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Genetics of host-pathogen interactions in the wheat *Stagonospora nodorum* pathosystem

Faris JD¹, Lu HJ², Zhang Z³, Reddy L³, Liu ZH², Xu SS¹, Chu CG³, Abeysekara N³, Fellers JP⁴, Cloutier S⁵, Keller B⁶, Scofield SR⁷, Friesen TL¹

¹ USDA-ARS Cereal Crops Unit, Northern Crop Science Laboratory, Fargo, ND 58105, ² Department of Plant Pathology, North Dakota State University, Fargo, ND 58105, ³ Department of Plant Sciences, North Dakota State University, Fargo, ND 58105, ⁴ USDA-ARS Plant Science and Entomology Research Unit, Manhattan, KS 66506, ⁵ Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg, MB R3T 2M9, ⁶ Institute of Plant Biology, University of Zurich, Switzerland, ⁷ USDA-ARS Crop Production and Pest Control Research Unit, West Lafayette, IN 47907

Stagonospora nodorum causes the disease *Stagonospora nodorum* blotch (SNB) in wheat. *S. nodorum* produces numerous host-selective toxins (HSTs), all of which interact with dominant host sensitivity genes to cause disease. These host-toxin interactions are mirror images of classical gene-for-gene interactions. The effects of compatible host-toxin interactions in the development of SNB are largely additive, and they play important roles in disease susceptibility of seedlings as well as adult plants. One of the first *S. nodorum* HSTs was SnToxA, which was recently involved in a lateral transfer from *S. nodorum* to the tan spot pathogen. The map-based cloning of the *Tsn1* locus on chromosome 5B, which confers sensitivity to SnToxA, reveals a complex evolutionary history of the locus when compared to rice, *Brachypodium*, and the homoeologous region of chromosome 5A. The isolation of *Tsn1* will allow the molecular characterization of interactions in the wheat-*S. nodorum* pathosystem, which may be an excellent toxin-based model for other necrotrophic pathosystems.

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Deciphering defense strategies that are elucidated in wheat containing different *Dn* resistance genes

Botha AM¹, Swanevelder ZH¹, Schultz T¹, Van Eck L², Lapitan NLV²

¹Department of Genetics, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa, ²Department of Soil and Crop Sciences, Colorado State University, Fort Collins, Colorado 80523-1170, USA

Russian wheat aphid (*Diuraphis noxia*, RWA, Homoptera: Aphididae) is a major pest problem in many wheat growing areas in the world. In the U.S. alone, the economic impact of RWA has been estimated at approximately \$900M from 1987-1993. However, this estimate may be conservative, since recently several new biotypes developed, presenting a difficult challenge for breeders. A lack of understanding of the interaction between the RWA and its host plant is a limitation in developing effective strategies for controlling the aphid. While our knowledge of plant-pathogen interaction has rapidly advanced in the last few years, our understanding of the interaction between insects and their plant hosts has lagged behind. It is generally assumed that the interaction between insect and plant is similar to that between plant and pathogen; that is, the product of a virulence factor from the insect is recognized by a protein from the host plant. Thus, RWA serves as a model organism for the study of molecular interactions between cereals and phloem-feeding insects, with the recognition and signalling events during the hypersensitive response (HR) and systemic acquired resistance (SAR) being of particular interest to plant breeders. Wheat lines containing different

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resistance genes to the RWA exhibit different resistance or tolerance responses. We investigated these responses at transcriptome level in near-isogenic wheat lines (NILs) containing the *Dn1*, *Dn2* and *Dn5* resistance genes, respectively. Affymetrix gene technology (i.e. Affymetrix GeneChip® Wheat Genome Array) and cDNA-amplified fragment length polymorphism (cDNA-AFLP) transcript profiling were utilized. Following these approaches, we have identified genes and pathways associated with different resistance phenotypes afforded by the *Dn* genes. Detailed expression analyses using qRT-PCR and Northern blots provided further supporting evidence that regulation of specific pathways is critical for the development of a specific mode of resistance.

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The role for basal resistance in non-host interactions

Tufan HA, Boyd LA

Disease and Stress Biology, John Innes Centre, Colney Lane, Norwich, UK

Species of the *Magnaporthe* complex include *M. oryzae*, which is able to infect small grain cereals such as rice, wheat and barley and *M. grisea*, able to infect grasses such as *Digitaria sanguinalis*. In rice, *M. oryzae* causes rice blast, an agronomically important disease of rice. In wheat, *M. oryzae* has only recently presented as a disease of wheat in the field, appearing in Brazil in the 1980's. In this study we examine the interactions between host isolates of *M. oryzae* and non-host isolates of *M. grisea* on the wheat cultivar Renan. Both the cellular and gene expression response of Renan are examined. At the cellular level a strong physical barrier response is observed in Renan towards the *Digitaria* isolates of *M. grisea*. Microarray expression screens have identified a number of candidate genes implicated in the differential responses seen between Renan and the different isolates of *Magnaporthe*. The results of these studies will be presented.

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Molecular analysis of fungal disease resistance in wheat

Keller B¹, Yahiaoui N¹, Brunner S¹, Cloutier S², Kaur N¹

¹*Institute of Plant Biology, University of Zurich, Zollikerstrasse 107, 8008 Zurich, Switzerland,* ²*Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg, MB R3T 2M9*

Obligate biotrophic fungi such as rust species and mildew are major pathogens of wheat. Although a large number of resistance genes have been identified in the wheat germplasm against these pathogens, only few genes have been isolated at the molecular level. We have cloned the *Lr1* and *Lr10* leaf rust resistance genes and the *Pm3* alleles conferring powdery mildew resistance. For all these genes we have studied resistance gene evolution by analysing alleles/orthologs in diploid, tetraploid and hexaploid wheat species as well as grasses related to wheat. These studies have revealed highly diverse evolutionary histories of the different resistance genes, reflecting in part the human interference by agriculture. The most comprehensive study on natural diversity was undertaken for *Pm3* alleles in wild tetraploid wheat as well as a set of wheat landraces. This indicated an unexplored pool of additional resistance alleles particularly in landraces which are currently analysed for their agricultural use. Finally, we are analysing at the molecular level the functionally important domains in the *Pm3* protein, allowing to perform the first experiments on molecular design of new resistance specificities.

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Fine-mapping of the durable leaf rust resistance gene *Lr34* using sequence information from *Brachypodium* and *Aegilops tauschii*

Krattinger SG², Lagudah ES¹, Spielmeier W¹, Keller B²

¹CSIRO Plant Industry, Canberra, Australia

²Institute of Plant Biology, University of Zurich, Zurich, Switzerland

One of the most relevant genes in wheat disease resistance breeding is *Lr34*, located on chromosome 7DS. The *Lr34* locus has contributed towards durable resistance to leaf rust, stripe rust and powdery mildew, making *Lr34* a unique resource for breeding and a model for understanding the molecular basis of partial horizontal resistance. To increase marker density around the *Lr34* locus, we used molecular information from the orthologous region in *Brachypodium sylvaticum*, a new model organism for temperate grasses. Four coding sequences cover the orthologous *Lr34* target interval in *Brachypodium*. Wheat ESTs were identified by homology with these genes. To derive new polymorphic markers, BAC clones representing a total physical size of ~2 Mb and belonging to three contigs were isolated from *Ae. tauschii* by hybridization screening using the wheat ESTs. Eight BAC clones were low-pass sequenced resulting in a total of ~480 kb of sequence. Using the sequence information from *Ae. tauschii* we developed microsatellite, SNP and RFLP-based molecular markers. Fine-mapping of *Lr34* on three high resolution backcross populations revealed close linkage of *Lr34* to the molecular markers csLVMS, E17, SWM10, and SWSNP3. The two microsatellite markers csLVMS and SWM10 showed the same allele in the three independent sources of *Lr34* 'Frontana', 'Chinese Spring' and 'Forno' as well as in many additional cultivars containing *Lr34*. Hence they are highly useful markers to assist selection for *Lr34* in breeding programs worldwide. Genetic mapping defined a 0.17 cM interval that corresponds to a 400 kb physical contig in *Ae. tauschii*. Sequence information from the target interval revealed the presence of a gene rich island containing at least ten candidate genes that are not conserved in *Brachypodium sylvaticum*, *Brachypodium distachyon* or rice.

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General disease resistance loci against biotrophic pathogens in wheat

Lillemo M¹, Asalf B¹, Singh RP², Huerta-Espino J³, Chen XM⁴, He ZH^{4,5}, Brown JKM⁶, Bjørnstad Å¹

¹Dept. of Plant and Env. Sci., Norwegian Univ. of Life Sci., Ås, Norway, ²CIMMYT, México, ³Campo Experimental Valle de México-INIFAP, Mexico, ⁴Institute of Crop Sciences/National Wheat Improvement Center, CAAS, Beijing, China, ⁵CIMMYT China Office, c/o CAAS, Beijing, China, ⁶Dept. of Disease and Stress Biology, John Innes Centre, Norwich, England.

There is increased interest in breeding wheat cultivars for partial and race non-specific resistance against diseases like leaf rust (LR), stripe rust (YR) and powdery mildew (PM) which are caused by biotrophic pathogens with high evolutionary potential. The CIMMYT bread wheat line Saar exhibits good partial resistance to all three pathogens, and a QTL mapping study was conducted in a cross with the susceptible line Avocet-YrA. The adult plant rust resistance loci *Lr34/Yr18* and *Lr46/Yr29* were shown to be major determinants of the PM resistance in Saar as well as conferring resistance to LR and YR. The PM resistance genes at these two loci on chromosomes 7DS and 1BL have been named *Pm38* and *Pm39*, respectively. Further characterization of the resistance was conducted with near-isogenic lines in the susceptible background of Avocet-YrA. Both loci were shown to confer partial

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resistance to PM, LR and YR in field trials, while a detached leaf assay with primary seedling leaves indicated a race non-specific nature of the resistance to PM. To determine the time point when the resistance of *Pm38* and *Pm39* becomes active, colony numbers and sporulation rate were measured after inoculating leaf segments taken from plants spanning the whole range of growth stages from 1st leaves of 12-day old seedlings to flag leaves of adult plants. A significant reduction in colony numbers associated with *Pm38* and *Pm39* was only detected in flag leaves around the time for the onset of leaf tip necrosis, while some reduction in the sporulation rate was observed on penultimate leaves. Both genes were associated with partial hypersensitive cell death at the seedling stage, which was observed only for a low proportion of the penetration attempts, and this did not result in any measurable effect on resistance components.