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Poster Abstract – D.38

GENETIC ENGINEERING FOR WHEAT PROTECTION AGAINST MYCOTOXIGENIC FUNGAL PATHOGENS

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Wheat Head Scab (WHS) or Fusarium Head Blight (FBH) caused mainly by *Fusarium culmorum* and *F. graminearum* is a destructive disease of wheat. The harvested grain is often contaminated by fungal-secreted mycotoxins which cause serious illness and immunorepression in humans and animals, as well as yield loss *per se*. In this respect, it is important the development of improved wheat genotypes with increased pathogen resistance using traditional breeding biotechnology strategies. A maize endosperm cytosolic albumin, with a molecular weight of 32 kDa, termed b-32, has homology with several previously characterized Ribosome-Inactivating Proteins (RIPs). It was found that b-32 is a functional RIP by the criteria of inhibition of *in vitro* translation in a cell-free rabbit reticulocytes system and specific *N*-glycosidase activity on 28S rRNA. Additional evidence indicated that transgenic tobacco plants expressing b-32 showed an increased tolerance against infection by the soil-borne fungal pathogen *Rhizoctonia solani*. In order to further explore the antifungal activity of the maize b-32, we have placed the b32.66 cDNA clone under the constitutive promoter 35S CaMV and introduced it into hexaploid wheat (*Triticum aestivum* L.) cv. Veery via particle bombardment. Six lines expressing b-32 were raised and brought to homozygosity through genetic analysis of progeny. Expression of b-32 protein was confirmed through four subsequent generations and throughout the plant life cycle. The six lines at T4 level were challenged for response against *Fusarium culmorum* (WHS).

The six transgenic homozygous progenies western-b32 positive, and cv.Veery, as negative control, were raised to maturity into a containment-greenhouse and used, at the flowering stage, for a detailed analysis of b-32 expression and for pathogenicity tests. A differential b-32 expression in both leaves and immature spikelets of the various progenies, was recorded. Preliminary experiments on cv.Veery, supported the choice of "single floret injection inoculation method" parameters (spore concentration, detection time) useful for a reliable evaluation of the genotypes. Plants at the early flowering stage were inoculated by injection of *F.culmorum* spore suspension ($5x10^5$ spore/ml) into a floret in a central spikelet of a spike. For each genotype, controls were non-inoculated and sterile water-inoculated spikelets. Fusarium scab is easily recognized by premature bleaching of spikelets on emerging heads. According to literature, a visual scale, based on percentage of infected spikeletes, was chosen to estimate scab disease severity. Visual inspection has been conducted 7 and 14 days after inoculation, counting discoloured spikelets per inoculated spike and recording other visible symptoms of infection such as mycelial growth. The cv.Veery, not expressing b-32, was the most susceptible to *F.culmorum* attack, in comparison to all the transgenic progenies tested. No significative resistance differences between the progenies have been noticed. At maturity, the spikes were manually harvested, glumes were removed and scab-infected kernel symptoms, were evaluated. The percentage of "tombstones" (shriveled, light weight, dull greyish or pinkish in colourof kernels), indicative of scab disease severity, was recorded. Transgenic plants constitutively expressing b32 have a higher level of resistance to scab because the percentage of

tombstones was significantly reduced in comparison to cv.Veery. Data obtained suggest that b-32 protein may provide protection to wheat against mycotoxigenic fungal pathogens, as *F.culmorum*. Experiments using higher spore concentrations applied to WHS inoculation method, are in progress to establish the proper conditions for better discriminating the response to Fusarium infection of the different genotypes.