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# TRANSGENE PYRAMIDING TO COMBINE DIFFERENT RESISTANCE MECHANISMS IN WHEAT AGAINST *FUSARIUM* DISEASES

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*Fusarium Head Blight, Fusarium Crown Rot, UDP-glucosyltransferase, DON-detoxification, CWDE inhibitors*

*Fusarium* diseases, including Fusarium head blight (FHB) and crown rot (FCR), represent major agricultural problems worldwide, causing reduction of grain yield and quality, hence negatively affecting food security and safety. Indeed, grain contamination by *Fusarium* mycotoxins, mainly deoxynivalenol (DON), is responsible for health problems in humans and animals. DON is a protein synthesis inhibitor, acting as a virulence factor during pathogenesis. The principal mechanism involved in enhancing plant tolerance to DON is glycosylation, forming DON-3-β-D-glucoside (D3G), performed by specific UDP-glucosyltransferases (UGTs). In this work, we aimed to assess the DON-detoxification potential in controlling *Fusarium* disease of wheat and, in addition, to evaluate a possible synergistic effect of different resistance mechanisms against these diseases.

In a first step, we produced transgenic durum wheat plants (Ubi-UGT) constitutively expressing the barley *HvUGT13248* and bread wheat plants (Lem-UGT) expressing it in flower tissues. We demonstrated that Ubi-UGT plants enhance both FHB and FCR resistance in durum wheat, conferring a broad-spectrum resistance against *F. graminearum* and *F. culmorum*. Notably, a marked reduction of total DON content was observed, as compared to wild type plants. In addition, the floral-specific expression, besides confirming enhanced FHB resistance, also highlighted a dose-dependent efficacy of the UGT detoxification mechanism.

Subsequently, we stacked genes controlling different resistance mechanisms in the same genotypes, in particular by pyramiding transgenes which control the DON-to-D3G conversion and the inhibition of cell wall degrading enzymes by glycosidase inhibitors. To this aim, we have developed cross combinations between: i) the Ubi-UGT plants and plants expressing the *AcPMEI* gene (Ubi-PMEI), coding for a kiwi pectin methyl esterase inhibitor, and ii) the Lem-UGT plants and plants expressing the *PvPGIP2* gene (Ubi-PGIP), coding for a bean polygalacturonase inhibitor protein. We demonstrated that Ubi-UGT+Ubi-PMEI and Lem-UGT+Ubi-PGIP progenies, of durum and bread wheat respectively, increased FHB resistance in different genotype and combination. By contrast, the AcPMEI contribution in the progeny resulted ineffective against the FCR disease, the double-transgenic seedlings exhibiting similar level of symptom reduction to the single UGT transgenic line.

In conclusion, our results demonstrate that DON-detoxification confers a broad-spectrum resistance against DON-producing fungi. Moreover, pyramiding genes controlling different

resistance mechanisms can further reinforce the host response. This approach may be particularly attracting for breeding programs aimed at improving and broadening the plant reaction to pathogen attacks in a sustainable manner.