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Research article

Characterization of the response of *in vitro* cultured *Myrtus communis* L. plants to high concentrations of NaCl

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ABSTRACT

Effect of salt stress was examined in *in vitro* shoot cultures of *Myrtus communis* L. a species of the Mediterranean maquis. To determine the effects of high salt concentrations on myrtle plantlets and contribute toward understanding the mechanisms adopted from this species to counteract soil salinity, *in vitro* rooted shoots were transferred to a liquid culture medium containing 0, 125 or 250 mM NaCl for 30 days. After 15 and 30 days of *in vitro* culture, shoot and root growth, chlorosis and necrosis extension, chlorophylls, carotenoids, proline, arginine, cysteine and total sugars content, as well as guaiacol peroxidase (G-POD, EC 1.11.1.7) and ascorbate peroxidase (APX, EC 1.11.1.11) activities were determined. In treated plants shoot and root growth, as well as chlorophyll content, significantly decreased, while carotenoids content was not affected by the NaCl treatment. Among osmolytes, proline did not significantly increase, arginine and cysteine decreased, while total sugars were found to be higher in the treated plants than in the control. Enhancement of G-POD and APX activities was positively related to increasing salt concentrations in the culture media, regardless of the exposure time. Salt-treated plants did not show significant changes in lipid peroxidation or DNA fragmentation after 30 days salt treatment, regardless of the NaCl concentrations applied. The results represent a contribution towards understanding the mechanisms adopted by this species to high salinity.

1. Introduction

Most salt affected lands evolve due to natural causes such as the release of various soluble salts from weathering of parental rocks, climate, evaporation and deposition of marine salts carried by wind and rain. An additional reason for soil salinity is related to human activities such as poor water management, use of fertilizers and industrial expansion. As a consequence, dissolved salts accumulate in the soil, leading to soil compaction, reduction in water infiltration, the acceleration of water flow which enhances the process of soil erosion, the destruction of soil structure and the reduction in soil fertility.

Based on their salt tolerance, plant species are classified as halophytes (plants that tolerate a high level of soil salinity) and glycophytes (plants which are sensitive to salinity). In most cases it is not possible to draw a clear line between these groups. A gradient from more to less salt tolerant species may be identified among the glycophytes, this is due to many of the salt tolerant glycophytes being descendants of halophytes.

Exposure to salt affects plants in different ways [1]. The excess of sodium is responsible for metabolic disturbances in processes in which low Na^+ and high K^+ or Ca^{2+} are required for optimal functioning [2]. A decrease in nitrate reductase activity, an inhibition of photosystem II [3] and a breakdown in chlorophyll [4,5] are associated with increased NaCl concentrations. Moreover, an accumulation of Cl^- causes consistent effects such as destruction of photosynthetic functions [6] and chlorosis in plant tissues. By reducing the osmotic potential of the soil solution, plants energetic cost for water uptake

Abbreviations: APX, ascorbate peroxidase; BAP, 6-benzylaminopurine; f.w., fresh weight; G-POD, guaiacol peroxidase; IBA, indole-3-butyric acid; LN, liquid nitrogen; MDA, malondialdehyde; MKI, McKinney Index; PCD, programmed cell death; ROS, reactive oxygen species.

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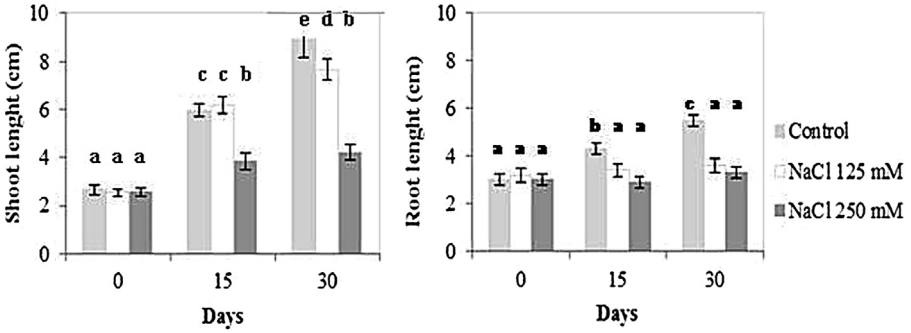

Fig. 1. Shoot and root length (cm) at the beginning of salt treatments and after 15 and 30 days of culture in presence of NaCl (0, 125 and 250 mM). Means with the same letter are not significantly different at $p < 0.05$, according to Tukey test.

increases. To restore the balance, osmotic adjustments are required by means of the uptake of salt and the compartmentalization of ions in plant tissues or in vacuoles and the synthesis of organic compounds, such as soluble sugars or compatible osmolytes. Furthermore, the oxidative damage, observed in plants exposed to salt, is related to the production of free radicals with one impaired electron. Such electron instability makes these atoms highly reactive with other molecules, i.e. membrane lipids, DNA, pigments and proteins which instigate cellular damage and oxidative stress [7].

Plant exposure to salt transmits a stress signal, first perceived by the receptors at the membrane level (ion channels, histidine kinases), which results in the generation of many secondary molecular signals such as Ca^{2+}, reactive oxygen species (ROS) and abscisic acid. Signal transduction induces stress responsive genes, leading to plant adaptation or stress tolerance. Plants react to salt stress in various ways, i.e. morphological adaptation, growth reduction, stomatal closure, which increase the activity and production of enzymatic and non-enzymatic antioxidants [8].

Myrtus communis L. (myrtle) is a Mediterranean shrub, belonging to the Oleo ceratonion vegetation, geographically distributed in South Europe, West Asia and North Africa till coastal areas, associated with *Pistacia lentiscus* L., *Phillyrea* spp. and *Arbutus unedo* L. [9]. Myrtle is gaining importance because of its antimicrobial, cosmetic and anti-tumor activity [10,11] and its commercial values, is also linked to the production of liqueur from its fruit and leaves. A previous study on *in vivo* grown *M. communis* plants [12] elucidated some response mechanisms to root-zone salinity stress of this species making evident changes in the ionic and water relation, photosynthetic activity, morpho-anatomical traits and the accumulation of polyphenols related to salt stress.

In this research the influence of NaCl on myrtle growth and development was studied in *in vitro* cultures with the aim to give a further contribution to the comprehension of the mechanism of the response of this species to high salt conditions particularly in view of its use in re-vegetation of salt affected lands.

The response was studied by investigating salt driven changes in growth, photosynthetic pigments, proline, arginine, cysteine and sugars contents and activities of some ROS scavenging enzymes (G-POD and APX). The presence of lipid peroxidation and of DNA fragmentation as an indicator of programmed cell death (PCD) was also evaluated.

2. Results

2.1. Growth response, chlorosis and necrosis

Salt treatments significantly influenced shoots length, depending on salt concentrations and the exposure time (Fig. 1). Exposure of plants to 125 mM NaCl did not reduce shoot elongation, with respect to the control, after 15 days while a significant limitative effect was only observed after 30 days of treatment. On the other hand, the highest concentration of NaCl (250 mM) negatively affected elongation after both 15 days and 30 days of exposure.

Root growth was significantly reduced by salinity (Fig. 1), in comparison to the control plants, regardless of the concentration and the period of application of the salt treatment.

Effects of NaCl were also detected by using McKinney Index (MKI) in the evaluation of chlorosis diffusion and necrosis (Table 1). In plants exposed to NaCl, MKI increased and was positively related to the length of exposure time and to salt concentrations: in samples exposed to 125 and 250 mM NaCl MKI for treated shoots were 1.62 and 3.36 after 15 days, and 2.3 and 6.2 after 30 days, respectively. Control plants maintained green leaves and stems after 15 days and MKI value was 0.7 at the end of the experiments. According to the MKI results, no necrosis was detected in plants treated with the lowest salt concentration, while the highest MKI values were determined in shoots exposed to 250 mM NaCl for 30 days, a clear indication that the number of chlorotic leaves increased significantly and that necrosis was present in the stems which were also bent.

2.2. Chlorophylls and carotenoids content

A significant reduction in total chlorophyll, compared to the control, was induced by salinity after 15 days, regardless of the amount of NaCl concentrations applied. Further significative reduction in chlorophyll content was observed after 30 days (Tables 1 and 2), particularly with 250 mM NaCl and this response was confirmed by calculation of the TI. Carotenoids content was not significantly affected by salt treatments.

2.3. Effect of salt treatments on osmolytes

No significant changes were detected in the amount of proline during the first 15 days of exposure to salt in both NaCl concentrations (Fig. 2). After 30 days of treatment a marked decrease in

Table 1
Myrtle plants treated with NaCl: McKinney Index (MKI) and Tolerance Index (TI) calculated on the basis of chlorophylls content.

Exposure time (days)	mM NaCl	MKI	TI
15	0	0.1	–
	125	1.62	68
	250	3.36	66
30	0	0.7	–
	125	2.3	51
	250	6.2	38