GENETIC ENGINEERING AND TRANSFORMATION

TRANSGENIC BARLEY WITH IMPROVED RESISTANCE TO *F. CULMORUM*.

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ABSTRACT

One strategy to reduce the amount of mycotoxins in small grain cereals would be the development of resistant cultivars. Cereal genes conferring resistance to *Fusarium* infection have not yet been identified, but in some wheat cultivars, resistance loci have been mapped. Trichoderma genes encoding chitinolytic enzymes involved in biocontrol have been introduced into several plant species and have been shown to increase the plants' resistance against fungal pathogens.

At Bioforsk we have produced GM-barley where a fungal endochitinase gene, ech42 from *T. atroviride* regulated by the barley promoter *Ltp2*, has been inserted resulting in increased resistance towards *Fusarium culmorum* infection. The advantage of the *Ltp2* promoter is that it permits a gene to be expressed only in the aleurone layer of developing seeds. One of the resulting transformed plant lines, PL9, seemed to be especially promising. The copy number was estimated by the real-time PCR method to be low. Study on the inheritance of the transgenes in T_1 progeny revealed a Mendelian 3:1 segregation pattern.

Some T_1 progenies showed very high *ech42* expression while others had either very low or no detectable expression at all. After inoculation with *F. culmorum*, all *ech42* containing T_1 progenies coming from PL9 showed high resistance. The amount of *F. culmorum* present after point inoculation of the spikes was quantified by real-time PCR analysis. Extremely low amounts or no *F. culmorum* could be detected in seeds located at the same spike close to the point inoculated grains compared to what was found in wild type control plants. The *F. culmorum* resistance was found to be stable when tested in the T_3 generation. Resistance towards *F. graminearum* however, could not be detected in the GM-barley.

EXPRESSION OF A TRUNCATED FORM OF RIBOSOMAL PROTEIN L3 IN TRANSGENIC WHEAT CONFERS RESISTANCE TO DEOXYNIVALENOL AND FUSARIUM HEAD BLIGHT.

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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. In previous studies, we have demonstrated that overexpression of a truncated form of yeast ribosomal protein L3 (L3Δ) in transgenic tobacco plants confers resistance to deoxynivalenol (DON). To determine if expression of the yeast L3Δ in transgenic wheat plants would provide resistance to FHB, the susceptible spring wheat cultivar, Bobwhite was transformed with the yeast L3Δ under the control of the barley floret-specific *Lem1* or the maize constitutive *Ubi1* promoter. Seeds from the homozygous lines containing each construct were able to germinate on media containing DON, unlike the seeds of the wild type Bobwhite plants. To determine if the lines that were resistant to DON were resistant to FHB, five different homozygous lines were evaluated for resistance to FHB in greenhouse tests by inoculating a single spikelet at the central node of the main spike of each plant with a macroconidial spore suspension of *F. graminearum*. All spikelets of inoculated wild type plants turned brown at 21 days after the inoculation. In contrast, only the inoculated spikelets of the transgenic lines turned brown; the uninoculated spikelets remained green in most of the transgenic plants. The disease severity was reduced by 48-56% in four different transgenic wheat lines compared to the untransformed Bobwhite plants. The reduction in disease severity correlated well with the level of expression of L3Δ mRNA. These results demonstrated that transgenic wheat plants expressing the yeast L3Δ showed improved resistance to FHB over the untransformed Bobwhite plants. To determine if resistance to FHB would result in a reduction in DON levels, the mature kernels above and below the inoculated spikelets were analyzed for DON levels. There was a 63-76% reduction in DON levels in the four different FHB resistant transgenic lines. The DON levels in one transgenic line were lower than the DON levels in the resistant line, Alsen. These results provide evidence that resistance to DON correlates with resistance to FHB and results in reduced accumulation of DON in transgenic wheat plants.

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A VIRUS-INDUCED GENE SILENCING SYSTEM FOR THE IDENTIFICATION OF GENES CONTRIBUTING TO FHB RESISTANCE IN WHEAT.

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ABSTRACT

Functional genomics analysis in hexaploid wheat is greatly impeded by the genetic redundancy of polyploidy and the difficulties in generating large numbers of transgenic plants required in insertional mutagenesis strategies. Virus-induced gene silencing (VIGS), however, is a strategy for creating gene knockouts that overcomes both of these impediments: 1) Being a homology-dependent silencing process, it can suppress expression any redundant gene copies that share at least ~85% sequence homology. 2) VIGS is initiated by viral infection, which is a rapid and easy process, unlike regenerating transformed wheat plants. For these reasons we have worked to develop a VIGS system for creating gene knockout phenotypes in hexaploid wheat. Our VIGS system is based on Barley stripe mosaic virus (BSMV). Data will be presented describing the general properties of this silencing system. We are particularly interested in functionally identifying genes that are essential for disease resistance responses in wheat. To this end, we have developed protocols for silencing candidate genes so that we can test if their expression is essential for resistance. We have successfully employed this system to analyze genes required in resistance pathways to leaf rust and several other foliar pathogens of wheat. Very recently, we have developed protocols for silencing genes in the spikes and heads of wheat and are now using BSMV-VIGS to identify genes essential for resistance to Fusarium head blight. The efficacy of this system has been validated in tests in which the wheat line Ning 7840, which has type II resistance to FHB, was first infected with control or experimental VIGS constructs, and then challenged by Fusarium graminearum. It was observed that viral constructs that target no wheat genes or the wheat phytoene desaturase gene, which is assumed to not be involved in FHB resistance, had no effect of Ning 7840's type II resistance. However infection with three different constructs that target wheat chitinase all result in loss of type II resistance.

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ENHANCING RESISTANCE TO *FUSARIUM GRAMINEARUM* BY EXPRESSION OF *ARABIDOPSIS THALIANA* NPR1 IN WHEAT.

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ABSTRACT

Fusarium head blight (FHB)/scab caused by the fungus *Fusarium graminearum* is a destructive disease of wheat and barley. We had previously demonstrated that constitutive expression of *Arabidopsis thaliana NPR1* (At*NPR1*), a key regulator of salicylic acid (SA) signaling, enhances scab resistance in the hexaploid wheat cv Bobwhite (Makandar et al. 2006). The transgenic wheat lines (125A & 192D), which express At*NPR1* from the *Ubi1* promoter, were found to be significantly more resistant to *F. graminearum* in comparison to the non-transgenic control plants in repeated greenhouse experiments. Defense responses (e.g. *PR1* expression) are primed to respond faster in response to challenge by *F. graminearum* in the transgenic *Ubi1*:At*NPR1* plants and the FHB resistant cv Sumai 3, than non-transgenic Bobwhite plants. The enhanced FHB resistance is associated with the faster and stronger expression of the *PR1* gene in *F. graminearum*-challenged spikes of the *Ubi1*:At*NPR1* transgenic plants. Furthermore, *PR1* expression in Sumai 3 and the *Ubi1*:At*NPR1* transgenic wheat was more sensitive to the exogenous application of SA and its analog BTH, than in the cv Bobwhite. Similar to Bobwhite, *PR1* expression in the FHB susceptible cvs Wheaton and Fiedler was also less sensitive to SA, than the FHB resistant Sumai 3 and the *Ubi1*:At*NPR1* transgenic plants, suggesting that sensitivity to SA maybe a useful marker for FHB resistance.

We have concluded two field trials in Manhattan and one in Minnesota in which we have monitored the impact of At*NPR1* on FHB resistance in the *Ubi1*:At*NPR1* transgenic plants. Results of these trials will be presented. In addition, we will present our progress on the introduction of the *Ubi1*:At*NPR1* construct into elite hexaploid wheat and durum cvs.

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Makandar, R., Essig, J. S., Schapaugh, M. A., Trick, H. N. and Shah, J. 2006. Genetically engineered resistance to Fusarium head blight in wheat by expression of *Arabidopsis* NPR1. Mol. Plant-Microbe Interact. 19:123-129.

TRANSGENIC WHEAT WITH ENHANCED RESISTANCE TO FUSARIUM HEAD BLIGHT.

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ABSTRACT

We are developing novel transgenic wheat germplasm resources for resistance to Fusarium Head Blight (FHB). We have developed and are testing transgenic wheat carrying ß-1,3-glucanase, thaumatin-like protein 1 (tlp-1), ribosome-inactivating protein (RIP), lipid transfer protein (LTP), glutathione-S-transferase (GST), thionin, and jasmonic acid inducible Myb transcription factor (JaMyb) genes. Transgenic lines over-expressing these genes were generated using micro-projectile bombardment of the wheat cultivar 'Bobwhite'. Both single and combinations of transgenes were generated. We developed 4, 1, 2, 1, and 4 lines carrying LTP, RIP/tlp-1, TRI 101/tlp-1, TRI 101/ ß-1,3-glucanases, and tlp-1/glucanase respectively. In multiple greenhouse screens of these lines, we identified five (one RIP, two TRI 101/tlp-1, and two tlp-1/glucanase) that exhibited statistically significant reductions in FHB severity compared to the non transgenic controls ($p<0.05$). We also identified 17 lines (1, 2, 1, 6, and 7 transgenic wheat lines carrying the RIP, chitinase/tlp-1, chitinase/RIP, RIP/tlp-1 and chitinase transgenes, respectively) showing reduced severity in comparison with non-transgenic Bobwhite in greenhouse screens. These lines were evaluated in field trials in 2005 and 2006. Three lines (one chitinase, one chitinase/RIP and one RIP) exhibited statistically significant reductions in FHB severity in the summer 2005 field trial compared to the nontransgenic control ($P<0.05$). In 2006, disease severity was extremely low because of the unusually hot and dry weather. We also crossed five transgenic wheat lines (one α-puro-thionin, one TLP and three ß-1,3-glucanase), that exhibited statistically significant reductions in FHB severity in the field, to the type II resistant cv. Alsen. In addition, we developed 9, 13 and 10 transgenic lines carrying LTP, GST and JaMyb genes. Greenhouse screening results will be presented from the transgenic lines derived from the Alsen crosses and the transgenic lines carrying LTP, GST and JaMyb.

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GREENHOUSE FHB REACTION OF DURUM WHEAT EXPRESSING *TRI101* AND A RICE *TLP*.

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum* (Schwabe), is a serious problem in small grain species such as durum wheat (*Triticum turgidum* L.) used for making pasta and semolina. One method being tested to incorporate FHB resistance is gene transfer technology. As previously reported, the cultivar Monroe was transformed by bombarding calli with the pathogenesis-related gene thaumatin-like protein (*tlp*) from rice, and the *Tri101* gene from *F. sporotrichioides*, along with the *bar* gene for selection. PCR and Southern blot analyses identified three insertion events in the 44 regenerated plants and western blot analysis confirmed the expression of both genes in the durum wheat cultivar Monroe. T_2 homozygous lines were identified and three lines from each event were tested for their response to FHB. Replicated greenhouse tests were conducted using spray inoculation with three *F. graminearum* isolates. Spikes from seven of the nine lines showed significantly reduced FHB spread compared to Monroe by 28 days after inoculation, but all had significantly more FHB spread than the resistant bread wheat cultivar Sumai 3. One line with increased FHB at 14 and 21 days after inoculation also showed increased DON. None of the lines showed reduced DON.