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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 F1 plants were backcrossed as females to the hexaploid KS05HW14 to generate BC1F1 progeny. Phenotyping of BC1F1 progeny with avirulent stem rust races identified stem rust-resistant progenies which were used as males to generate euploid BC2F1 plants. A bulked segregant analysis of BC1F1 or BC2F1 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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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 RKQCC). 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 RKQCC, 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


*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 15*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 15*sh* and putative orthologs in barley and *Brachypodium*, using Single Nucleotide Polymorphisms (SNPs) indentified 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. monococum (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. cylindrica (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. speltoides 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. speltoides segments were identified based on marker analysis and genomic in situ hybridization. We concluded that the Ae. speltoides 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 homoallelic to Sr9 or Sr28.

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 were observed at very early stages of infection, particularly at apressorium 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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