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 Sn-Tox3, 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 chitinbinding 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-