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Eco-physiological adaptations of *Panicum antidotale* to hyperosmotic salinity: Water and ion relations and anti-oxidant feedback

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ABSTRACT

Threshold of salt resistance of plants is determined by their response to osmotic and ionic stress (primary constraints) imposed upon them. However, recent reports emphasize the importance of secondary constraints like oxidative stress. The aim of this study was to determine the effect of salinity on growth, mineral nutrition, water relations, compatible solutes, and the antioxidant system in *Panicum antidotale*.

Five levels of salinity (0, 125, 250, 375 and 500 mM NaCl) were applied using a quick check system in a fully randomized greenhouse study. Plant growth parameters, water relations, organic (proline and soluble sugars), inorganic osmolytes (Na^+, K^+, $Ca^{++}$and Mg^{++}), and macronutrients such as carbon or nitrogen were measured beside the activities of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APx) and glutathione reductase (GR) and non-enzymatic antioxidant metabolites (oxidized and reduced ascorbate).

Sodium chloride concentrations above 125 mM substantially inhibited growth. This inhibition was attributed to high energy costs needed for osmotic adjustment, ion compartmentalization, synthesis of organic osmolytes (such as proline and sugars), ROS scavenging and the maintenance of ionic homeostasis. The plants resisted against oxidative stress by increasing activities of antioxidant enzymes such as SOD, APx, GR and CAT and elevating levels of oxidized and reduced ascorbate (DAsA and AsA) at higher salinity. They also maintained a low redox ratio of ascorbate/dehydro-ascorbate and therefore a high capacity to manage oxidative stress. Thus *P. antidotale* is capable of managing ROS stress at high salinity and therefore can be sustainably grown as a fodder crop in saline arid regions.

Introduction

Soil salinity is one of the most threatening abiotic factors especially for arid and semiarid regions where it decreases conventional agricultural productivity (Gleick et al., 2011) through reducing growth and survival of plants (Shi and Wang, 2005; Sobhanian et al., 2010). Halophytes have adapted to counter harmful effects of soil salinity and are able to complete their life cycle in saline habitats (Flowers and Colmer, 2008; Munns et al., 2010). The utilization of salt resistant plants as non-conventional alternate sources of food, fodder, wood, medicine, oil, biofuel and industrial raw material, and for land reclamation and ornamental purposes is well established (Abideen et al., 2012; Khan et al., 2009; Qasim et al., 2010). *Panicum antidotale* has a considerable agrarian potential to be used as cattle feed and it can be grown with saline water irrigation of salinized and/or waste land. The plant produces about 60 t ha^{-1} $year^{-1}$ fresh biomass on saline soils with brackish water irrigation and can be used to replace maize as cattle feed (Khan et al., 2009). However, saline water irrigation requires precision, and accurate knowledge about sustainable growth conditions needs to be assured. Therefore this study develops arguments regarding underlying mechanisms of salt resistance of *Panicum* (Koyro et al., 2013) up to levels of seawater salinity.

Hyperosmotic salinity limits plant growth primarily by low water availability (osmotic effect) and high salt concentrations (ionic effect) (Munns and Tester, 2008). The scarcity of water can reduce leaf cell expansion and stomatal conductance and is frequently accompanied by a reduced CO_2 fixation. Latter one can contribute to oxidative stress (Koyro et al., 2013). Halophytes reduce the osmotic potential by absorbing Na^+ and they avoid its toxicity by compartmentalizing Na^+ in the vacuole (Lee et al., 2007). High concentrations of Na^+ in the medium may alter the transport and uptake of K^+ (Marcum, 2008; Zhou and Yu, 2009) leading to K^+ starvation and/or $[K^+]/[Na^+]$ imbalance (Ahmed et al., 2013; Hauser

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and Horie, 2010). However, Na^+ toxicity and/or K^+ deficiency in the cytoplasm and its organelles (ionic effect) may reduce or inhibit plant growth (Sobhanian et al., 2011) and can be an additional reason for oxidative stress. Down-regulation to low cytoplasmic water potential, ionic homeostasis and the protection of proteins, protein complexes and membranes against oxidative stress are frequently ensured for instance by the accumulation of compatible organic solutes, e.g. amino acids (proline and citrulline), onium compounds (glycinebetaine, 3-dimethylsulfonoproprionate), carbohydrates and polyols (Subudhi and Baisakh, 2011; Verbruggen and Hermans, 2008). Some of these compatible osmotic substances such as proline act as ROS scavenger because they use protons during their synthesis and therefore help in reducing oxidative stress (Kocsy et al., 2013). The reduction of CO_2 assimilation via ionic and osmotic effects can disturb this balance due to over-reduction of the photosynthetic electron transport chain. Consequently production of active oxygen species (ROS) such as superoxide or hydrogen peroxide may be stimulated (Koyro et al., 2013).

ROS have a high affinity to react with proteins, lipids and nucleic acids and cause the malfunctioning of these macromolecules (Kocsy et al., 2013). However, several antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidases (APx) and glutathione reductase (GR) along with non-enzymatic ROS scavengers like nitrate, ascorbate, glutathione, carotenoids and tocopherols can detoxify ROS (Jithesh et al., 2006). A strong antioxidant response mechanism is of vital significance for plants coping with low soil water potential, hyperosmotic salinity and nutrient imbalance (Jithesh et al., 2006). It was shown for instance that the excessive production of ROS in *P. antidotale* at high salinity can lead to an increased lipid peroxidation of membranes causing leakage (Koyro et al., 2013).

The aim of this study was to determine the effect of salinity on growth, mineral nutrition, water relations, compatible solutes, and the antioxidant system in *P. antidotale*. These information will be used to analyze interactive responses on salinity constraints.

Materials and methods

Plant material and treatments

Surface sterilized seeds of *Panicum antidotale* (wild type) were germinated in soil type LD 80 (Fa. Archut, Vechta, Germany) in an environmentally controlled green house (Giessen, Germany). After 2 weeks young seedlings were transplanted to a soil less (gravel/hydroponics) quick check system (Koyro 2006). The plants were irrigated with a basic nutrient solution as modified by Epstein (1972) under 16 h light/8 h dark photoperiod for 3 more weeks. Temperatures were $27 \pm 2\,^{\circ}C$ during the day and $17 \pm 2\,^{\circ}C$ during the night. Relative humidity ranged from 45 to 65%. Irradiation intensity was in the range of 190 $\mu mol\, m^{-2}\, s^{-1}$ at the plant level. NaCl concentrations increased daily stepwise by 50 mM NaCl (25 mM each at the beginning and at the end of the light period daily) until the final concentration was achieved after 2 weeks: 0 (control), 125, 250, 375 and 500 mM NaCl. Plants were irrigated at 4 h intervals for half an hour every day and solutions were allowed to drain freely from the pots. Solutions were changed every 2 weeks to maintain nutrient levels. The experiment was conducted for a total period of 12 weeks.

Growth measurements

Three plants were harvested after 5 weeks of the NaCl treatment and were divided into three parts, leaves (juvenile leaves–upper 4 to 5 nodes, and adult leaves–lower 4 to 5 nodes from top), stem and root. The shoot length, number of leaves and leaf area were measured. Plants were dried in an oven at 70 °C until constant weight was obtained.

Water relations

Water potential was measured on intact leaves by the dew point method with HR-33 T Dew Point Microvolt meter, using a L-51-SF leaf chamber (Wescor, USA). Relative water content (RWC) was measured using 3 discs of 1 cm diameter from a leaf (avoiding margins and midrib) and fresh weight (FW) was determined (Sharp et al., 1990). Discs were placed in 1.5 ml of deionized water for 4 h at 4 °C to let the tissue absorb water. Thereafter weight of the discs was termed as turgid fresh weight (TFW). Tissues were dried at 70 °C for 48 h and dry weight (DW) was determined. RWC (%) was calculated as:

$$RWC\,(\%) = \frac{FW - DW}{TFW - DW} \times 100$$

Proline and sugar determination

Proline was estimated by using powdered plant material (50 mg) which was mixed with 4 ml of 3% sulphosalicylic acid and sonicated at 30 °C for 15 min and then centrifuged at 3000 × *g*. This extract was used for measuring proline content. One ml acid ninhydrin and 1 ml of glacial acetic acid were added to the reaction mixture which was boiled at 100 °C for 1 h. The reaction was terminated in ice and 2 ml of toluene were added to the reaction mixture which was vortexed for exactly 30 s. The absorbance of the upper phase was measured at 520 nm and proline concentration was estimated against a standard curve using L-proline (Bates et al., 1973). Soluble sugars were determined by the anthrone method (Ludwig and Goldberg, 1956). Press sap was used for measuring sugars. Samples were heated for 11 min with anthrone reagent (in 95% H_2SO_4) and the reaction was terminated in ice. The absorbance was recorded at 630 nm and sugars were estimated by using sucrose as a standard.

Cations, carbon and nitrogen analysis

200 mg of dried leaves (adult and juvenile), stem and root were ashed in an oven at 600 °C for 8 h. The ash was dissolved in 20% HNO_3 on Bunsen burner at gentle flame. The mixture was filtered through ash-less filter paper (Whatman no. 40). Na^+, K^+, Ca^{++} and Mg^{++} were measured with an atomic absorption spectrophotometer (AAS 2100, PerkinElmer). The carbon and nitrogen content was determined in powdered dry plant material using an element analyzer VarioMAX CNS (Elementar Analysen Systeme GmbH, Hanau, Germany).

Protein extraction

Leaf tissues (0.5 g) were ground to a fine powder in liquid N_2 and then homogenized in 5 ml of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, 1 mM ascorbic acid, 2% (w/v) PVPP and 0.05% (w/v) Triton X-100 using a chilled pestle and mortar (Gossett et al., 1994). The homogenate was centrifuged at 12,000 × *g* for 20 min at 4 °C and the supernatants were collected and used for the assays of catalase, ascorbate peroxidase, glutathione reductase and superoxide dismutase. Protein concentrations in the enzyme extract were determined by Bradford (1976) method and bovine serum albumin (BSA) was used as standard.