GENETIC ENGINEERING AND TRANSFORMATION

Chairperson: Ron Skadsen

FUNCTIONAL ANALYSIS OF PUTATIVE GENES FOR FHB-RESISTANCE/SUSCEPTIBILITY IN WHEAT USING RNAI

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ABSTRACT

Fusarium head blight (FHB) significantly reduces grain yield and quality. Identification of genes that govern FHB resistance/susceptibility will facilitate biotechnology-assisted development of superior wheat varieties with FHB resistance. Our study on global expression profiling using microarray and real-time PCR identified three genes that are significantly highly expressed (up-regulated) in the resistant cv. Ning 7840 in comparison with the susceptible cv. Clark. Since these genes were always expressed to a greater extent in the resistant cultivar than in the susceptible cultivar, these could be very important genes for FHB resistance. A cluster of genes with significantly higher expression levels (down-regulated) in the susceptible cultivar Clark relative to the Ning 7840 was also discovered. These genes may facilitate fungal growth in a spike. Further investigation of the functions of these genes may provide useful information for understanding the mechanisms of FHB resistance. To investigate the biological function of these candidate genes, RNAi-mediated gene silencing will be used to knock out their corresponding homologous mRNAs in wheat. Double stranded hairpin RNA molecules corresponding to up-regulated genes will be targeted to resistant cultivars while dsRNA from down-regulated genes will be targeted to susceptible cultivars to create loss-of-function plants. RNAi constructs using pANDA vector are underway.

ACCUMULATION OF TRANSGENE-ENCODED DEFENSE-ASSOCIATED ENZYMES IN TISSUES VULNERABLE TO INITIAL *FUSARIUM* INFECTION

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ABSTRACT

Our goal is to achieve Fusarium Head Blight resistance by employing genetic transformation to introduce new genes into wheat. Of particular interest are anti-*Fusarium* genes that could provide protection in the early stages of infection, thus improving Type I resistance. To achieve this, we have employed an expression vector carrying the barley *Lem1* promoter, which we had previously shown to be active in the outer organs of transgenic wheat florets from anthesis to the soft dough stage of kernel development. Into this vector, we inserted coding regions from three candidate anti-*Fusarium* genes that have been associated with naturally occurring plant defense mechanisms: *Aspergillus* glucose oxidase (*GO*) and barley peroxidases *Prx7* and *Prx8*. GO is an apoplastic enzyme that catalyzes oxidation of β-D-glucose, generating H_2O_2, a compound with multiple functions in plant defense. Induction of the peroxidases Prx7 and Prx8 has been correlated with the appearance of antifungal compounds and papillae structures, respectively, in barley leaves exposed to powdery mildew. We have analyzed several transformed wheat plants for inheritance and expression of the Lem1::PRX and/or Lem1::GO transgenes. Peroxidase and glucose oxidase enzymes were detected *in situ* in the outer tissues of the floret, where they accumulated either in the extracellular space (GO and Prx8) or inside the cells (Prx7). In some of the lines, lignin content was increased in the outer floret tissues. The potential for synergistic effects of the transgene-encoded enzymes in improving host resistance to initial fungal infection and pathogen spread will be discussed.

OVEREXPRESSION OF ANTIFUNGAL PROTEINS INCREASES RESISTANCE OF WHEAT TO FUSARIUM HEAD BLIGHT IN THE FIELD

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ABSTRACT

We are developing and testing transgenic wheat for resistance to Fusarium Head Blight (FHB). Anti-fungal proteins (AFPs), such as ß-1,3-glucanases, thionins, chitinases, thaumatin-like proteins (TLPs), ribosome-inactivating proteins (RIPs) and lipid transfer proteins (LTPs), are thought to inhibit fungal growth via different mechanisms. Transgenic wheat lines over-expressing these AFPs were generated using micro-projectile bombardment of the cultivar 'Bobwhite'. Both single transgenes and combinations of two transgenes were generated, either through co-bombardment or later crossing between transgenic materials. Transgenic materials were screened for Type II resistance in the greenhouse using single floret inoculation. Lines showing reduced severity in comparison with non-transgenic Bobwhite in three to four greenhouse trials were evaluated in field trials in 2004 and/or 2005. Of the seven lines that were evaluated in field trials in both 2004 and 2005 (one a-puro-thionin line, two TLP lines and four ß-1,3-glucanase lines), four showed reduced FHB severity, two showed reduced visually scabby kernels (VSK), and three showed reduced levels of DON (ppm) ($p<0.05$). Of the seventeen additional lines evaluated in the field in 2005 alone, six showed reduced FHB severity, five showed reduced VSK, and two showed reduced levels of DON (ppm) ($p<0.05$). In addition, we developed and tested transgenic wheat carrying LTP, RIP. RIP/TLP, TLP/*TRI101*, ß-1,3-glucanase/*TRI101*, and ß-1,3-glucanase/TLP combinations in the greenhouse using single floret inoculation. Results of these greenhouse screens will be presented.

A RAPID ASSAY SYSTEM FOR TRANSGENES CONFERRING RESISTANCE TO DON

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ABSTRACT

There is considerable interest in using transgenic approaches to enhance resistance to Fusarium Head Blight (FHB). Production of the tricothecene deoxnivalenol (DON) is thought to be a virulence factor for FHB, at least during some stages of infection, and it has been proposed that engineering resistance to DON might make plants less susceptible to infection with FHB. Testing transgenes for efficacy against DON in wheat or barley germplasm is time consuming and labor intensive. It would be extremely useful to be able to assay transgenes for efficacy against DON or FHB, before they are introduced into wheat or barley.

We have developed a rapid and efficient whole-plant system based on the recombinogenic plant *Physcomitrella patens*, an emerging model system for functional genomics. Physcomitrella is sensitive to physiological levels of DON. Toxicity of DON is almost completely abolished in Physcomitrella plants that overexpress the cell death regulator BI-1. These transgenic plants also display almost complete resistance to several necrotrophic pathogens. DON toxicity is also substantially attenuated in transgenic Physcomitrella plants that express a modified ribosomal L3 gene (the L3Ä mutant) or in Physcomitrella plants in which other endogenous pathogen-induced transcripts have been deleted through gene targeting.

These results confirm that the sensitivity to DON is under genetic control in plant cells and demonstrate that sensitivity can be affected at a number of genetic control points. Current efforts are focused on establishing whether genes effective against DON act through similar or independent pathways. Our results form the basis for future studies designed to understand the mechanism of action of mycotoxins. The rapid assay system also provides a means of assessing transgenes for efficacy, prior to their introduction into wheat or barley. The system could also be adapted for medium- to high-throughput screens for novel sources of mycotoxin resistance.

A VIRUS-INDUCED GENE SILENCING SYSTEM FOR THE ANALYSIS OF DISEASE RESISTANCE PATHWAYS IN WHEAT AND BARLEY

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ABSTRACT

Systems for virus-induced gene silencing (VIGS) that can rapidly and efficiently create gene knockout phenotypes, have proven to be very useful tools for the analysis of plant gene function. VIGS is a form of RNA-mediated gene silencing. All forms of RNA-mediated gene silencing involve the production of large amounts of dsRNA that activates a host defense mechanism that results in the degradation of all RNAs with homology to the sequences within the dsRNA. In VIGS, certain RNA viruses are used to produce dsRNAs that trigger the silencing mechanism. If the virus has been engineered to contain sequences from a plant gene of interest, mRNAs from that gene are degraded as well, thus creating knockout phenotype for the chosen gene. Given a validated VIGS system and a 200-500bp fragment from a gene of interest as starting material, it is possible to assemble the VIGS construct, infect plants and observe the knockout phenotype within one month. The first VIGS systems were effective in only a few dicot species however, recently a VIGS system based on Barley stripe mosaic virus has been demonstrated to efficiently trigger VIGS in barley and wheat. The creation of gene knockouts in polyploid plants, such as wheat, is very difficult using conventional mutagenesis strategies because expression of homeoloci mask mutations. However, since VIGS operates through a homology-dependent mechanism, it promises to be particularly useful in polyploids, because mRNAs from homeoloci should be degraded as well, provided they share sufficient sequence homology. This talk will describe the development of the BSMV-VIGS system and demonstrate its utility in the functional analysis of genes required in a range of wheat and barley disease resistance pathways.

ENGINEERING SCAB RESISTANCE IN WHEAT WITH PLANT DEFENSE SIGNALING GENES

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ABSTRACT

Fusarium Head Blight (FHB)/scab is a devastating disease of wheat and barley that severely limits crop productivity and grain quality. We have developed a plant-pathogen system consisting of *Arabidopsis thaliana* and *Fusarium graminearum* to identify genes that are involved in plant defense against *F. graminearum*. We have shown that constitutive expression of the Arabidopsis *NPR1* gene (At*NPR1*) in transgenic Arabidopsis plants enhances resistance to *F. graminearum*. Likewise, the wheat cultivar Bobwhite, which is normally susceptible to scab by *F. graminearum*, when engineered to constitutively express the Arabidopsis *NPR1* gene (At*NPR1*) exhibited heightened resistance to the pathogen. The NPR1 protein is a key regulator of salicylic acid (SA) signaling in plant defense and the activation of systemic acquired resistance (SAR). Resistance against *F. graminearum* in the At*NPR1* expressing wheat plants correlated with a rapid and strong activation of expression of the pathogenesis related, *PR1* gene, in the pathogen-challenged plants. This rapid response of *PR1* expression in the At*NPR1* expressing Bobwhite plants was similar to that of the scab-resistant cultivar, Sumai 3. Application of benzothiadiazole (BTH), a functional analog of SA, also induced *PR1* gene expression faster and to a higher level in Sumai 3 and the At*NPR1* expressing transgenic wheat than in non-transgenic Bobwhite plants, suggesting that a SA/BTH-regulated signaling mechanism regulates plant defense against *F. graminearum*. Indeed, BTH treatment was sufficient to enhance scab resistance in non-transgenic Bobwhite plants. These results suggest that scab resistance in Sumai 3 and the At*NPR1* expressing transgenic plants may result from increased responsiveness of these plants to an endogenous defense signaling molecule. In order to further understand the molecular and physiological basis of scab resistance in these At*NPR1* expressing plants we have carried out microarray studies. We are further developing the Arabidopsis-*F. graminearum* system to rapidly identify other genes and signaling pathways involved in plant defense against *F. graminearum*. In the future some of these genes could be targeted to enhance scab resistance in wheat and barley.

REFERENCE

Makandar, R., Essig, J. S., Schapaugh, M. A., Trick, H. N. and Shah, J. Genetically engineered resistance to Fusarium head blight in wheat by expression of *Arabidopsis NPR1*. Mol. Plant-Microbe Interact. (in press).

TRANSGENIC BARLEY CO-EXPRESSING ANTIFUNGAL AND ANTITOXIN GENES

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ABSTRACT

Overexpression of pathogen response proteins in plants could lead to enhanced resistance against diseases and reduce economic losses. Plants expressing combinations of these genes have shown synergistic action against fungal diseases. We have transformed a commercial malting barley cultivar (*Hordeum vulgare* cv. Conlon) to co-express antifungal and antitoxin genes by particle bombardment. In the past, we have produced T_2 homozygous transgenic barley lines that co-express antifungal genes such as thaumatin-like protein (*tlp*) or chitinase (*chi*) genes from rice, and *Tri101*, an antitoxin gene. Backcross lines with these genes are being tested in the field and greenhouse against Fusarium head blight. More than 250 transformed plants carrying antitoxin and antifungal genes have been developed from 72 transformation events including 11 different gene combinations. PCR analysis of T_0 and T_1 progenies from 45 of these events indicated the presence of the transgenes while western blot analysis confirmed expression of the proteins. T_2 homozygous lines have been selected and are currently being tested against FHB.

MODIFICATION OF RIBOSOMAL PROTEIN L3 CONFERS RESISTANCE TO DEOXYNIVALENOL

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ABSTRACT

Wheat and barley scab, also known as Fusarium head blight (FHB) is a devastating disease worldwide, caused mainly by *Fusarium graminearum*. The *Fusarium*-infected grain is contaminated with potent mycotoxins, especially deoxynivalenol (DON), which poses a great threat to human and animal health. DON belongs to the group of trichothecene toxins, which target ribosomal protein L3 at the peptidyltransferase site of eukaryotic ribosomes and inhibit protein synthesis. The goal of our work is to identify mutations in L3 that confer resistance to DON and to determine if FHB resistance can be engineered in transgenic wheat plants by expressing DON resistant L3 genes. We have demonstrated that overexpression of a truncated form of yeast ribosomal protein L3 (L3Δ) in transgenic tobacco plants confers resistance to deoxynivalenol (DON). Expression of the yeast L3Δ also confers resistance to a ribosome inactivating protein, pokeweed antiviral protein (PAP), which binds to L3 to depurinate the ribosomes. Transgenic plants expressing PAP and L3Δ are phenotypically normal and ribosomes are not depurinated in these plants. These results demonstrate that expression of yeast L3Δ leads to *trans*-dominant resistance to PAP and DON, providing evidence that both toxins target L3 by a common mechanism. The goal of our project is to translate the success we had in engineering DON resistance in tobacco to wheat and to generate wheat lines resistant to FHB. In collaboration with Dr. Ann Blechl, we have introduced the yeast L3 genes into wheat and identified transgenic lines containing the maize *Ubiquitin1* promoter and L3Δ, barley *Lem1* promoter and L3Δ, barley *Lem1* promoter and the full length yeast L3. We have used PCR to identify two to five stable transformants per construct and obtained seed from the homozygous lines. The primary wheat transformants and their homozygous progeny are phenotypically normal and fertile. Real time PCR analysis was used to confirm expression of the transgenes in the T2 progeny. The T2 plants, which express the transgenes, are indistinguishable from the non-transformed plants in their growth and morphology. We are in the process of evaluating the transgenic wheat lines for resistance to trichothecenes and FHB.

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