Poster 67. Gene expression differences in wheat induced by six Puccinia triticina races.

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Next generation sequencing provides a unique opportunity to understand gene expression. Solexa RNAseq was used to look into gene expression differences that occur in the wheat variety Thatcher when infected with different races of leaf rust. Six races were selected from the North American 5 linage, single pustule purified, and inoculated individually onto 2–3 leaf stage wheat seedlings. At six days post inoculation, leaves were sampled and total RNA was isolated and sent to Cofactor Genomics. A single lane of paired end read Illumina GAIIx sequencing was performed for each race. Each race averaged 27.4 million reads and 3.295 million base pairs. Reads were aligned to the Puccinia triticina genome and a wheat EST data set. Pairwise comparisons were made. Thirty-one genes were found to have expression differences. These genes are currently being verified by real-time PCR.

Poster 68. Functional analysis and localization of SnTox1, a necrotrophic effector produced by the wheat pathogen Stagonospora nodorum.

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Stagonospora nodorum, the causal agent of wheat Stagonospora nodorum blotch, is known to produce multiple necrotrophic effectors, each of which specifically interacts directly or indirectly with a corresponding host gene product to induce disease. SnTox1/*Snn1* was the first fungal effector–host gene pair to be identified in this pathosystem by using ITMI population. We recently cloned the fungal gene encoding for SnTox1 and found it to be a highly cysteine-rich protein that can trigger PCD-like reactions in sensitive plants as well as having a significant role in fungal penetration. In the present work, we are investigating the mode of action and cellular localization of SnTox1. Using yeast culture filtrates containing SnTox1, we have demonstrated that widespread necrotic lesions form after directly spraying SnTox1 onto the leaf surface of lines harboring the corresponding sensitivity gene *Snn1*. However, this is not the case for SnToxA or SnTox3, suggesting a unique mode of action for SnTox1. Furthermore, the recognition of SnTox1 on the leaf surface takes place within a few minutes, which was demonstrated by SnTox1 application to the leaf surface followed by washing at different time points. Based on a Prosite motif search of SnTox1, multiple predicted sites including a putative chitin-binding domain were targeted for site-directed mutagenesis. SnTox1 activity was significantly reduced when mutations were produced at a casein kinase II phosphorylation site and a predicted helical region where lysine residues are abundant. Using a fungal strain expressing an SnTox1GFP fusion protein, we examined the location of the SnTox1 protein during fungal growth and infection. SnTox1 was observed in higher concentration on parts of several fungal structures, including the surface of conidia and mycelium, hyphal septa, and hyphal tips. The accumulation of SnTox1GFP is particularly obvious at hyphal regions where new hyphae are arising. *In planta*, SnTox1 is highly expressed in the hyphopodia where the penetration is initiated, providing further evidence that SnTox1 plays a role in penetration. Currently, we are using immunolocalization methods to determine if SnTox1 is internalized into the plant cell.

Poster 69. Dissecting the cytoplasmic component of plant-pathogen interaction pathways.

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The interactions between genomes present in the cytoplasm and the nucleus are critical to all eukaryotic organisms. These interactions trigger changes in gene expression, production of ATP, and control a large number of morpho-physiological functions. Wheat alloplasmic lines are plants where the cytoplasmic genomes of one wheat species (*Triticum*) are substituted by those of a wild relative (*Triticum* or *Aegilops*), while the original nucleus is maintained. Under these con-

ditions, the interaction between the nucleus and different cytoplasm can be evaluated at the morphological and molecular level. Cytoplasmic organelles (mitochondria and chloroplast) have been implicated in multiple plant-pathogen interaction pathways.

Our project is to measure differential responses of various alien cytoplasms in a specific nuclear background to various pathogens to provide a better understanding on the effect of nuclear-cytoplasmic interactions on biotic stress tolerance. In this study, we analyzed the nuclear donors of 56-1 (tetraploid), and Chris and Selkirk (both hexaploid), alloplasmic lines. Fifty selected alloplasmic lines were tested for their diseases response to *Pyrenophora tritici-repentis* (Ptr) isolates BR15 (produces ToxA, B, and C) and Pti2 (produces ToxA and C), one of the more virulent isolates in our collection. Results indicated that *Aegilops bicornis* cytoplasm provided reduced sensitivity to isolate BR15 as identified in alloplasmic lines of Selkirk and Chris, whereas *Ae. variabilis* cytoplasm provided reduced sensitivity to isolate BR15 as identified in alloplasmic lines 56-1 and Chris. Selkirk alloplasmic lines showed a similar reaction to the euplasmic parent to isolate Pti2. These alloplasmic lines, and others that showed significant increases in resistance or susceptibility, are being screened with additional Ptr isolates. Further investigations determining the mechanism of increased resistance or sensitivity by measuring the changes in the production of reactive oxygen species in those selected alloplasmic lines are underway. In conclusion, cytoplasm variability can improve resistance to plant diseases.

Poster 70. The function of scs, Rf, and vi for proper compatibility of durum wheat nucleus in alien cytoplasms.

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Interactions between alien cytoplasms and particular species cytoplasm specific, *scs*, genes from *Triticum timopheevii* or common wheat (*T. aestivum*) transferred to the nuclear genome of durum wheat (*T. turgidum*) produce male-sterile, A lines that are maintained by crossing to normal counterparts (B lines). Our objective was to study the function of fertility restorer (*Rf*), *scsti*, and *vitality* (*vi*) genes in alloplasmic lines of durum wheat to provide partial (sterile) and full compatibility (fertile) between alien cytoplasm and nucleus. We crossed A lines of durum wheat with cytoplasms from different related species, including *T. timopheevii* (Tt), *T. araraticum* (Ta), and *Aegilops speltoides* (Spt) to the (*Ae. longissima*) double-ditelosomic 1B ((lo) dDt 1B) having *scsti scsti* and *vi vi* and the durum lines having *scsti scsti* or *vi vi*. The crosses of (lo) dDt 1B with the *scsti scsti* and *vi vi* gene pairs produced fertile F_1s, showing that *scsti* and *vi* produce fertile F_1s, whereas crosses to durum lines having a *scsti scst*i or *vi vi* produced male sterile F_1s, showing that *scsti* or *vi* alone do not produce fertility in A lines with alien cytoplasm. Also, crosses of (lo) *scsti*–durum to the R lines having *Rf* genes and cytoplasm of Tt, Ta, spt, or other related species produced plump and viable seeds having *scsti* and *Rf* and fertile F_1s, but seeds having *Rf* genes alone were shriveled and inviable, like those from a cross to control durum. In summary, the (lo) *scsti vi* or (lo) *scsti Rf* produced plump seeds and fertile F_1,whereas (lo) *vi* produced plump seeds with greatly reduced fertility and plant vigor, and *Rf* produced shriveled and inviable seeds.

Poster 71. Homoeoallelic relationship of two speciation genes involved in the evolution of allopolyploid wheat.

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The evolution of wheat is a very complex process involving both nuclear and extranuclear genomes. Here we present work on homoeologous relationship between two speciation genes affecting nuclear-cytoplasmic (NC) interactions, critical in the development of allopolyploid wheat. Disruption of such NC interactions leads to multiple incompatibilities, such as lack of seed viability or low vigor. These incompatibilities create genetic barriers, playing an important role in the speciation process, and preclude the commercial production of hybrid wheat and the full exploitation of the secondary and tertiary gene pools in breeding of *Triticum* ssp. The species-cytoplasm specific (*scs*) genes restore adequate compatibility between durum wheat nucleus and *Aegilops longissima* (S^1S^1; 2n=2x=14) cytoplasm. Classical mapping using