



Germination and growth of *Atriplex prostrata* and *Plantago coronopus*: Two strategies to survive in saline habitats



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ABSTRACT

We investigated the effects of NaCl on germination, germination rate, growth, proline content, polyamine levels (putrescine, spermidine and spermine) and total polyamines in *Atriplex prostrata* and *Plantago coronopus*. The seeds collected from Brujuelo (salt marshes, Jaén, southern Spain) were germinated under control (water) and saline conditions (50, 100, and 200 mM NaCl) for 20 days at 25 °C. Salinity decreased germination percentage and the germination rate in *A. prostrata*, and *P. coronopus* with increasing salt concentrations. In seedlings at day 6 and 10 of the experiment, both the length and fresh mass increased in *A. prostrata* for all saline treatments with respect to control, whereas in *P. coronopus*, only fresh mass increased with 50 and 100 mM NaCl, indicating its lower salinity tolerance. With respect to proline, the maximum increase occurred with 200 mM NaCl in *A. prostrata*; however in *P. coronopus* the values were low and without significant differences between treatments. The polyamine levels changed, in *P. coronopus* spermidine and spermine increased with salt, while in *A. prostrata* putrescine and spermidine decreased. These results indicate that the increase of proline was accompanied by a decrease of total polyamines (putrescine + spermidine + spermine) in *A. prostrata* while *P. coronopus* had high total polyamine levels with low values of proline, demonstrating that these plants use different strategies to adapt to saline environments.

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1. Introduction

Salinity is one of the most significant abiotic factors that affect the germination, growth, and productivity of plants (Parida and Das, 2005; Gupta and Huang, 2014). However, a small category of plants, known as halophytes, survive and are distributed in a wide variety of saline habitats, including inland or coastal areas, salt-marshes, dunes, and deserts (Gul et al., 2013). These plants, which include annuals as well as perennials, present different degrees of tolerance to salinity (Zhang et al., 2015). Saline soils contain a variety of salts, being NaCl one of the most harmful for vegetation in general (Flowers and Colmer, 2008; Soriano et al., 2014). Soil salinity affects plant development in two-ways: the osmotic effect, whereby soil particles retain water, hampering its absorption by

plants; and the toxic effect, due to the excessive concentration of ions such as Cl⁻ and Na⁺, triggering ionic imbalances and nutritional deficiency, which alter seed germination and plant growth (Türkan and Demiral, 2009; Li et al., 2010). As a result, halophytes have developed a wide range of morphological, physiological, and biochemical adaptations (Gupta and Huang, 2014).

Although halophytes are naturally adapted to salinity, their tolerance is greatly influenced by their genetics and developmental stage (Megdiche et al., 2007). Seed germination and the early seedling growth are the most vulnerable stages and the most important for the development and distribution of species that inhabit saline environments (Ungar, 1996; Malcolm et al., 2003). Detrimental effects of salinity on seed germination and seedling growth have been well characterized (Koyro and Eisa, 2008; Zhang et al., 2015), but the physiological and biochemical mechanisms require a fuller understanding.

One of the main consequences of NaCl stress is the loss of intracellular water. Plants accumulate many metabolites that are also known as “compatible solutes” in the cytoplasm to bolster their hyperosmotic tolerance against salt-stress-induced water loss from the cells. This process is also necessary in order to balance the osmotic potential of Na⁺ and Cl⁻ being sequestered into the vac-

Abbreviations: PAs, Polyamines; SAM, S-adenosyl-L-methionine; Put, Putrescine; Spd, Spermidine; Spm, Spermine.

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uole (Liu et al., 2010; Zhang et al., 2015). Various compounds such as proline, sugars, quaternary amino acid derivatives, tertiary amines, and sulfonium compounds have been suggested to serve this function in halophytes (Türkan and Demiral, 2009). Proline synthesis occurs mainly from glutamate and ornithine and its accumulation is due to an increase in synthesis enzymes and/or the decrease in oxidation enzymes (Kavi Kishor et al., 2005). Proline is a compatible solute, cytosolic pH buffer, protects enzymes and subcellular structures, acts as a free-radical scavenger, stimulates antioxidant enzyme activities, and cell redox balance (Gupta and Huang, 2014; Iqbal et al., 2014). Proline is one of the most abundant osmolytes, enabling the survival of plants under saline-stress conditions without interfering with normal cell metabolism (Sleimi et al., 2015).

Plant-growth regulators play a very important role in germination, seedling growth, and response to abiotic and biotic stress (Miransari and Smith, 2014; Fahad et al., 2015), including polyamines (PAs) (Hussain et al., 2011). Their biological activity is attributed to their cationic nature at physiological pH and therefore PAs interact with negatively charged macromolecules such as DNA, RNA, proteins, and phospholipids, stabilizing their structure. The most abundant PAs are: putrescine (Put) (diamine), spermidine (Spd) (triamine), and spermine (Spm) (tetraamine) and can exist in free and conjugated form (associated with small molecules or bound to macromolecules) (Kusano et al., 2007; Martin-Tanguy, 1997; Alcázar et al., 2010). They have multiple functions in the growth and development of plants, such as the regulation of gene expression, translation, and cell proliferation, the modulation of cell signalling, and membrane stabilization (Kusano et al., 2008). They are also involved in a wide range of environmental stress, including drought, salinity, low temperature, oxidative stress, and metal toxicity (Hussain et al., 2011; Tiburcio et al., 2014; Pál et al., 2015). PAs might act as compatible solutes in specific organs, cells or organelles (Wi et al., 2006). Plants can also accumulate free PAs to function as scavengers of free radicals and to be efficient antioxidants (Tang and Newton, 2005). Additionally, PAs can affect ion channels conductivity related to Ca^{2+} and homeostasis (Kusano et al., 2007; Hussain et al., 2011). Proline and putrescine have two common precursors, i.e. glutamate and ornithine (Álvarez et al., 2003; Iqbal et al., 2014).

Plants of the genus *Atriplex* (Amaranthaceae) are considered halophytes for their ability to survive in saline environments. These annual plants grow on land that has been altered or nitrified as well as on saline sites in Europe, North Africa, South-West Asia, and North America. These species are of great interest both in the restoration of eroded areas, to provide livestock feed, and they constitute an alternative to boost the productivity of these marginal areas (Le Houérou, 1992). *Atriplex prostrata* Boucher ex DC is an annual salt marsh species that grows on altered and nitrified soils, whether saline or not. These plants are able to take up water by maintaining a low osmotic potential through the accumulation of inorganic and organic solutes such as proline, glycinebetaine or soluble sugars (Hassine et al., 2008; Gomes Silveira et al., 2009). The genus *Plantago* (Plantaginaceae) represents a group of plants which have different levels of salt sensitivity and which can be used in comparative studies with other species regarding the response to salt stress (Vicente et al., 2004). These plants are distributed in the Mediterranean-Atlantic areas of Europe and the West Irano-Turanian and Saharo-Arabian phytogeographic regions (Zaady et al., 1997). *Plantago coronopus* L. is an herbaceous rosette that lives on uncultivated land and even in some salt marshes. It can be found in markedly different habitats, being common along roadsides, trampled areas, nitrified and degraded pastures, and near the sea (Yoshida and Tanaka, 1997), in addition, they can grow under conditions of variable salinity (Vicente et al., 2004). This species is efficient in water-use and the accumulation of compat-

ible solutes in response to increasing external salt concentrations (Koyro, 2006).

The present study was undertaken to gain new insights into the ecophysiology on two wild plant species, *A. prostrata* and *P. coronopus*, with different tolerance to salinity, and growing in the same salt marsh. The aim was to evaluate germination, growth, changes in the level of proline, total and free polyamines with high and low NaCl concentrations in order to determine the importance of each compound in the response to salt stress. Several studies on these responses have been conducted on crop species, but little is known in the literature on wild species.

2. Material and methods

2.1. Study sites and seed collection

Mature seeds from many plants of natural populations of *A. prostrata* Boucher ex DC and *P. coronopus* L. in Brujuelo salt marsh, Jaén, southern Spain (37°52'46"N, 3°40'11"W) were randomly collected in September 2010. The salt marsh occupies 0.008 km² and has an annual average temperature of 16.9°C; the average annual rainfall is 578 mm (<http://www.globalbioclimatics.org/station/es-jaen2.htm>). The plants are located at the edge of the salt marsh, undergoing temporary flooding of the roots during the seasons of maximum precipitation (Pérez Hurtado de Mendoza, 2004; Lendínez, 2010). In both species the seeds have dormancy, so that dry storage and cold stratification for several months is needed to break dormancy (Baskin and Baskin, 2001). Therefore, the clean seeds were stored in air-tight containers with silica gel and refrigerated (4°C) until used in germination tests and other determinations (Easton and Kleindorfer, 2009).

2.2. Germination experiments

Seeds of *A. prostrata* and *P. coronopus* were washed with sterile distilled water and incubated in 90 mm diameter Petri dishes on two layers of Whatman No. 1 filter paper moistened with 5 ml of saline solution or distilled water (control). The experimental treatments consisted of four levels of salinity (0, 50, 100, and 200 mM) of sodium chloride (NaCl) (Merck). Each Petri dish contained 50 seeds. All Petri dishes were wrapped (to prevent evaporation and change in salinity) and incubated for 20 days. For germination, a growth chamber was used with a thermoperiod of 25/16°C and photoperiod of 16 h light/8 h dark (450 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, 400–700 nm) provided by Sylvania Cool White (Osram Sylvania Inc., Danvers, MA, USA) and Osram Dulux Superstar (Osram Sylvania Inc., Danvers, MA, USA) lamps and relative humidity 55–75%. The Petri dishes were examined every two days to monitor the number of germinating seeds. Seeds were considered to have germinated when radicle emergence was visible. The Germination Rate (GR) was estimated using a modified Timson index of germination velocity: $\text{GR} = \sum G/t$, where G is the percentage of seed germination at 2-day intervals and t is the total number of days of the germination period. A greater value of G indicates a faster germination rate (Easton and Kleindorfer, 2009).

2.3. Seedling growth

At day 6 and 10 of the experiment, seedlings were chosen to study growth (measured as length and fresh mass of the seedling). At day 2 and 4, the percentage of germination and seedling fresh mass were so small that it was difficult and laborious to collect sufficient plant material for all the determinations. Therefore, studies were made with seedlings from day 6 to day 10 of the experiment. The tables and figures include data for day 6 and 10, since day 8 had

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