

Reconstruction and cloning of wheat UDP-glucosyltransferases with the putative ability to inactivate the *Fusarium* toxin deoxynivalenol

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Abstract

Fusarium graminearum frequently infects wheat and barley causing severe contamination of the harvested material with mycotoxins, such as deoxynivalenol (DON). Wheat lines harboring the *Fhb1* resistance QTL have been shown to accumulate higher levels of the non-toxic DON-3-glucoside (LEMMENS et al. 2005). The compound is generated by the activity of UDP-glucosyltransferases (UGT), which consequently have been implicated with playing a role in the resistance of wheat and other plants to *Fusarium* head blight. Previous transcriptomic studies in our group have led to the identification of 16 candidate UGT genes in wheat that are specifically upregulated in response to *Fusarium* stress. Among them we identified a UGT gene (Ta.12887.1.S1) which is a close homolog to a UGT gene from barley, which was shown to exhibit the ability to transform DON into DON-3-glucoside (SCHWEIGER et al. 2010). Another candidate UGT (Ta.22565.1.S1) is closely related to the *Arabidopsis* UGT73C5 gene, which also accepts DON as substrate. The group of upregulated UGTs comprises also a gene (EX982036) similar to a UGT previously identified by STEINER et al. (2009) in a wheat cDNA-AFLP analysis. As probe sets from microarrays derive from expressed sequence tags (ESTs) whose sequence

is not fully known, the starting point of our work was to reconstruct the full-length genes for subsequent cloning. For this purpose we have employed the Unigene-EST databases provided by NCBI and TIGR, public resources for large-scale plant gene expression such as Plexdb and next sequencing data from the UK wheat sequencing project (<http://www.cerealsdb.uk.net/>). We managed to reconstruct Ta.22565.1.S1 and EX982036 in silico, PCR-amplified both genes and cloned into a pGEM-T-easy cloning vector. Ta.22565.1.S1 was subcloned into a yeast expression to transform toxin-sensitive yeast strains and test the transformed strains for enhanced resistance against DON. These tests have not been performed yet. Capturing Ta.12887.1.S1 so far has been unsuccessful as the N-terminal region of the gene harbors a large GC-rich region, which complicates cloning of the gene. Also the hexaploid nature of wheat makes it extremely difficult to distinguish targets whose sequence is only partially known from homoealleles. To speed up our tests Ta.12887.1.S1 was ultimately custom synthesized and is currently tested in yeast.

Keywords

Deoxynivalenol, *Fusarium*, UDP-glucosyltransferases, wheat

References

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