Mechanisms of Response to Salt Stress in Oleander (*Nerium oleander* L.)

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Abstract

Elucidating the mechanisms of abiotic stress tolerance in plants will help to develop more resistant crop varieties, improving agricultural production in a climate change scenario. Basic responses to salt stress (osmolyte accumulation, activation of antioxidant systems), have been studied in *Nerium oleander*, a xerophytic species widely used as ornamental. Salt strongly inhibited growth, but the plants survived one month in the presence of 800 mM NaCl, indicating that the species is also relatively resistant to salt stress. Salt induced a slight increase in the levels of proline, glycine betaine and soluble sugars; the latter compounds showed much higher absolute contents, suggesting a functional role in osmotic adjustment, and the presence of constitutive mechanisms of response to salt stress. High salinity generated oxidative stress in the plants, as shown by the increase of malondialdehyde levels. Antioxidant systems, enzymatic and non-enzymatic, are generally activated in response to salt stress; in oleander, they do not seem to include total phenolics or flavonoids, antioxidant compounds which did not accumulate significantly in salt-treated plants.

Keywords: *climate change; Nerium oleander; salt stress; soil salinisation*

Introduction

Soil salinity is one of the most important causes of reduction of crop yields worldwide, an increasing problem due to climate change. Genetic improvement of salt tolerance of our crops has become an urgent need for the future of agriculture, especially in arid and semiarid regions (Fita *et al.*, 2015). Although all plants activate the same basic responses to salt stress (control of ion transport, osmotic adjustment, activation of antioxidant systems, etc.), the relative relevance of these conserved responses for the tolerance of a given species is largely unkown. Elucidating the most efficient mechanisms of tolerance in different plants will contribute to increasing agricultural production and to global food security.

Aims and objectives

This work reports an initial characterisation of the responses to salt stress in the Mediterranean shrub *Nerium oleander*, a xerophyte (drought-tolerant plant) widely used as ornamental, which has been also described as moderately resistant to salinity.

Materials and methods

One-year-old *N. oleander* plants were treated for one month with 400 and 800 mM NaCl in half-strength Hoagland nutritive solution, while control plants were grown in parallel in the absence of salt. After harvesting, several growth parameters were measured, and the contents of common osmolytes [proline (Pro), glycine

Tab. 1. Salt stress-induced growth inhibition in *Nerium oleander*. Growth parameters: fresh weight (FW), stem length (LS), in control and salt stressed plants, after one-month treatments with the indicated NaCl concentrations

NaCl treatments (mM)	FW (%)	SL (%)
0 (control)	100	100
400	30.7 ± 6.8	14.3 ±2.3
800	24.6 ± 3.3	10.0 ± 2.5

Tab. 2. Relative changes in leaf levels of proline (Pro), glycine betaine (GB), total soluble sugars (TSS), malondialdehyde (MDA), total phenolic compounds (TPC) and total flavonoids (TF), in *Nerium oleander* plants, after one-month salt treatments with the indicated NaCl concentrations, as compared to non-stressed control plants (considered as 100% for each measured compound)

NaCl treatments (mM)	Pro (%)	GB (%)	TSS (%)	MDA (%)	TPC (%)	TF (%)
0 (control)	100	100	100	100	100	100
400	158 ± 12	170 ± 6	126 ± 4	157 ± 15	93 ± 4	104 ± 7
800	125 ± 12	171 ± 5	126 ± 4	189 ± 9	113 ± 5	135 ± 4

betaine (GB) and total soluble sugars (TSS)] and of some non-enzymatic antioxidants [total phenolic compounds (TPC), total flavonoids (TF)], as well as those of malondialdehyde (MDA, an oxidative stress marker), were determined using spectrophotometric assays.

Results and discussion

Salt inhibited growth of *N. oleander* in a concentration-dependent manner, as shown by a marked decrease of stem length and fresh weight, already observed in 400 mM NaCl-treated plants and stronger at 800 mM NaCl (Tab. 1.). Nevertheless, the very fact that the plants survived for one month these high salinity conditions indicates that this species is relatively resistant to salt stress, not only to drought.

The relative levels of common osmolytes (Pro, GB and TSS), increased in plants treated with 400 mM NaCl, as compared to the controls, but only slightly – by less than two-fold, in all cases – with the smallest variation observed in TSS. Increasing salt concentration to 800 mM did not lead to higher osmolyte accumulation (Tab. 2.). Yet, absolute TSS contents were much higher than those of the other osmolytes, both in salt-treated and in control plants, reaching nearly 100 mg eq. glucose g^{-1} DW. Therefore, soluble carbohydrates are probably the major functional osmolytes in oleander, accumulating at high concentrations even in the absence of stress; this suggests the presence of constitutive mechanisms of defence against abiotic stress, supporting the hypothesis of the 'pre-adaptation' to stress in salt-tolerant plants.

The salt treatments induced oxidative stress in oleander plants, as revealed by the relative increase in MDA levels (Tab. 2.). Changes in the contents of non-enzymatic antioxidants, such as TPC or TF, were however very small or not significant (Tab. 2.), indicating that other antioxidant systems, enzymatic and/or non-enzymatic, should be activated to counteract the salt-induced oxidative stress.

Conclusions

TSS appear to be the main contributors to osmotic adjustment in *Nerium oleander*, and their presence at high levels in non-stressed plants may represent a constitutive mechanism of response to salt stress. High external salt concentrations generate oxidative stress in oleander plants; antioxidant systems, which could be activated to counteract it, do not seem to include phenolic compounds or flavonoids.

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