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The effects of salt stress on growth, water relations and ion accumulation in two halophyte *Atriplex* species

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ABSTRACT

Atriplex halimus is found in the Mediterranean Basin along the coastal areas of Sardinia, but few data are available on its adaptability to salinity. The effects of drought and salinity under controlled conditions on two clones of *A. halimus*, designated MOR2 and SOR4, originating from southern and northern Sardinia, respectively, were compared with those of seedlings of *A. nummularia*, an Australian species widely used in the restoration of arid areas. The effects of increasing NaCl salinity above seawater concentrations and of increasing the KCl concentration gradient were tested. Plants were harvested and analysed after 10 and 20 days of NaCl and KCl treatments. All plants remained alive until the end of treatment, although growth was strongly reduced, mainly for the *A. halimus* MOR2 clone, under increasing concentrations of KCl. The leaves and roots of both species responded positively to increasing NaCl concentrations up to 600 mM NaCl for *A. halimus*, whereas the optimal growth of *A. nummularia* was recorded at 300 mM NaCl. SOR4 was more sensitive to KCl toxicity. The Na^+ concentration in the plants increased with increased salinity and was higher in *A. halimus* than in *A. nummularia*, suggesting that *A. halimus* is an ion accumulator and may be used for phytoremediation. The sodium accumulation in the roots of the *A. halimus* MOR2 clone was far greater than in its leaves. This suggests that MOR2 is an Na^+ excluder, either by minimising the entry of salt into the plant or by an excretion mechanism via the vesiculated hairs that play a significant role in the removal of salt from the remainder of the leaf, thereby preventing its accumulation to toxic levels in the leaves, whereas SOR4 acted as an Na^+ includer. Higher levels of proline were detected in the MOR2 clone under NaCl treatments, suggesting a more developed adaptative mechanism for the selection of this characteristic in the southern part of the island, which is more exposed to abiotic stresses, particularly water stress that is either generated by salinity or by other causes.

1. Introduction

Salinity and water deficits are among the most important agricultural problems in semi-arid and arid regions (Flowers et al., 1977; Glenn and Brown, 1998). These conditions cause significant yield reductions on cultivated lands, inducing a wide range of perturbations at the cellular and whole-plant levels that can lead to plant death and decreases in productivity. However, it has been well established that plants growing naturally in arid and semi-arid areas have evolved many adaptations to counteract salt stress (Khan et al., 2000). The ability to maintain turgor despite the lack of water induced by salt stress may preserve the metabolic processes, and therefore the growth, of a plant (Martinez et al., 2004).

On farms in arid areas, fodder shrubs are commonly used to buffer against annual and seasonal fluctuations in productivity (Le Houerou, 2000; Martinez et al., 2003). These shrubs are used as emergency livestock fodder during very dry years in addition to their use as a source of fuel wood in poor countries. In these arid climates, plants have had to adapt to periodic and unpredictable environmental stresses that arise during their growth and development (Khan et al., 2000). Surviving such stresses over a long evolutionary scale has led plants to acquire mechanisms by which they can sensitively perceive the onset of stress-inducing conditions and regulate their physiologies accordingly. Sources of periodic water stresses to plants include unpredictable rainfall and salt stress in areas where soils are naturally high in salts or are subject to seawater invasion in coastal areas (Martinez et al., 2003).

Salt deserts (caused by drought or a lack of fresh water) and salt marshes are often considered wastelands. However, these habitats are national assets and harbour many salt-tolerant species with a high economic potential for increasing food and fibre production as well as species of potential medicinal importance (Khan et al., 2000; Parida and Das, 2004). These salt-affected areas may also be utilised for raising cash-crop halophytes, which are well

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equipped to survive and complete their life cycles in saline soils (De Araùjo et al., 2006; Ungar, 1991). Additionally, these halophytes play a crucial role in sustaining the complex natural ecosystems in which they exist. Currently, all over the world, a critical goal is the sustainable development of non-conventional crops and agricultural practices suited to local environmental conditions. Natural habitats are being preserved and disturbed communities are being restored to preserve biodiversity. These agricultural and ecological goals can best be accomplished by understanding the distribution and eco-physiology of species already adapted to diverse saline environments (Chinnusamy et al., 2005).

In the arid and semi-arid regions of the world, shrubs that can survive in saline soils have been the subject of considerable research (Ayala and O'Leary, 1995; Khan et al., 2001; Naidoo and Rughhunanan, 1990; Parida and Das, 2004). These plants have been evaluated as alternative sources of forage for livestock. It is necessary to evaluate the potential domestication of plants that grow in saline environments. This evaluation will help determine whether it is possible to establish and manage these plants in a given environment and to develop, in the long term, a product of a quantity and quality that justify the effort invested in its production. Some modest early results indicate that halophytes can represent a profitable crop on soils unable to grow other plants (Chen et al., 2009; Lindhauer et al., 1990). It should therefore be possible to facilitate the rehabilitation of saline lands by defining these sites in terms of plant's requirements for growth and designing plantings based on the plants adapted to such conditions (Le Houerou, 2000).

Plant salt tolerance is generally considered to be the inherent ability of a plant to withstand the effects of high salt concentrations in the rhizosphere or in the leaves without significant adverse consequences. Maintaining their growth rates, preserving nutrients, avoiding ion toxicities, and inducing metabolite changes that improve their water balances are probably the most common and universal characteristics of salt-tolerant plants (Chen et al., 2009). Many plants develop mechanisms either to exclude salt from their cells or to tolerate its presence within the cells (Cramer et al., 1985; Mozafar and Goodin, 1970). During the onset and development of salt stress within a plant, all of the major processes, such as photosynthesis, protein synthesis, and energy and lipid metabolism, are affected (Parida and Das, 2004).

Because salt resistance is a complex trait involving several interacting properties, there has been increasing interest in studying the physiological behaviour of halophytic species to identify and understand salt resistance mechanisms (Bajii et al., 1998). Salts naturally affect a myriad of cellular activities, primarily because they alter the water content of the cell (Ndimba et al., 2005). Salt tolerance is the ability of plants to grow and complete their life cycles on substrates that contain high concentrations of soluble salts. This tolerance is not exclusively correlated with adaptation to Na^+ toxicity, which is reached at high levels of salt in the medium; at lower salt levels, salt tolerance mainly reflects adaptation to water deficit induced by the high salinity (Marschner, 1995; Maathius and Amtmann, 1999). Therefore, the uptake mechanisms and ion accumulation patterns in different plant organs are important factors determining a plant's salt tolerance (Ashraf and Ahmed, 2000). Plants develop biochemical and molecular mechanisms to cope with salt stress, and these mechanisms act additively and probably synergistically (Mäser et al., 2002; Parida and Das, 2004).

The use of *Atriplex* spp. has long been considered suitable for the restoration of degraded lands with precipitation of lower than 200 mm/year, and thus, several million hectares have been planted throughout the world. The species of the genus *Atriplex* are partly spontaneous in the WANA (West Asia and North Africa) area and have partly been introduced to determine their adaptability for use as fodder species (Le Houerou, 2000). Several species belonging to the genus *Atriplex* are well adapted to extreme environmental conditions and may possess interesting properties for use as fodder. *Atriplex halimus* L. is the native halophyte species that has been planted most often. Belonging to the *Chenopodiaceae* family and possessing the C_4 metabolic pathway, *A. halimus* is widely distributed in non-saline as well as highly saline soils. *Atriplex nummularia*, an exotic species from Australia, is species that has been planted in the widest area of WANA, South America, Australia and other countries (Le Houerou, 2000). As in most species within the genus *Atriplex*, *A. halimus* and *A. nummularia* are able to accumulate high levels of sodium under high salt-stress conditions, and their growth is even stimulated by moderate salt levels. The particular characteristics of *A. halimus*, a species typically found in the Mediterranean, in promoting both littoral and arid areas in an ecologically sustainable way has aroused the interest of many researchers throughout the world. The adaptations of this species to salinity and drought conditions make it a particularly invaluable species for use in the rehabilitation of degraded lands at risk of desertification (Bajii et al., 1998). The effective use of this species first requires a thorough knowledge of its characteristics, particularly its adaptations to fluctuating environments, which have allowed it to develop a high plasticity, both at the individual level and within species and across ecotypes. We focused our investigation on populations of this species growing under natural conditions on the island of Sardinia.

The main objectives of the current study were thus to determine the salt tolerance of *A. halimus* native species in comparison to *A. nummularia*, in terms of whole plant responses to water and salt stress in relation to the accumulation of organic and inorganic solutes.

2. Materials and methods

2.1. Plant materials and culture conditions

A. halimus L. and *A. nummularia* cuttings were taken from cultivated plants at the experimental station of the University of Sassari located in Oristano (Sardinia). The *A. halimus* plants came from selected clones of wild populations from different parts of Sardinia, whereas the *A. nummularia* clones came from a collection of Moroccan seedlings. Stem cuttings of approximately 10 cm were taken from a selected plant of *A. nummularia* and from 2 *A. halimus* clones from Is Mortorius in the south and Marina di Sorso in the north of Sardinia, named MOR2 and SOR4, respectively.

Cuttings, with the leaves removed to minimise transpiration and the resulting desiccation effect, were planted in a bed of perlite in a cold greenhouse with a basal temperature of 28 °C and with a "mist" irrigation system. When they reached a stage of root development allowing for their transplantation (after 30 days), the plants were transplanted in pots with a substrate composed of 1/3 soil, 1/3 perlite and 1/3 organic compost. The substrate had a pH of 7.05 and a total percentage of humus of 1.76%, and the mineral composition was estimated in ppm as follows: P: 19, Ca: 2120, K: 210, Na: 60, Mg: 90.

Pots were put in a greenhouse and were well irrigated daily under a photoperiod of 14–15 h. After 20 days, plants of equal size (approximately 10 cm in height) designed to the salt stress, were transferred in polyethylene pots (10 cm × 10 cm) containing inert perlite substrate after washing soil from the roots under running water. Potted plants were grown in plastic trays containing 1/4 strength modified Hoagland nutritive solution (Hoagland and Arnon, 1950) and arranged in a completely random design within treatment trays (the positions of the trays were changed daily) and were sub-irrigated. The level of water was adjusted each day with distilled water to correct for evaporation. After 26 days of acclimatisation, 5 salinities (0, 300, 600, 800 and 1000 mM NaCl; 0, 300,