More durable partial or non-specific resistance may possess different mechanisms from those involved in specific resistance. We studied the histological components of durable adult plant leaf rust resistance present in the Brazilian variety Toropi, as well as in the susceptible variety BRS 194 and a line possessing the specific resistance gene *Lr9* at both the seedling and adult plant stages of development. We evaluated the processes of colonization and infection of the fungus in each of the genotypes by scoring the occurrence of cell death, accumulation of phenolic compounds, autofluorescence and formation of hydrogen peroxide. After inoculation, samples were taken at time intervals of 6, 12, 18, 24, 36, 48 and 120 hours, for evaluating the percentages of spores germinated, appressoria over stomata, sub-stomatal vesicles, infective hyphae, haustorial mother cells and haustoria. The effects of Toropi resistance was observed at very early stages of infection, particularly at appressorium formation over stomata. Among all the components evaluated, this seems to be a major effect of Toropi resistance, although late cell death may also be involved at some infection sites.

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