

## FUNCTIONAL ANALYSIS OF THE RICE *Osmyb4* GENE IN STRESS TOLERANCE

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The *Osmyb4* rice gene, encoding a R2/R3 Myb transcription factor, was isolated as a cold induced gene involved in cold acclimation. Its constitutive expression in transgenic *Arabidopsis thaliana* plants results in improved cold and freezing tolerance.

A comparison of the transcriptome profile of wild-type and *Osmyb4* expressing plants identified more than 250 *Osmyb4* up-regulated genes, 22% of which encoding proteins involved in gene expression regulation and signal transduction, suggesting a Myb4 upstream action in the stress response. Most of the up-regulated genes are known to be involved in tolerance not only to cold but also to other abiotic and environmental stresses. Moreover, a substantial proportion of them has known functions in resistance to pathogen attacks. The biochemical and physiological analysis of the transgenic plants showed that the *Osmyb4* ectopic expression induced multiple metabolic changes commonly observed in plants during stress response, such as the accumulation of a higher amount of osmolytes/osmoprotectants and of aromatic compounds.

When exposed to different adverse conditions, transgenic plants indeed resulted not only in an improved tolerance to all the abiotic stresses assayed (drought, salt, u.v. irradiation, ozone fumigation) but also to different biotic stresses (viruses, bacteria and fungi), suggesting that *Osmyb4* represents a crucial knot in the cross talk of stress signalling cascades.

The ability of the rice (a monocot) *Osmyb4* gene to confer a coherent phenotype in *Arabidopsis* (a dicot) suggests that its action is evolutionary conserved. On this basis, in collaboration with other research groups, our gene was expressed in several crops, namely maize, tomato, apple, *Osteospermum*, tobacco and sage. Moreover, in order to better understand its physiological role, we have constitutively expressed *Osmyb4* in rice plants.

We found that *Osmyb4* overexpression in heterologous species actually resulted in a phenotype analogous to that obtained in *Arabidopsis*. Transgenic plants accumulated several metabolites known to be essential in stress response and actually acquired multiple stress tolerance, although the specificity of action depended on the host plant species. For example transgenic maize plants showed an enhanced tolerance to drought and cold, although this trait depended on the genetic background into which the gene was introduced. In the *Osteospermum* species, the *Osmyb4* expression gave rise to a significant increase in freezing tolerance, but not in drought tolerance, possibly because this species is already quite tolerant to this stress per se. On the contrary, transgenic tomato plants acquired a higher tolerance to drought (but not chilling) treatment and to viral (but non fungal) infection.

Among the metabolites accumulated in transgenic plants, we found that *Osmyb4* increased the secondary metabolites content in all the assayed species, most of which accumulated phenylpropanoids. However, within this class of compounds, the specific molecules accumulated depended on the species: for example, maize and tobacco mainly accumulated chlorogenic acid and not sinapoylmalate, as was the case for Arabidopsis.

Our results suggest that *Osmyb4* might represent a powerful tool both to confer an increased tolerance to several stress conditions in many crop species and to improve the production of secondary metabolites of interest.