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Poster Abstract – D.39

THE MAIZE RIBOSOME-INACTIVATING PROTEIN (b-32): ROLE IN THE DEFENCE AGAINST FUNGAL PATHOGENS

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The development of improved maize genotypes with increased resistance to fungal pathogens is one of the major objectives of breeding biotechnology strategies. *Fusarium* species are widespread pathogens in cereals. *F. verticillioides* attacks maize causing root, stem, and ear rot diseases, and produces mycotoxins (fumonisins) which can be formed in infected plants before harvesting, or in grains during post-harvest storage. The occurrence of mycotoxins in cereal grains is a great concern worldwide, because their presence in feed and foods is often associated with mycotoxicoses in livestock and also in humans. In the maize endosperm, a cytosolic albumin with a molecular weight of 32 kDa, termed b-32, is synthesized in temporal and quantitative coordination with the deposition of storage proteins. Both cDNA and genomic clones encoding b-32 have been isolated. It was shown that the *b-32* genes form a small gene family. The *b-32* gene, as well as the 22 kDa storage protein zeins, are under the control of the seed-specific transcriptional activator *Opaque-2 (O2).* In *opaque-2* mutants the b-32 protein is expressed at very low levels. Although, the role of b-32 in maize endosperm remains unclear, this protein displays structural and functional homology with other 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 evidences indicated that transgenic tobacco plants expressing b-32 showed an increased tolerance against infection by the soil-borne fungal pathogen *Rhizoctonia solani*.

Research is in progress in our laboratories to verify if maize plants expressing b-32 in various organs and tissues display an increased resistance against fungal pathogens in comparison with normal control plants. For these purposes transgenic plants were obtained via genetic transformation using the vector pSC1b32 containing the *b-32* coding sequence under the constitutive promoter *35SCaMV* and the cassette *ubi1-bar* for L-glufosinate resistance as a selectable marker. A set of six homozygous progenies *PCR-b32* and western-b32 positive, and a progeny *PCR-b32* positive and western-b32 negative (as negative control) were raised to maturity into a containment-greenhouse and used, at flowering stage, for a detailed analysis of b-32 expression in leaves and for pathogenicity tests. Various progenies, characterized by a differential b-32 expression in the leaves, were identified; these ones were used for setting up pathogenicity experiments, in order to evaluate a possible differential response to *Fusarium* attack in leaf tissue colonization bioassays. Plants were raised to maturity into a containment-greenhouse. Preliminary experiments supported the choice of bioassay parameters (spore concentration, detection time) useful for a reliable evaluation of genotypes. Leaves of progeny not expressing b-32 protein were surface sterilized and square segments dissected and plated on PDA (Potato Dextrose Agar); they were inoculated with 5µl spore suspensions at four different concentrations; control leaf squares were non-infected and treated with sterile water. Infection progression was daily monitored measuring fungal colony diameter around inoculated leaf squares. A concentration of 10^5 and/or 10^6 spores/ml and 3-7 days following inoculation as detection time, were the parameters adopted for pathogenicity experiments. All progeny, previously

quantified for b-32 expression in leaves, were tested with this method. As a result, the negative control was the most susceptible to *F.verticillioides* attack, whilst in all progenies expressing b-32, a differential resistance was detected.