Proceedings of the 50th Italian Society of Agricultural Genetics Annual Congress
Ischia, Italy – 10/14 September, 2006
ISBN **88-900622-7-4**

Poster Abstract – C.38

THE MAIZE RIBOSOME-INACTIVATING PROTEIN B-32: GENETIC ENGINEERING FOR MAIZE PROTECTION AGAINST MYCOTOXIGENIC FUNGAL PATHOGENS

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Zea mays L, *ribosome-inactivating-protein, resistance gene, b-32, fungal pathogens*

The development of improved maize genotypes with increased resistance to fungal pathogens is one of the major objectives of breeding biotechnology strategies. *F. verticillioides* attacks maize, causing root, stem, and ear rot diseases, and produces mycotoxins (fumonisins), their presence in feed and foods is often associated with mycotoxicoses in livestock and also in humans.

In 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. Although, the role of b-32 in maize endosperm remains unclear, this protein has homology with several previously characterized Ribosome-Inactivating Proteins (RIPs). It was found that b-32 is a functional RIP and shows anti-fungal activity by *in vitro* and *in vivo* experiments.

Research is in progress in our laboratories to verify if maize plants expressing b-32 in various organs and tissues have an increased defence against fungal pathogens in comparison with plants expressing b-32 only in the kernel. For these purposes transgenic plants were obtained through genetic transformation using the vector *pSC1b32* containing the *b-32* coding sequence clone under the constitutive promoter *35SCaMV* and the cassette *ubi1-bar* for Basta herbicide resistance as 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.

A differential b-32 expression in the leaves of various progenies was recorded.

Proteomic experiments on protein leaf extracts have been set up. The 2DE map matching clearly showed the presence of additional spots in a progeny b-32 western positive, in comparison to a progeny Basta-sensitive and b-32 western negative.. These spots have been cut and digested with trypsin to achieve protein identification by MALDI-TOF MS. Both induced b-32 and herbicide resistance in multiple spots have been successfully identified. The identification of progenies with a differential b-32 expression in the leaves was useful 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. The negative control was the most susceptible to *F.verticillioides* attack, in comparison to all the progenies expressing b-32. Experiments are in progress to extend pathogenicity tests to other plant tissues and to evaluate the specificity of b-32 role in the defence against other fungal pathogens (i.e., *Aspergillus*, *Penicillium*).