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Poster Abstract – C.03

FUNCTION OF THE RICE *OSMYB4* GENE AND OF ITS PUTATIVE ORTHOLOGUES

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The rice *Osmyb4* gene was isolated from a coleoptile cDNA library and encodes a Myb transcription factor. Its expression is induced at 4°C and it is able to transactivate cold-inducible promoters. Its overexpression in *Arabidopsis thaliana* plants increases both chilling and freezing tolerance and leads to an improved tolerance/resistance to several abiotic, environmental and biotic stresses (drought, salt, UV irradiation, ozone fumigation, viruses, bacteria and fungi). It has also been introduced into other mono and dicotyledonous plants, such as maize, tomato, apple and *Osteospermum* and in all of them its ectopic expression confers improved tolerance to several stresses.

To elucidate the *Osmyb4* role in stress tolerance pathway, we transformed rice (*Oryza sativa* cv Arborio) with *Osmyb4* cDNA under a constitutive promoter. To this purpose, an expression vector carrying the cassette *Ubi1-Osmyb4* (Ubi-Myb4) was constructed. This plasmid was utilized for transformation with the biolistic system, using rice mature embryo-derived primary calluses as target tissue. A total number of 300 calluses was co-bombarded with a mixture of Ubi-Myb4 and a plasmid carrying the *hph* gene, that confers resistance to hygromycin, as a selectable marker. We obtained two independent events of transformation, but only one gave rise to mature transgenic plants. The low percentage (0,67%) of transgenic lines obtained suggests that the high constitutive overexpression of *Osmyb4*, that has an important and early role in the cascade of events leading to stress response, is detrimental for rice growth, and only a low level of overexpression is compatible with plant survival. We analysed some of the transformed plants for *Osmyb4* expression, distinguishing between endogenous and exogenous transcripts. In normal conditions the expression level of the exogenous form was higher than that of the endogenous. No changes were found in the transcript level of the endogenous *Osmyb4* between wild type and transgenic plants.

T1 plants deriving from this event of transformation presented different levels of exogenous mRNA, possibly due to the segregation of multiple copies introduced in T0 plants. Plants with different *Osmyb4* expression levels seem not to have any phenotypic differences in normal growth conditions.

The data reported about *Osmyb4* expression in other plants indicate that it activates stress response pathways in all assayed species, both mono and dicotyledonous, although the specifity of action depends on the host plant. The pathway of abiotic and biotic stress response cross talk seems to be conserved among species and Myb4 seems to represent a crucial knot in the stress signalling network. We have made a phylogenetic tree on the basis of multiple sequence alignments of different *myb* genes from various plant species. *Osmyb4* belongs to a small Myb subfamily of three members and analogous families are present in several species. In Arabidopsis a family of three

members is present, whereas other species show a different number of members (two or four). Our investigations about the expression of these genes in different stress conditions, such as cold, drought, and wounding, indicate that, although some of them have a specific role in stress response, there is not a direct correspondence between individual members of the family. These findings suggest that what is maintained is the function of the family as a whole rather than the function of the single genes.