Environmental and Experimental Botany 102 (2014) 27–36

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Saline tidal flooding effects on *Spartina densiflora* plants from different positions of the salt marsh. Diversities and similarities on growth, anatomical and physiological responses

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ARTICLE INFO

Article history:
Received 16 October 2013
Received in revised form 4 February 2014
Accepted 9 February 2014

Keywords:
Allocation
Ion regulation
Leaf growth
Photosynthesis
Salinity
Senescence

ABSTRACT

Spartina densiflora is a halophytic grass present in many salt marsh ecosystems where it dominates throughout topographical stress-gradients. This work aimed at studying diversities and similarities in ecophysiological responses of *S. densiflora* plants from two contrasting positions in the salt marsh. We simulated a natural tide by exposing plants of *S. densiflora* from upland and lowland sites of a salt marsh to saline intermittent flooding (9 h day^{-1}) during 60 days. Responses in plant growth, biomass allocation, anatomy, ion regulation, and photosynthetic performance were assessed. Saline intermittent flooding caused changes in anatomical and morphological traits of plants from both sites associated with increased root aerenchyma and decreased mass allocation to leaf blades in relation to leaf sheaths, concomitant with reductions of blade size and changes in blade shape. Similar negative effects of saline intermittent flooding were found on physiological traits related to photosynthetic functioning of plants from both sites, like decreases in chlorophyll fluorescence, quantum efficiency and $\delta^{13}C$. However, lowland plants presented unaffected leaf length, better ion regulation (higher Cl^- exclusion, higher K^+ concentration, and lower Na^+/K^+ ratio), as well as later leaf senescence with respect to upland plants, when subjected to saline intermittent flooding. Accordingly, plant biomass production decreased by 15% and 32% for lowland and upland plants, respectively. These results indicate that plants of *S. densiflora* inhabiting in the lowland positions have a better acclimation capacity to the harsh environment imposed by the tide than plants from the upland.

1. Introduction

Coastal salt marshes are important natural environments that provide relevant ecosystem services like wildlife conservation, coastal protection, erosion control, and carbon sequestration (Barbier et al., 2011). These environments are mainly shaped by two restrictive environmental factors, soil salinity and flooding, whose magnitude is directly associated with the frequency and intensity of tides. Elevation above sea level directly determines a gradient of abiotic stress: daily tides in lowland sites imposes higher salinity and more frequent flooding events than in more elevated uplands, subjected to less frequent tides. *Spartina densiflora* is a perennial tussock grass with C_4 metabolism. In contrast to other species (e.g. *S. anglica*), *S. densiflora* is able to inhabit all along salt marsh spatial gradients, including areas with different tidal regimes (Bortolus, 2006; Maricle et al., 2009). This can be attributed to the existence of different ecotypes, large phenotypic plasticity, or both (Loreti and Oesterheld, 1996; Sultan, 2000). This paper assesses the effect of simulated tides on upland and lowland plants of *S. densiflora*, a halophyte that dominates the entire topology of many temperate salt marshes worldwide, including those on the Atlantic coast of southern South America (Bortolus, 2006; Isacch et al., 2006).

Previous studies have determined ecophysiological plant responses to saline flooding on natural halophytes species (Colmer and Flowers, 2008; see Wetson et al., 2012 for *Suaeda maritima*). These species can deal with flooding because they are able to generate aerenchyma in their roots, which favours internal oxygenation, thereby preventing an energy crisis typical of anoxic tissues. In

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http://dx.doi.org/10.1016/j.envexpbot.2014.02.009

this way, these species can maintain their roots well aerated and, therefore maintain an adequate regulation of ions (i.e. Na^+, Cl^- and K^+) delivered from roots towards shoots (Colmer and Flowers, 2008). However, most studies have focused on relatively tolerant annual crops (as rye, barley or wheat), and bushy/woody perennials species (e.g. *Atriplex amnicola*, *Acacia* ssp., *Eucalyptus* ssp.), reporting that flooding under saline conditions increases Na^+ and Cl^- concentration in plant shoots, which can have adverse effects on plant growth and survival (Barrett-Lennard, 2003). In addition to the damage by salt toxicity (ion excess effect), salinity generates dehydration of plant tissues through osmotic or water-deficit effect (Munns and Tester, 2008). Therefore, plants inhabiting saline environments usually have traits and develop responses for minimizing water loss, for example through changes in leaf morphology and anatomy (Maricle et al., 2009). Poorter et al. (2012) stated that flooding generally increases biomass allocation to shoots, whereas salinity minimally affects allocation but, in turn, it negatively affects physiological parameters. Furthermore, salinity negatively affects physiological processes mainly due to the generation of osmotic stress in the roots and ion toxicity (i.e. excessive Na^+ or Cl^-) at cellular level (Munns and Tester, 2008). Tolerant halophyte species cope with salinity through controlled uptake and compartmentalization of toxic ions, synthesis of organic compatible solutes, and secretion of toxic ions towards the outside of leaves through salt glands (Flowers and Colmer, 2008).

Photoinhibition of photosynthesis can occur in response to several environmental stresses (Takahashi and Murata, 2008), including flooding (Mateos-Naranjo et al., 2007) and salinity (Redondo-Gómez et al., 2007). The damage to the photosynthetic apparatus at PSII level – evaluated through chlorophyll fluorescence (F_v/F_m) and quantum efficiency (θPSII) – leads to a reduction in the leaf photosynthetic capacity (Maxwell and Johnson, 2000) and, therefore such variables can be used as indicatives to infer species tolerance. While highly tolerant to salinity stress, *S. densiflora* is known to show decreases in growth and photosynthesis rates as well as in photochemical efficiency when subjected to high salinity levels (Castillo et al., 2005; but *cf.* Maricle et al., 2007). In C_4 plants, increases in ^{13}C discrimination during CO_2 assimilation indicate higher CO_2 leakiness (the proportion of the CO_2 pumped into bundle sheath cells by the photosynthetic carbon reduction cycle that is not fixed by Rubisco and diffuses back to the mesophyll) and/or a higher ratio of intercellular to atmospheric CO_2 (for ^{13}C discrimination greater than 4.5‰; Farquhar et al., 1989). Since the latter is an unlikely response to saline intermittent flooding in *Spartina* (Maricle et al., 2007), a higher ^{13}C discrimination would indicate greater CO_2 leakiness.

The objective of our study was to determine whether *S. densiflora* plants from upland vs. lowland sites in a salt marsh on the coast of the Rio de la Plata river exhibit significant intraspecific variation according to tidal simulation and whether they differ or not in morphological, anatomical, and physiological traits in response to saline flooding. To achieve this objective, we exposed plants of *S. densiflora* from upland and lowland sites to simulated tidal inundation (saline intermittent flooding treatment) in a greenhouse, and explored diversities and similarities in their response in biomass production and allocation, leaf length, root and leaf anatomy, ion regulation, and photosynthetic performance in relation to control plants. We hypothesized that saline intermittent flooding negatively affects some of the studied parameters in a different extent between plants from both sites, being the lowland plants more tolerant to saline intermittent flooding than the upland ones. These differences would reflect different acclimation capacity of plants from both sites across the topographical gradient of the salt marsh, ecosystems of increasing vulnerability due to sea level rise predictions for the present century.

2. Materials and methods

2.1. Study site and plant material

Individual plants of *S. densiflora* (Brongn.) from two different topographic positions – upland and lowland – were extracted from a salt marsh within the National Park "Campos del Tuyú" (56° 50′ W – 36° 19′ S) on the west margin of the Rio de la Plata estuary (Argentina). This environment is characterized by a surface hydrological system with a subtle topographic gradient that originates a network drainage influenced by tidal fluctuation of the estuary (Carol et al., 2008). Tides are predominantly semidiurnal, with salt water usually ranging less than 2 m into the land, but tidal intensity can vary in relation to seasonality and weather conditions. Tidal water contains NaCl (Carol et al., 2008) with an average electrical conductivity of ca. $26 \pm 0.3\,dS\,m^{-1}$ (this study). Soils belong to the Vertisols order with clay texture, smectite expansible clays, low permeability, and ca. 7% organic matter. According to the information above, flooding and salinity appear as important factors determining a topographic stress gradient, which leads to halophytic plant communities zonation (Cagnoni and Faggi, 1993). In addition, the presence of reduced phytotoxins should not be neglected as they might also affect plant performance in salt marshes (King et al., 1982). However, we focused our attention on the combined effects of flooding and salinity as major stress factors. The upland site is located ca. 20 cm higher than the lowland one, which means that upland positions experience less frequent flooding events (very few times per year). In relation to salinity, upland soils have an electrical conductivity that ranges between 9.1 ± 2.9 and $14.4 \pm 2.4\,dS\,m^{-1}$, in winter and summer, respectively. Upland site vegetation is dominated by *S. densiflora* in association with *Juncus acutus* and *Cortaderia selloana*, whereas *Apium sellowianum*, *Limonium brasiliense*, *Distichlis spicata* and *Agropyron scabrifolium* are present as subordinate species (Di Bella et al., 2014). Lowland site, where floods occur on a daily basis due to its location beside the tidal channels, is characterized by higher soil salinity, which ranges between 9.3 ± 1.3 and $25.3 \pm 2.3\,dS\,m^{-1}$, in winter and summer, respectively. Vegetation at this site is almost exclusively dominated by *S. densiflora* and *Sarcocornia perennis*, with ca. 25% bare soil (Di Bella et al., 2014).

In December 2010, we collected 50 plants from both upland and lowland sites. These sites are located at a distance of ca. 85 m apart in the salt marsh under study. To avoid sampling the same individual (because of rhizome growth) collected plants were separated at least 2 m from each other. Plants were transported to an experimental garden at the University of Buenos Aires and they were vegetatively propagated twice (January and August 2011; four–six individuals per original plant) to minimize any influence from the original environment (as in Loreti and Oesterheld, 1996). Afterwards, plants were cultivated in a greenhouse until the beginning of the experiment in October 2011.

2.2. Experimental design and growth conditions

The experiment had two factors: "site" as the classification factor and "flooding" as the treatment factor. Site levels were individuals collected from upland and lowland positions, while flooding treatment levels were control and saline intermittent flooding. Each combination site × flooding had 20 replicates, with 80 experimental units. Plants were grown on washed sand in 2 L plastic pots with drainage holes. The experiment began after 7-days acclimation period, where half the plants were subjected to increasing saline concentration (adding 100 mM NaCl every two days until the final saline concentration was reached, avoiding osmotic shock by sudden imposition of saline conditions). To simulate saline intermittent flooding, we placed pots in plastic containers, simulating