

Poster Abstract - E.16

OVEREXPRESSION OF RICE *OSMYB4* GENE IN APPLE (*MALUS PUMILA* MILL.) AFFECTS PLANT RESPONSE TO DROUGHT AND COLD STRESSES

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apple, Osmyb4, stress tolerance, transgenic plants

The rice *Osmyb4* gene is a putative Myb transcription factor constitutively expressed in rice and strongly induced by cold treatments at 4°C. Overexpression of *Osmyb4* gene in *Arabidopsis thaliana* plants demonstrated a significant increase in cold and freezing tolerance.

In this work, *Osmyb4* gene was introduced into the temperate woody species *Malus pumila* Mill., cv. Greensleeves, clone GS92 under the control of the constitutive CaMV35S promoter by *Agrobacterium*-mediated genetic transformation. Transgenic plants were studied in order to determine the effects of *Osmyb4* expression on chilling and drought tolerance.

Low temperature effects on respiration were studied *in vivo* in wild-type and transgenic plants. Arrhenius plots show that from 30°C to 12°C, O₂ release decreases linearly for both mature and young leaves of wild-type apple. Below 12°C O₂ release exhibited a rapid decline, whereas in transgenic plants, breaks were observed at 10°C for mature leaves and at 6°C for young leaves. Values for activation energy and the (Q₁₀) factor differed significantly.

Drought tolerance was evaluated by relative water content (RWC) and ion leakage of detached leaves of wild-type and transgenic plants after different periods of treatment. Control plants start with a higher water content than transgenic plants and 6 days of drought (soil water potential at -10 MPa) are enough to produce a 10% reduction of the RWC, whereas in transgenic leaves reduction is significantly lower (0.5%). After 15 days of treatment (soil water potential > -30 MPa) a 15% reduction of the RWC is detected for control plants, whereas in transgenic leaves no significant reduction is shown.

After 9 days of drought, ion leakage of control leaves doubled from 25% to 50%, whereas for transgenic plants it was about 30%. After 15 days of treatment, control plants showed a 70% of released ions, whereas for transgenic leaves no significant differences are detected, demonstrating a higher membrane stability than control.

Furthermore transgenic plants overexpressing *Osmyb4* gene showed under non-stressed conditions higher concentration of soluble sugars and proline than control plants and it is known that a plant strategy that may confer stress tolerance is the accumulation of compatible, low-molecular weight osmolytes, such as sugars and amino acids.

The obtained results suggest that overexpression of *Osmyb4* gene might confer an increased tolerance to cold and drought stress conditions in plants reinforcing the hypothesis that *Osmyb4* represents a key gene of stress signalling processes.