TOLERANCE OF PINUS PINASTER AIT. TO SALT: RESPONSES TO OXIDATIVE STRESS

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Introduction: *Pinus pinaster* Ait. (maritime pine) is a two-needle pine distributed throughout the Western Mediterranean basin. Its characteristics make it economically important for pulp and timber industries. *P. pinaster* is a pioneer species thriving in dune systems, well adapted to growth in sandy silicate soils. Its increased adaptability to shoreline ecosystems should result from tolerance to salt stress, both at plant and cellular levels. We investigated the responses of *P. pinaster* suspension cells and seedlings to salt, in terms of ROS production and anti-oxidant systems activation.

Mat&Meth: Responses over time were analysed in suspension cells subjected to 100mM NaCl and seedlings subjected to 350mM NaCl. Seedlings were also subjected to different NaCl concentrations up to 600mM. ROS was quantified using XTT (Able *et al.*, 1998), H_2DCFDA (Civoli, 1994) and KI (Loreto and Velikova, 2001). Lipid peroxidation was quantified using MDA (Loreto and Velikova, 2001). Chlorophyl was determined according to Hipkins and Baker (1986). Superoxide dismutase (Sod) activity was evaluated by in-gel assay (Beauchamp and Fridovich, 1971). Chloroplastic *Cu,Zn-SOD* and *Fe-SOD* genes were isolated from a cDNA library and used in expression analysis, according to standard procedures.

Results: Growth curves of suspension cells indicate 100mM NaCl as the fittest concentration for stress induction studies. Cells suffer an initial burst of superoxide anion, coupled with increase in lipid peroxidation. Chloroplastic Fe-Sod is the only SOD isoenzyme up-regulated in suspension cells as a response to salt. Seedlings suffer a transient increase in ROS but adapt to surpass the oxidative stress caused by NaCl up to 450 mM. Lipid peroxidation also increases. The reduced decrease in chlorophyll content of seedlings suggests capacity for maintaing photosynthetic activity. Chloroplastic *Fe-SOD* and *Cu-Zn-SOD* are differently expressed during salt stress.

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