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BIORES* PROJECT: Use of bioactive proteins in plant protection against pathogens

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Plants respond to attack by pathogenic fungi with a complex network of active responses such as the production and accumulation of proteins that are toxic or inhibitory to pathogens such as RIP (Ribosome Inactivating Protein). The role of RIP in the pathogens defense has been documented. In maize endosperm, a cytosolic albumin termed b-32 is synthesized in temporal and quantitative coordination with the deposition of storage proteins. In the past years b-32 was shown i) to enzymatically inactivate ribosomes modifying rRNA inhibiting protein synthesis in vitro (Maddaloni et al., Genet. and Breed 45:377-380, 1991) ii) to inhibit the growth of *Rhizoctonia solani* mycelia in an in vitro bioassay and plant assays (Maddaloni et al., Transgenic Research 6:393-402, 1997). In this context, we have recently shown and that maize b-32 was effective in wheat transgenic lines as an anti fungal protein by reducing *Fusarium culmorum* head blight (FHB) (Balconi et al., European Plant Pathology 117: 129-140, 2007) and in maize transgenic lines reducing *Fusarium verticillioides* attack symptoms in leaf tissues assays (Lanzanova et al., European Journal of Plant Pathol. 124: 471-482, 2009). Similarly to other RIPs, maize RIP is accumulated in the seed as an inactive precursor, which is converted into an active form by proteolytic processing which removes peptide segments from the N (residues 1-16 of pro-RIP) and C (residues 295-301) termini and also from the center of the polypeptide (residues 162-186 Hey et al., Plant Physiology 107: 1323-1332, 1995).

Aims of the BIoRES project are devoted to deepen our knowledge about relationships between structure and substrate specificity of b-32 protein, in order to clarify the role of the processed segments of b-32 gene on the ability of maize RIP to inhibit fungal growth.

Thereby, a series of genetic constructions was made by selectively deleting the N-terminal, or C-terminal or internal linker domain. Genetic deletions of the b-32 gene, that is apparently responsible for suppressing enzymatic activity in the precursor, will be primarily expressed in *Escherichia coli* to produce sufficient quantities of modified proteins. To assess the role of bioactive b-32 modified protein protection against fungal pathogens (*F. verticillioides*, *Aspergillus flavus*), a series of *in vitro* bioassay will be performed to analyze their effect on the fungal growth and on mycotoxins accumulation.

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