Effect of salt stress on the superoxide dismutase activity in leaves of *Citrus limonum* in different rootstock-scion combinations

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Abstract

The effect of salinity on leaf superoxide dismutase (SOD) activity of lemon trees of different rootstock-scion combinations was studied. In leaves from *Citrus limonum* cv.Verna scions on *Citrus macrophylla* and *C. reticulata* rootstocks, salinity treatment clearly caused a significant depression in both Fe-SOD and Mn-SOD activities and an increase in Cu,Zn-SOD activity. However, in leaves from *Citrus limonum* on *Citrus aurantium* rootstock, the reduction observed in the activity values of Fe-SODs and Mn-SODs was not statistically significant. Salt stress also produced a decrease in the content of soluble proteins and chlorophylls. However, this drop was greater in *C. limonum* leaves on *C. macrophylla* than for other combinations.

Additional key words: chlorophyll, *Citrus aurantium*, *Citrus limonum*, *Citrus macrophylla*, *Citrus reticulata*, mineral elements, proteins.

Introduction

In south-eastern Spain (Murcia), irrigation water, usually containing chloride concentrations higher than 10 mM, is used to irrigate a variety of horticultural crops, including *Citrus*, which are generally reported as salt-sensitive plants (Cerdá *et al.* 1990). Salt tolerance of *Citrus* plants varies depending on the rootstock-scion combination and water and soil quality (Cerdá *et al.* 1990).

Plants exposed to salt stress undergo changes in their metabolism in order to cope with the changes taking place in their environment. Salt stress, in addition to its known components of osmotic stress and ion toxicity, is also manifested as an oxidative stress, all of which contribute to its deleterious effect (Hernández *et al.* 1993a, 1995, 2000, Gómez *et al.* 1999). Superoxide dismutases (SOD; E.C. 1.15.1.1) are a group of metalloenzymes that catalyse the disproportionation of $\text{O}_2^-$ radicals to molecular oxygen and $\text{H}_2\text{O}_2$, and play an important role in protecting cells against superoxide-derived oxidative damage (Halliwell and Gutteridge, 1989).

*Citrus* rootstocks differ widely in their ability to exclude sodium and/or chloride from scion foliage (Walker and Douglas 1983). While the influence of salinity and *Citrus* rootstock-scion combinations in the leaf gas exchange, water relation, ion content and concentration of compatible solutes is reasonably well documented, at present there is no information about the effect of salinity and rootstock combination on the isozyme pattern of SODs. In addition, changes in some stress parameters such as ion content, protein concentration and leaf chlorophyll level were also studied.

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Abbreviations: AOS - activated oxygen species; Cu,Zn-SOD - copper, zinc-containing superoxide dismutase; Fe-SOD, iron-containing superoxide dismutase; Mn-SOD, manganese-containing superoxide dismutase; $\text{O}_2^-$ - superoxide radicals.

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Materials and methods

Plants and treatments: Uniform seedling of sour orange (Citrus aurantium L.), Cleopatra mandarinate (Citrus reticulata B.) and Citrus macrophylla rootstock were transplanted from a nursery to the experimental site. Two years later they were grafted with Verna lemon (Citrus limonum R. cv. Verna) scion as described previously by Cerdà et al. (1990).

Two NaCl concentration (12 and 28 mM), applied through the irrigation water, were used over a five year period. Tap water, having 6 mM NaCl, was considered as control. Each experimental plot had 4 trees of each rootstock planted 6 x 5 m apart with no guard trees.

Preparation of extracts and enzyme assays: All operations were performed at 4 °C. Samples of leaves (8 - 10 months old) were blended and total SOD activity was determined as described by Almansa et al. (1989). SOD isozymes (Cu,Zn-SOD, Mn-SOD and Fe-SOD) were separated by isoelectric focusing and identified according to Almansa et al. (1989). The isozyme activity was quantified by recording the transmittance of the gels in a Shimadzu CS-9000 densitometer (Kyoto, Japan).

Ion analysis: Leaf samples were oven-dried at 65 °C and ground to a fine powder. Nutrient concentrations of intact leaves were determined by atomic absorption spectrometry, except for chloride contents, which were measured by a potentiometric method (Hernández et al. 1993b).

Chlorophylls were extracted with N,N-dimethylformamide and estimated by the methods of Inskemp and Bloom (1985). Proteins were determined by the Bradford (1976) procedure.

Statistics: Each experiment was replicated four times with four different batches of leaves. The average values were calculated and the data included in the tables were analysed according to Duncan’s Multiple Range Test.

Results and discussion

Citrus reticulata and C. macrophylla rootstock have been considered as salt sensitive and C. aurantium rootstock as salt tolerant (Cerdà et al. 1990).

We have previously described that leaves from C. limonum cv. Verna contain nine SOD isozymes: 4 Cu,Zn-SOD, 3 Fe-SOD and 2 Mn-SOD (Sevilla et al. 1984, Almansa et al. 1989). Under normal growth conditions Cu,Zn-SOD and Fe-SOD are the most abundant SOD isozymes.

Total SOD activity shows a decrease in salt-treated C. limonum trees, but it was only significant in trees on C. reticulata rootstock treated with 28 mM NaCl (Fig. 1). In C. limonum trees on Citrus macrophylla and C. reticulata combinations, salinity treatment clearly determined a significant depression in both Fe-SOD and Mn-SOD activities and an increase in Cu,Zn-SOD activity (Fig. 1). However, in leaves from C. limonum on C. aurantium rootstock treated with 12 mM NaCl only Cu,Zn-SOD activity decreased. It is important to note that leaves from C. limonum trees on C. aurantium rootstock had a higher SOD isozymes constitutive activity than the other combinations (Fig. 1). In pea plants, the induction of antioxidant defences, among other factors, seems to be important in the tolerance mechanisms of peas to long term salt treatment (Hernández et al. 1993a, 1995, 2000). However in C. limonum trees, it seems that high constitutive SOD levels could be important in the protection against salt stress.

Mn-SOD has been localized in mitochondria and peroxisomes (del Río et al. 1989). An increased production of O$_2^-$ has been described in mitochondria from salt-treated pea cultivars (Hernández et al. 1993a), whereas an induction in mitochondrial Mn-SOD was only found in the NaCl-tolerant pea cultivar and not in the sensitive one, in which Mn-SOD even decreased (Hernández et al. 1993a). In this sense, in C. limonum leaves on C. reticulata and C. macrophylla rootstocks treated with 12 and 28 mM NaCl the Mn-SOD activity decreases (Fig. 1), indicating that an oxidative stress mechanism mediated by O$_2^-$ could be involved in mitochondrial and peroxisomal levels.

In plants, Cu,Zn-SODs and Fe-SODs are present mainly in chloroplasts (Bowler et al. 1992). Chloroplasts are important AOS generators like O$_2^-$ and H$_2$O$_2$ under salt-stress (Hernández et al. 1995). In C. limonum leaves on C. macrophylla and C. reticulata combinations, the decrease on Fe-SOD activity was compensated with an increase in the Cu,Zn-SOD activity to cope with the O$_2^-$ radicals produced during saline conditions. However, this increase would not be sufficient to avoid oxidative stress because Cu,Zn-SOD are sensitive to H$_2$O$_2$. However, in C. limonum leaves on C. aurantium, Cu,Zn-SOD activity decreased slightly. This decrease may be compensated by maintaining Fe-SOD activity. It has been described that H$_2$O$_2$ completely deactivates Cu,Zn-SOD, whereas Fe-SOD is deactivated to a limit of 90 % (Kamematsu and Asada 1994) and that this residual H$_2$O$_2$-resistant activity may play an important role during stress conditions.

Salt stress produced a decrease in soluble proteins from leaf extracts especially with the highest salt level used (Table 1). However, this drop was stronger in C. limonum leaves on C. macrophylla reaching a 40 %