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Growth responses and physiological traits of seashore paspalum subjected to short-term salinity stress and recovery

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ABSTRACT

The objective of the present research is to draw a comprehensive picture of the integrated response mechanisms of 'SeaSpray' seashore paspalum, a recently released seeded cultivar, to controlled short-term salinity conditions. A solution culture study was performed during a two weeks time-course experiment. In addition its ability to recover in terms of photochemistry activity was examined, at the highest salinity level after seven days of rewatering with distilled water only. To quantify growth and physiological responses to salinity (NaCl), plants were divided into five treatments and grown across a range of salinities (0–600 mM). Although exposure to severe salinity stress 14 days after treatment (DAT) was sufficient to affect biomass fresh weight, dry weight production was not affected. The photon yield of PSII and non-photochemical quenching responded to the strength of the stress applied, whereas the maximum efficiency of PSII photochemistry declined only in the highest salinity stress level after 14 DAT. However, the rapid and full recovery of the main chlorophyll *a* fluorescence parameters upon rewatering confirms the hardy tolerance of the species to such stress conditions. Functional changes were observed in pigment and carbohydrate content and composition among different treatments, especially after 14 DAT. The use of multicanonical analysis revealed the canonical relationship between the treatment fingerprints obtained from biometric and physiological data. Overall, our dataset suggests the use of straight seawater or brackish water for this salt tolerant species, creating the opportunity to develop turfgrass landscapes in arid and seashore regions.

1. Introduction

In higher plants, salinity can cause hyperosmotic and hyperionic stress, inducing significant morphological, physiological and biochemical changes which under persistence may contribute to plant demise (Hasegawa et al., 2000). Water availability decreases under salinity stress (Chaves et al., 2009; Munns, 1993), primarily due to the osmotic or water deficit effect that reduces the ability of the plant to take up water, leading to a slower growth rate. The ion-excess effect of salinity stress occurs when salt accumulates toxic concentrations in mature and old leaves, which enter the transpiration stream required to maintain the water status of the plant.

Under high salinity conditions, or in sensitive species that lack the ability to control Na^+ transport, the ionic effect dominates the osmotic effect and the photosynthetic capacity of the plant will no longer be able to supply the carbohydrate requirement of the young leaves, which further reduces their growth rate (Munns and Tester, 2008). One dramatic plant response to salinity is a decrease in stomatal aperture, and although there are strong correlations between increases in leaf ion concentrations and reductions in photosynthesis or stomatal conductance, there is as yet no unequivocal evidence for causal relationships (Munns, 2002). The induction of a stress-related decline in PSII photochemistry with consequent PSII photoinhibition and/or photodamage is likely to occur (Degl'Innocenti et al., 2009). Reduced leaf expansion resulting in a buildup of unused photosynthate in growing tissues may generate feedback signals to downregulate photosynthesis. Important stress adaptation effectors are categorized as those that mediate ion homeostasis, and osmolyte biosynthesis, achieved by the synthesis and cytoplasmic accumulation of organic solutes. Frequently

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observed metabolites with an osmolyte function are sugars (mainly sucrose and fructose), sugar alcohols (glycerol, methylated inositols), and complex sugars (trehalose, raffinose, fructans) (Hasegawa et al., 2000). Soluble sugars that are altered by a water deficit and salinity, also act as signalling molecules under stress (Chaves and Oliveira, 2004) and interact with hormones as part of the sugar sensing and signalling network in plants (Rolland et al., 2006).

The ecological benefits expected from the use of deep-rooted perennial grasses is the ability to lower the water-table (Munns et al., 2006). In addition, as a plant transpires 50 times more water than it retains in leaves, Na^+ exclusion is a primary determinant of variability in salinity tolerance, even more so for perennial than for annual species, because the leaves of perennials live and transpire for longer (Munns and Tester, 2008). In an ecological context, especially for perennial species, survival is often more important than growth, thus the emphasis on growth maintenance as an adaptive response is less pronounced. For these species under prolonged stress conditions, there is the opportunity to become dormant, and thus survive a period of stress that will be relieved later by rainfall (Munns et al., 2006).

The morphological and physiological traits of perennial rhizomatous warm-season turfgrasses in warm and transition zones for sports fields and recreational areas offer a high potential to overcome a range of abiotic stresses such as extremes of temperature and rainfall and edaphic factors such as increased soil salinity (Pompeiano et al., 2014). Seashore paspalum (*Paspalum vaginatum* Swartz), belonging to the subfamily Panicoideae, has been found to exhibit an excellent halophytic response. It has recently gained attention for use on saline turfgrass sites and forage production, drainage water reuse schemes, and land reclamation under saline conditions (Lee et al., 2008). Probably originating from Western Africa, its native habitat includes sand and mud near the seashore and in swampy ecosystems (Duncan, 1997). Intra-specific variations in morphological and physiological responses to salinity have been observed (Lee et al., 2005b; Lee et al., 2008; Shahba et al., 2012), and many ecotypes have exhibited the typical halophytic response of increased total plant growth with increasing salinity within the 5–20 dS m^{-1} EC range, followed by a decrease in growth (Lee et al., 2005a). 'SeaSpray' is a medium-textured, recently released high shoot density seeded cultivar which can provide an economical way to establish a high-quality seashore paspalum stand. Compared to other warm-season grasses, 'SeaSpray' germination has been shown not to be inhibited under moderate salinity conditions, although it has the lowest germination (Johnson et al., 2007). Slow establishment rates have been observed, whereas in the transition zone, 'SeaSpray' provides fast spring green-up, and excellent growth performance under deficit irrigation (Bañuelos et al., 2011). However, to the best of our knowledge, limited information is available on the effects of salinity on growth of this cultivar and even less is actually known on the ability of the species to recover in terms of photochemistry activity induced by high level of NaCl.

The aim of the present research was therefore (*i*) to characterize the biometric and physiological response of 'SeaSpray' to prolonged salinity exposure by evaluating contributions of soluble carbohydrates to the osmotic adjustment, (*ii*) to assess the temporal variation in potential and actual PSII photochemical efficiency, non-photochemical quenching and photosynthetic pigments within the leaf lamina of the plant suffering salinity stress, (*iii*) to evaluate its ability to recover the photochemistry activity and the electron transport capacity of PSII upon cessation of the stress, and (*iv*) to provide guidance to turf managers interested in expanding seashore paspalum cultivation in saline soils and in coastal areas irrigated with seawater or brackish water.

2. Materials and methods

2.1. Plant material and growth conditions

The research was carried out at the department of Agriculture, Food and Environment, University of Pisa, Italy. On April 4, 2014, plants of *Paspalum vaginatum* Swartz cv. 'SeaSpray' (selected as a seeded cultivar standard in the warm and transition zone of the Mediterranean basin) were collected and clonally propagated as phytomers (1–2 cm segments of stolon, containing root tissue, crown, and shoot material) into a sphagnum moss-based peat growing medium, mixed with volcanic sand (80:20 in volume), into 160-hole seed trays, with a single cell volume of 5 cm^3. Plants were established at 23 °C (±7 °C) day/night temperatures for 21 weeks in a greenhouse. The fertilization program was adjusted according to the physiological age and state of the grass, but during the active growing season, from May to August 2014, mineral solutions with 8N–7P–19.9K + 4Ca–2Mg (1.3 g L^{-1}) were supplied three times per week, while other nutrients were maintained at sufficiency levels as determined by periodic analysis. Irrigation was applied as needed to prevent wilting, and plant material was maintained at a cutting height of 2.5 cm throughout the entire pre-treatment phase. Mature plants were then acclimated in a growth chamber for four weeks before treatments, and maintained at 22 ± 1 °C with a 12-h photoperiod and a light intensity of 100 μmol m^{-2} s^{-1}.

A solution culture study was performed. Uniform plants cores were secured in polyvinyl chloride (pvc) cylinders with coarse screen bottoms, and allowed to acclimate under controlled conditions for two weeks. To prevent salinity shock, NaCl levels were gradually increased by 75 mM NaCl per day until final levels of 0, 75, 150, 300, and 600 mM NaCl (control, S12.5, S25, S50, and S100) were reached (Dudeck and Peacock, 1985). After final salinity levels were attained, shoots were mown to 2.5 cm above the soil surface and discarded.

The salinity treatments were applied for two weeks, after which a final harvest marked the end of the sample collection. An additional set of S100 plants were again watered daily with distilled water only for a further seven days to evaluate the recovery responses (R100). Turfgrass cores were placed in pots suspended in nutrient tanks, each containing 25 L of a continuously aerated, half-strength modified Hoagland nutrient solution (pH 6.50 ± 0.05), containing the appropriate salinity level (Epstein and Bloom, 2005). The solutions were changed three times a week in order to leach out any accumulated salt. Salinity levels were monitored with the same frequency by measuring the electrical conductivity of the solution (ECw) at 22 °C with a Crison conductivity meter (Pt-100, Barcelona, E).

Leaf firing, based on percentage of leaves exhibiting visual symptoms of chlorosis or tissue desiccation, was rated during the experimental period. In order to conduct destructive measurements, for each treatment 10 plants were removed at each time point (0, 3, 7, and 14 DAT with an additional time point set at the recovery for the highest salinity level). Plants were then separated into root and verdure fractions (crowns plus stems up to the mowing height of 2.5 cm). Root tissues were washed with water to remove the soil, separated, and immediately dried (at least 72 h at 60 °C), then later weighed. Their fresh and dry weight (FW and DW) were also recorded. Additional sets of plants not used for testing biometric traits, were collected and immediately processed or ground in liquid nitrogen, then stored at −80 °C for further biochemical analyses.

2.2. Chlorophyll a fluorescence measurements

Chlorophyll fluorescence was measured using a miniaturized pulse-amplitude-modulated fluorometer (Mini-PAM; Heinz Walz