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Roots are necessary for the responses of *in vitro*-cultured citrus plants to high salinity but not to osmotic stress

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In the field, plants are exposed simultaneously to variable biological and environmental conditions that can make physiological studies very difficult. The *in vitro* tissue culture techniques can overcome some of these limitations. In the present work, this methodology was applied to the study of salt and osmotic stress conditions on 'Carrizo' citrange. The stress conditions were generated by adding either NaCl or polyethylene glycol, to the culture medium. Micropropagated shoots, growing under salt- or osmotic-stress, shown symptoms of leaf damage very similar to those found in intact plants, which confirmed the incidence of the imposed stress on plant physiology. In whole plants, it has been reported that physiological responses to water and salt stress are essentially identical; on the contrary, in shoots cultured *in vitro*, levels of stress markers such as malondialdehyde and proline increased only under water deficiency but not under elevated salt conditions. Differences were also observed in the hormonal regulation of the shoots subjected to each abiotic stress condition. Abscisic acid concentration increased in shoots grown under osmotic stress conditions whereas no differences with the controls were observed in salt-stressed ones. It can be concluded that, at least when culture *in vitro*, citrus roots are necessary for the perception and signaling of the salt stress conditions. On the contrary, the presence of this organ is not necessary to modulate the response of shoots to osmotic stress.

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Physiological analysis of salt stress behaviour of citrus species and genera: low chloride accumulation as an indicator of salt tolerance

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Tolerant citrus rootstocks are defined as Cl⁻ excluders. However, little is known about the salt tolerance of cultivars used as scions, particularly the tolerance of monoembryonic citrus genotypes. To enhance the genetic resources for generating improved hybrid rootstocks, the evaluation of large samples of citrus species, including both monoembryonic and polyembryonic genotypes, is necessary. In this study, 12 citrus genotypes representing the major *Citrus* species and all the three genera of the *Rutaceae* family were subjected to moderate salt stress (75mM) for 12 weeks to characterise their physiological response to salt stress. Various symptoms and physiological parameters were evaluated to characterise their salt sensitivity. These included plant growth (stem diameter), leaf chlorophyll content, leaf flavonoid content, maximum quantum yield of PSII $[(Fm-F0)/Fm]$, net photosynthesis, stomatal conductance and leaf Na and Cl⁻ contents. The results clearly demonstrated that the most salt sensitive genotypes accumulated high concentrations of Na and Cl⁻ and maintained a fair growth and photosynthetic rate. By contrast, salt-tolerant genotypes accumulated less Na and Cl⁻ and decreased their growth and gas exchange. 'Poncire commun' citron and 'Marumi' kumquat were the most sensitive species, while mandarins, pummelo and 'Australian' sour orange were the most tolerant species. Among the genotypes, 'Engedi' pummelo presented a specific trait for salt tolerance that has not been previously reported. Taken together, the results suggest that low leaf chloride content can be used as an indicator of salt stress tolerance in citrus genotypes. Exploitation of this indicator will enable the improved evaluation of citrus genetic resources and should lead to the identification of new sources of tolerance for rootstock breeding.