

CRA-MAC

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USE OF BIOACTIVE PROTEINS IN PLANT PROTECTION AGAINST PATHOGENS*

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One of the main topics of maize breeding is the improvement of plant protection against pathogens. 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 pathogen defense has been documented (Balconi et al., 2010, *Plant Cell Monographs* 18, Springer-Verlag Berlin Heidelberg : 149-166).

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., 1991, *Journal of Genetics and Breeding*, 45: 377-380); ii) to inhibit the growth of *Rhizoctonia solani* mycelia in an *in vitro* bioassay and plant assays (Maddaloni et al., 1997, *Transgenic Research*, 6, 393-402). 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., 2007, *European Journal of Plant Pathology*, 117, 129-140) and in maize transgenic lines reducing *Fusarium verticillioides* attack symptoms in leaf tissues assays (Lanzanova et al., 2009, *European Journal of Plant Pathology*, 124: 471-482).

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., 1995, *Plant Physiol.*107: 1323-1332).

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 and/or mycotoxin accumulation.

Thereby, a series of genetic constructions was made by selectively deleting the N-terminal, or C-terminal or internal linker domain (Figure 1). 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 in the protection against fungal pathogens (*F. verticillioides*, *Aspergillus flavus*), a series of *in vitro* bioassays are in progress, to analyze their effect on the fungal growth and on mycotoxin accumulation in comparison with i) the unmodified b-32 (control); ii) a commercial RIP (Saporin).

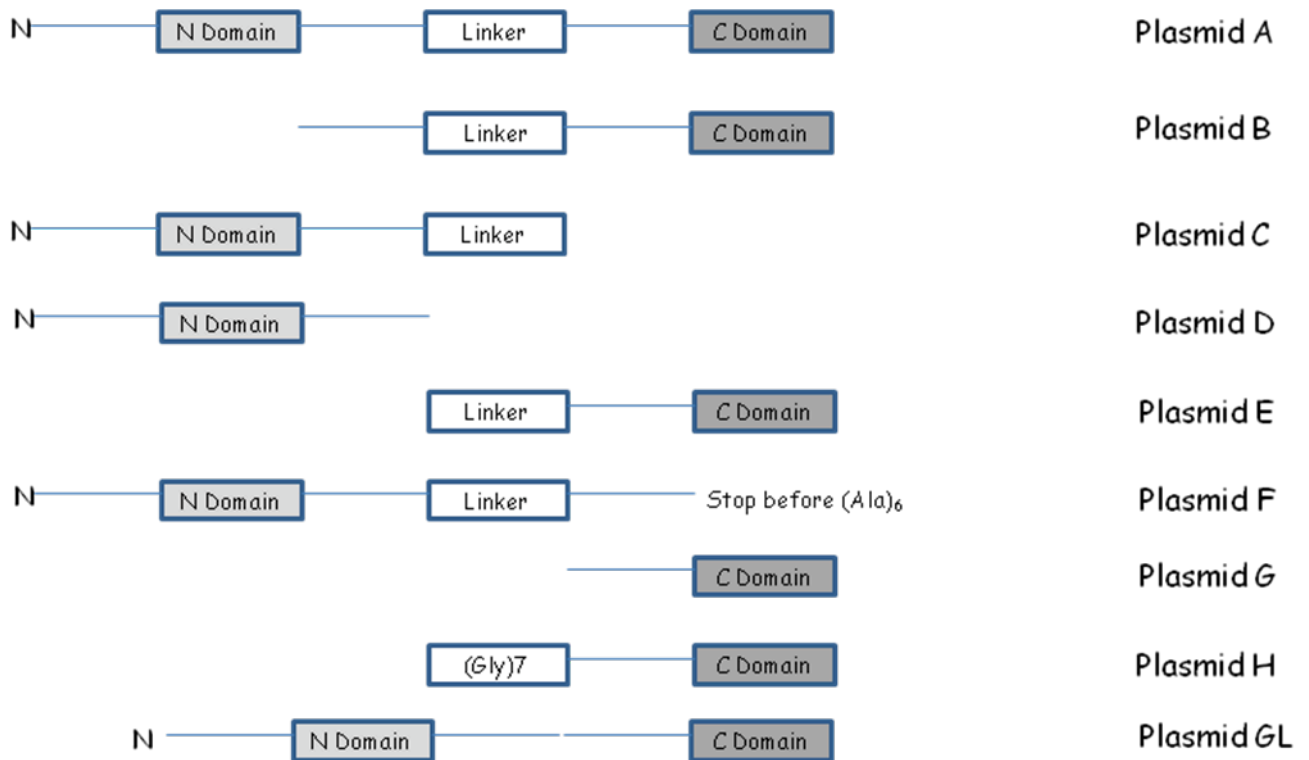


Figure 1: Genetic deletions of the *b-32* gene

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