INTERACTION OF PATHOGENIC AND SYMBIOTIC FUNGI WITH TRANSGENIC RICE EXPRESSING THE MAIZE RIP b-32

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The maize gene b-32, normally expressed in the maize (Zea mays L.) endosperm, encodes for a RIP (Ribosome Inactivating Protein) exhibiting antifungal activity. Transgenic rice plants, in which the b-32 gene driven by the Ubi-1 promoter was inserted in association with the bar gene as a selectable marker, were obtained via biolistic transformation. A set of b-32 expressing homozygous progenies and the non-transgenic parental cv. Selenio as a negative control, were raised to maturity into a containment greenhouse. All transgenic plants exhibited a normal phenotype and were fully fertile and set seeds. Three homozygous b-32 rice progenies (SE7.1, SE7.2 and SE7.15) were characterized for the level of expression of the b-32 protein in various plant tissues, and for their interaction with pathogenic and symbiotic fungi. The different level of b-32 expression exhibited by the three transgenic lines allowed to set up pathogenicity experiments, in order to evaluate the level of response to Fusarium attack in leaf tissue colonization bioassays. The results obtained showed that the three transgenic progenies tested were more resistant than the cv. Selenio to Fusarium verticillioides, when leaves were inoculated with 10^6 spores/ml and evaluated 2-4 days after inoculation. The same progenies were tested with Magnaporthe oryzae, a major rice fungal pathogen in the European area. Seedling tests, conducted on seedlings at the three leaf stage, did not confirm difference in response between the transgenic and non-transgenic rice plants. Antifungal proteins expressed in genetically modified organisms (AMPs) have the potential to affect non-target organisms. From the perspective of environmental risk-assessment, the effect of each new integrated gene on soil-borne microbiota must be evaluated. The effects of these AMPs on mycorrhizal fungi are accepted as good indicators of their effect on soil microbiota in general. Therefore, the arbuscular fungal (AM) species Glomus intraradices was tested for its ability to form mycorrhizal associations in the three transgenic rice lines and the control wild-type cv. Selenio. The experiments of colonization were performed by means of the sandwich system. Roots of young seedlings were carefully spread on a cellulose nitrate disc. Clumps of mycelium with spores were placed in direct contact with the roots; roots were subsequently covered with a second membrane, forming a “sandwich”. The inoculated seedlings were planted in sand in plastic pots, grown at 25°C and 16-hr photoperiod and supplied with water or Long Ashton solution. Five weeks after inoculation, the roots were stained with Cotton Blue and the presence of AM fungal infection was detected under light microscope. Results obtained showed that the mean percentage of
colonization by *G. intraradices* in the b-32 expressing lines was comparable with those observed in the wild type non-transgenic variety, showing a non-significant effect of the transgene on the mycorrhizal association. The results of the study are discussed in view of the effect of transgenic plants expressing antifungal genes on the rhizosphere microbiota population.

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