A mutation in the ribosomal protein L3 confers resistance to Deoxynivalenol in transgenic tobacco

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Introduction

Mycotoxins belonging to the class of trichothecenes act primarily as inhibitors of eukaryotic protein synthesis. Gene disruption studies indicate that production of deoxynivalenol (DON), a member of the trichothecenes, is a virulence factor of *Fusarium graminearum*. The probable role of the toxin is to inhibit active defense responses of the plant by interfering with the expression of defense related proteins and to condition the host for colonization. Correlative evidence is available that toxin resistance significantly contributes to field resistance of wheat.

It was shown in yeast that trichodermin (also a member of the trichothecenes) binds to a component of the 60S subunit of the wildtype ribosome (Barbacid and Vazquez, 1974) and that the trichodermin resistance gene *TCM1*, which was found to encode the ribsomal protein L3 (*RPL3*), is responsible for semidominant resistance to the toxin (SCHULTZ and FRIESEN, 1983).

Materials and Methods

We used yeast as a model system to explore molecular mechanisms responsible for deoxynivalenol resistance and identified semidominant mutations in the L3 gene.

In order to save expensive toxin, all yeast strains employed contain a disruption of the gene *PDR5*, which encodes a plasma membrane localized ABC transporter protein with specificity for trichothecenes (Adam & Lemmens, 1996). For the shuttle mutagenesis of yeast *RPL3*, a strain was constructed containing a deletion of the chromosomal *RPL3* gene. This strain (YZGA315) is viable on galactose medium due to a plasmid allowing expression of *RPL3* under control of the glucose repressible *GAL1* promoter. A plasmid, which contains a wildtype copy of *RPL3* and the *TRP1* and *ADE2* genes as selectable markers, was mutagenized by passage in the *E. coli* mutator strain XL1-Red (Stratagene) and by hydroxylamine-mutagenesis. Upon transformation of YZGA315, colonies were selected on glucose based medium and transferred to plates containing 100 ppm DON. Plasmid was recovered from resistant colonies, and the alterations responsible for resistance identified by subcloning and sequencing.

A tomato cDNA clone *LeRPL3* was isolated from a phage lambda cDNA library and sequenced. One of the alterations identified in yeast was introduced into the gene by overlap extension PCR. Furthermore, a *c-myc* epitope was added at the C-terminus. The constructs were cloned into a binary plasmid behind the 35S promoter and introduced into tobacco by *Agrobacterium*-mediated transformation. Transgenic plants were characterized by Southern and Western blotting and tested for alterations in toxin resistance.

Results and Discussion

Semidominant mutations in *RPL3* (formerly known as *TCM1*) conferring resistance to the trichothecene trichodermin had been described previously (Fried & Warner, 1981), but the nature of the mutation was not determined. We have performed a random mutagenesis of yeast *RPL3* and characterized 100 plasmids conferring resistance to DON. Five single amino-acid alterations in four different positions of the protein which lead to DON resistance were identified by subcloning and sequencing. In addition, we have also identified the amino-acid change in two of the original *TCM1* mutants.

One of the changes (W255C in *ScRPL3*) was engineered into the highly homolo gous tomato *RPL3* cDNA and introduced into yeast and tobacco. The tomato gene, without and with a C-terminal epitope tag is able to complement a yeast mutant containing a deletion of *RPL3*. In addition, the engineered version of the tomato cDNA also confers DON resistance in yeast. Subsequently we have introduced the wildtype and the engineered versions of *LeRPL3*, with and without the c-Myc tag into tobacco.

Characterization of the several transgenic plants in different assays (seed germination on toxin containing medium, regeneration of leaf disks, gravitropism response in the presence of DON) revealed at first that, at best, a disappointingly small increase in toxin resistance could be achieved. Analysis of protein extracts of the transgenic plants furthermore showed that, in contrast to the wild type protein, only traces of the tagged *Rpl3p* containing the mutation are detected. Our hypothesis is that the mutant *RPL3* protein has a disadvantage during assembly into the ribosome, and that this protein is rapidly degraded. A similar phenomenon was observed in yeast heterozygous for $RPL3_{WT}/RPL3_{W255C}$. Despite being present in unstressed yeast in a low amount, the mutant form of the protein confers semidominant resistance. Pretreatment of yeast with sub-inhibitory amounts of DON led to a rapid accumulation of the mutant form of the protein and dramatically improved toxin resistance.

We applied those findings to tobacco by adapting the transgenic plants to low concentrations of DON before transfering them to media containing higher amounts of toxin. Using this approach we obtained a significant increase of DON resistance of the transgenic tobacco plants.It remains to be tested whether the improved ability to adapt to the fungal toxin leads to enhanced *Fusarium* resistance in transgenic wheat.

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Acknowledgments

This project was made possible by an APART fellowship of the Austrian Academy of Sciences of Gerhard Adam, a DOC fellowship of Brigitte Poppenberger and by funding from the Austrian Science Foundation (FWF P11551-MOB).

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