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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.

### Enzyme assays

Catalase (CAT, EC 1.11.1.6) activity was determined using an assay mixture containing 50 mM potassium phosphate buffer (pH 7.0), 25 mM $H_2O_2$ and 50 μl enzyme extract in a reaction mixture of 3 ml and measuring the OD at 240 nm (Aebi, 1984). This assay measures the rate of consumption of $H_2O_2$ ($\epsilon = 39.4\ mM^{-1}\ cm^{-1}$). The reaction mixture for determining ascorbate peroxidase (APx, EC 1.11.1.11) activity consisted of 50 mM potassium phosphate (pH 7.0), 0.2 mM EDTA, 0.5 mM ascorbate ($\epsilon = 2.8\ mM^{-1}\ cm^{-1}$), 2 mM $H_2O_2$ and 50 μl enzyme extract with a final volume of 3 ml. The absorbance was measured at 290 nm (Nakano and Asada, 1981). Glutathione reductase (GR, EC 1.6.4.2) activity was determined following oxidation of NADPH at 340 nm (Foyer and Halliwell, 1976) in a mixture (final volume of 3 ml) containing 100 mM Tris-HCl (pH 7.8), 0.5 mM GSSG, 0.03 mM NADPH ($\epsilon = 6.2\ mM^{-1}\ cm^{-1}$), 5 mM EDTA and 100 μl enzyme extract. To determine the activity of superoxide dismutase (SOD), a reaction mixture was used which contained 10 mM of L-methionine, 0.05 mM of nitroblue-tetrazolium salt and 0.75% of Triton X-100 in 50 mM potassium phosphate pH 7.8 (Beyer and Fridovich, 1987). One ml of the above reagent was added in a glass tube followed by 40 μL of enzyme extract and 10 μL of 0.12 mM riboflavin. After thorough mixing the solution was put under a 40 W fluorescent tube for 7 min before the measurement of absorbance at 560 nm against a blank kept in dark. The enzyme activity was calculated as the percentage inhibition per minute.

### Ascorbate determination

The determination of ascorbate (AsA) and dehydroascorbate (DAsA) was carried out by dipyridyl assay based on the reduction of $Fe^{3+}$ to $Fe^{2+}$ by ascorbic acid (Kampfenkel et al., 1995). Half g of fresh leaf material was ground to fine powder in liquid nitrogen and homogenized in 2 ml pre-chilled 6% TCA. The mixture was centrifuged at 16,000 × *g* for 20 min at 4 °C and the supernatant was collected. Ascorbate was determined by adding 0.2 ml supernatant to the reaction mixture, 0.6 ml Na-phosphate buffer (pH 7.4; 200 mM), 0.2 ml deionized water, 1 ml 10% TCA, 0.8 ml phosphoric acid (42%), 2,2′-dipyridyl (4%) and finally adding the freshly prepared 0.4 ml of 3% ferric chloride immediately followed by vigorous mixing. All samples were incubated in a water bath maintained at 42 °C for 40 min. and then 10 mM dithiothertol (DTT) was added to reduce DAsA to AsA. Subsequently excess DTT was removed with 0.5% N-ethyl maleimide (NEM). Absorption of the $Fe^{+2}$-dipyridyl complex was recorded at 525 nm. A standard curve was prepared for the estimation of total ascorbate (with pretreatment DTT) and DAsA (subtracting AsA from total ascorbate).

### Statistical analysis

Data were arcsine transformed before statistical analysis to ensure homogeneity of variances. Data were analyzed by using SPSS 11.0 for Windows and means of 3 replicates were compared using LSD post-hoc test at the 5% level of significance.

## Results

### *Growth measurements*

Plants grown at low salinity (125 mM NaCl) had a 125% increase in juvenile leaf biomass, while all other growth parameters were similar to that of non-treated control (Figs. 1 and 2). Biomass production of *P. antidotale* along with leaf number, plant height, and

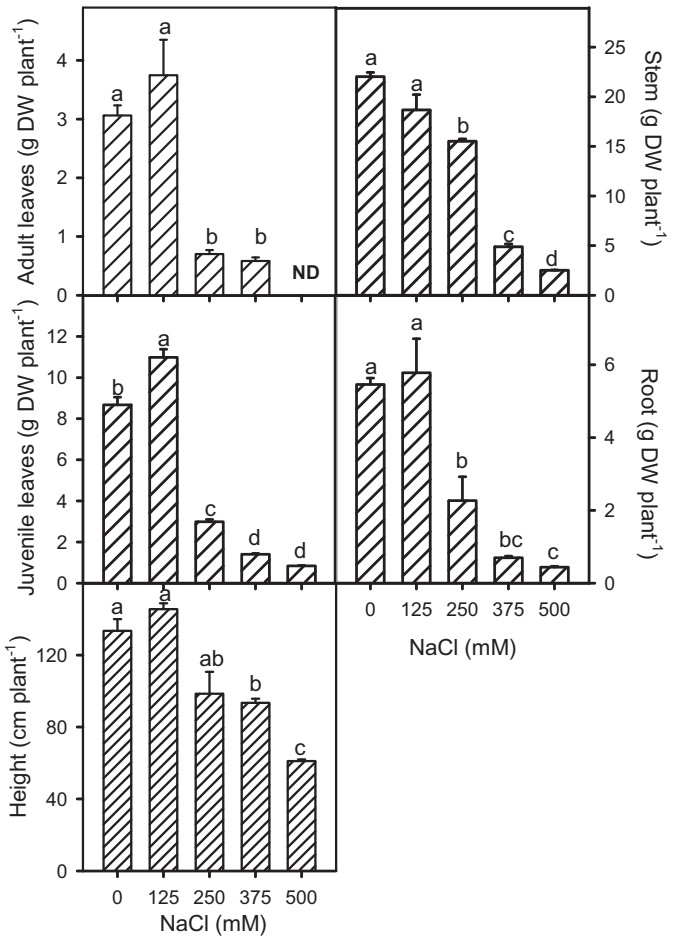


**Fig. 1.** Effect of various NaCl concentrations on biomass of *Panicum antidotale*. Bars represent dry weight of leaves (adult and juvenile), stem, roots and height of the plants. Values represent mean ± SE ($n = 3$) and different letters indicate significant difference between treatments at $P < 0.05$ using LSD Post hoc test. ND: Not determined due to lack of plant material.

leaf area linearly declined with the increase in salinity and a substantial inhibition was recorded at 500 mM NaCl (Figs. 1 and 2).

### *Water relations, soluble sugars and free proline*

Water potential of leaves progressively decreased with the decrease of soil water potential and the increase in NaCl concentrations of the substrate solutions, and was about 5-fold lower at 500 mM NaCl compared to control (Fig. 3). The water potential of the irrigation solutions was higher in the entire range of salt treatments in comparison to leaf water potential (Fig. 3). Relative water content (RWC) remained unaffected up to 375 mM NaCl, whereas it decreased by about 17% at 500 mM NaCl (Fig. 3). Concentration of soluble sugars was substantially increased at or above 375 mM NaCl (Fig. 4). Proline concentration increased significantly in all plant parts at higher salinities and was substantially elevated with respect to control plants in stem followed by roots, juvenile and adult leaves (Fig. 5).

### *Cation, carbon and nitrogen accumulation*

Accumulation of cations in *P. antidotale* varied with the level of salinity and the organ (Table 1). Sodium concentration in plants was higher in all saline treatments in comparison to control. Sodium concentration in root and stem was 7–8-fold higher at 500 mM NaCl treatment compared to control but in juvenile and adult leaves this

**Table 1**
Changes induced by NaCl solutions in cations ($Na^+$, $K^+$, $Ca^{++}$ and $Mg^{++}$) of leaves (adult and juvenile), stem and roots of *Panicum antidotale*. Data represent analysis of 3 independent replicates. Different letters indicate significant difference between treatments at $P < 0.05$ using LSD Post hoc test.

| NaCl (mM) | | | | | | |
|---|---|---|---|---|---|---|
| Ions | Tissues | 0 | 125 | 250 | 375 | 500 |
| $Na^+$ | Ad. leaves | 69.55 ± 10.23c | 242.91 ± 6.00a | 177.47 ± 17.00b | 201.27 ± 29.30a | 266.21 ± 33.88a |
| | Jv. leaves | 63.48 ± 5.54d | 172.29 ± 17.24c | 116.17 ± 8.67b | 215.41 ± 4.13a | 217.86 ± 18.86a |
| | Stem | 37.77 ± 6.35c | 189.46 ± 2.10ab | 164.74 ± 8.91b | 213.56 ± 2.35a | 260.21 ± 68.35a |
| | Root | 22.82 ± 3.56c | 89.63 ± 3.22b | 146.25 ± 9.93b | 207.37 ± 8.98a | 184.43 ± 28.86a |
| $K^+$ | Ad. leaves | 42.4 ± 2.14b | 39.36 ± 1.65b | 41.86 ± 6.27b | 25.59 ± 5.48c | 105.55 ± 11.20a |
| | Jv. leaves | 60.73 ± 2.55b | 95.79 ± 13.76a | 94.21 ± 12.22a | 99.35 ± 7.97a | 115.8 ± 32.74a |
| | Stem | 39.06 ± 1.40b | 23.33 ± 2.89c | 23.93 ± 2.06c | 61.61 ± 1.80a | 55.83 ± 6.58a |
| | Root | 17.18 ± 0.99b | 9.16 ± 1.22c | 17.33 ± 1.76b | 19.5 ± 1.32b | 118.51 ± 1.85a |
| $Ca^{++}$ | Ad. leaves | 23.1 ± 5.64a | 10.52 ± 0.98c | 10.43 ± 0.03c | 9.64 ± 0.78c | 17.3 ± 2.36b |
| | Jv. leaf | 19.53 ± 5.07a | 11.18 ± 1.62b | 9.5 ± 0.92b | 11.77 ± 0.85b | 11.39 ± 1.17b |
| | Stem | 2.1 ± 0.39a | 2.3 ± 0.34a | 3.01 ± 0.95a | 4.24 ± 0.28a | 3.23 ± 0.67a |
| | Root | 5.49 ± 1.06b | 2.57 ± 0.38b | 2.58 ± 0.15b | 4.11 ± 0.26b | 16.57 ± 2.28a |
| $Mg^{++}$ | Ad. leaves | 21.27 ± 1.14b | 20.41 ± 0.84b | 19.75 ± 0.26b | 17.03 ± 1.07b | 32.92 ± 1.47a |
| | Jv. leaf | 23.69 ± 3.32a | 17.24 ± 1.58a | 22.84 ± 0.20a | 21.68 ± 1.57a | 23.26 ± 3.63a |
| | Stem | 15.65 ± 0.87a | 14.1 ± 0.81a | 14.88 ± 0.06a | 13.05 ± 1.59a | 13.78 ± 1.20a |
| | Root | 32.4 ± 0.81a | 14.48 ± 0.53c | 19.77 ± 1.15b | 15.5 ± 0.22c | 22.69 ± 2.80b |

increase was lower (4-fold). At all salinities sodium chloride had increased $K^+$ only in juvenile leaves and at high salinity in adult leaves and roots. $Ca^{++}$ and $Mg^{++}$ showed minor and inconsistent changes in the entire range of salt treatments in leaves and stem while $Ca^{++}$ increased 3-fold in root at 500 mM NaCl (Table 1).

Nitrogen content decreased in stem but remained unchanged in the root and leaves in the entire range of NaCl treatments (Fig. 6). Carbon content was also unchanged (Fig. 6). The C/N ratio of leaves was not influenced by salinity but significantly increased in the stem and root under NaCl salinity (Fig. 6).

### *Antioxidant enzymes and substrates*

All enzyme activities remained unchanged at 125 mM NaCl, however, their activity increased substantially with a further increase in salinity and the highest activity of all enzymes was

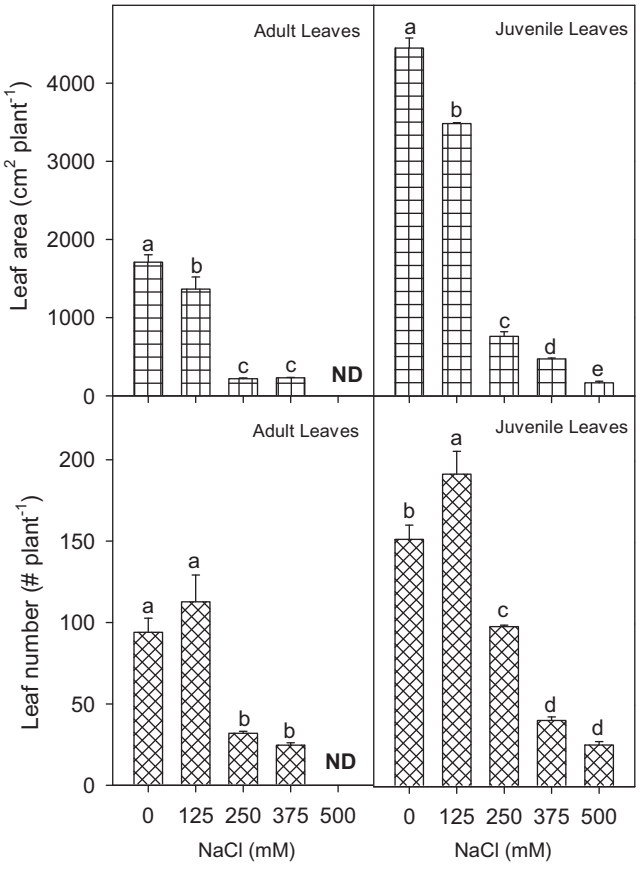


**Fig. 2.** *Panicum antidotale* leaf area and leaf number (adult and juvenile leaves) changes in response to various NaCl concentrations. Other details as for Fig. 1.

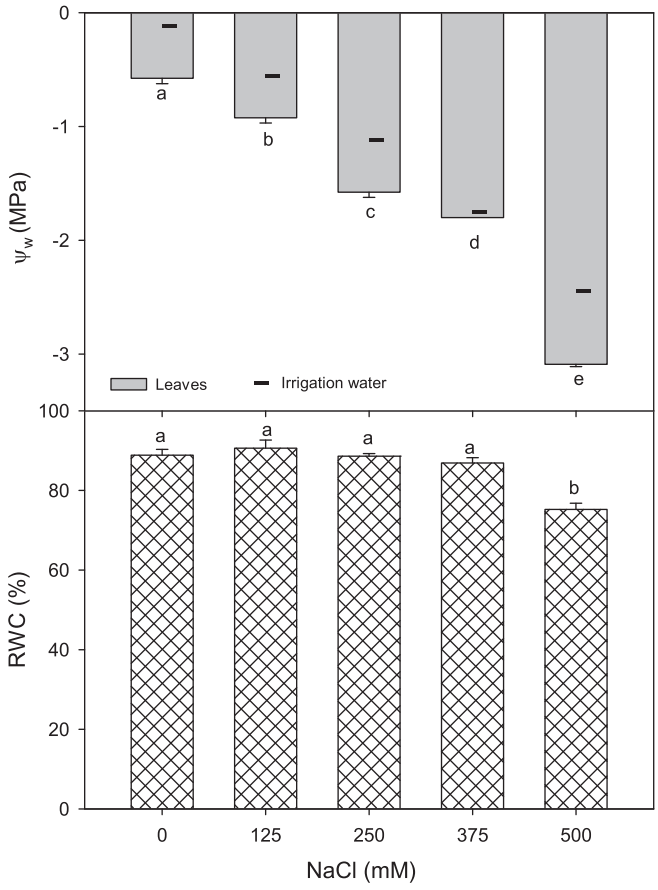


**Fig. 3.** Water potential ($\Psi_W$) and relative water content (RWC) of fully expanded (3rd and 4th node) leaves of *Panicum antidotale* in response to various NaCl concentrations. Other details as for Fig. 1.

**Table 2**
Salt induced changes in the ascorbate pool in fully expanded (3rd and 4th node) leaves of *Panicum antidotale*. Data represent analysis of 3 independent replicates. Different letters indicate significant difference between treatments at $P < 0.05$ using LSD Post hoc test.

| NaCl (mM) | AsA (μmol $g^{-1}$ FW) | DAsA (μmol $g^{-1}$ FW) | Total ascorbate (μmol $g^{-1}$ FW) | AsA/DAsA (μmol $g^{-1}$ FW) |
|---|---|---|---|---|
| 0 | 2.32 ± 0.33d | 1.42 ± 0.08b | 3.75 ± 0.40d | 1.62 ± 0.15 |
| 125 | 3.15 ± 0.17bc | 2.34 ± 0.22ab | 5.49 ± 0.28c | 1.34 ± 0.16 |
| 250 | 3.10 ± 0.15b | 2.24 ± 0.59ab | 5.33 ± 0.65bc | 1.38 ± 0.47 |
| 375 | 3.73 ± 0.12ac | 2.79 ± 0.52a | 6.53 ± 0.41ac | 1.35 ± 0.31 |
| 500 | 3.85 ± 0.07a | 2.99 ± 0.30a | 6.85 ± 0.30a | 1.28 ± 0.13 |

recorded at 500 mM NaCl (Fig. 7). Total ascorbate content increased by 1.8 times compared

to the control in plants exposed to 500 mM NaCl. Similar trends were also observed with respect to reduced and oxidized ascorbate (Table 2). Reduced to oxidized ascorbate ratio was slightly decreased by salinity compared to the control.

## *Discussion*

Biomass production of *Panicum antidotale* at low NaCl concentration (125 mM) was similar to the non-saline control; however, number of juvenile leaves was increased at this concentration. Al-Khateeb (2006) recorded growth stimulation in early seedlings of *P. turgidum* under low salinity (<100 mM NaCl) at a temperature regime of 10–20 °C whereas plants could not survive in 400 mM NaCl. However, in the present study the 12 week old plants were more resistant compared to early seedlings and survived in up to seawater salinity (Fig 1). Previous reports also indicated little growth stimulation in monocotyledonous halophytes at low salinity (Mateos-Naranjo et al., 2013; Yu et al., 2011). However there are exceptions in a few grasses (Bell and O'Leary, 2003; Muscolo et al., 2013; Wang, 2005).

One of the major challenges is to avoid water deficit in plants imposed due to osmotic stress under saline conditions (Flowers and Colmer, 2008). *Panicum antidotale* was able to maintain leaf water potential at all salinities significantly lower than in the soil (Fig. 3). The reduction in area and number of leaves with increase of salinity contributed to minimizing water loss just like the previously reported increase of stomatal resistance (Koyro et al., 2013). This interaction contributed to maintaining leaf turgidity in an acceptable range at the expense of a reduced $CO_2/H_2O$ exchange rate and the subsequent reduction in biomass production (Koyro et al., 2013).

The salt induced decrease in leaf water potential of *P. antidotale* agrees with results observed for other salt resistant grasses such as *Sporobolus virginicus* (Bell and O'Leary, 2003), *Odyssea paucinervis* (Naidoo et al., 2008), *Posidonia oceanica* and *Cymodocea nodosa* (Sandoval-Gil et al., 2012), and *Aeluropus lagopoides* (Ahmed et al., 2013) by a decrease in leaf water content and by an increased accumulation of ions, mainly $Na^+$ (Table 1).

The accumulation of $Na^+$ and $K^+$ ensures a reduction of the osmotic potential which supports the uptake of water from the soil and prevents the plants from drastic effects of water deficit under

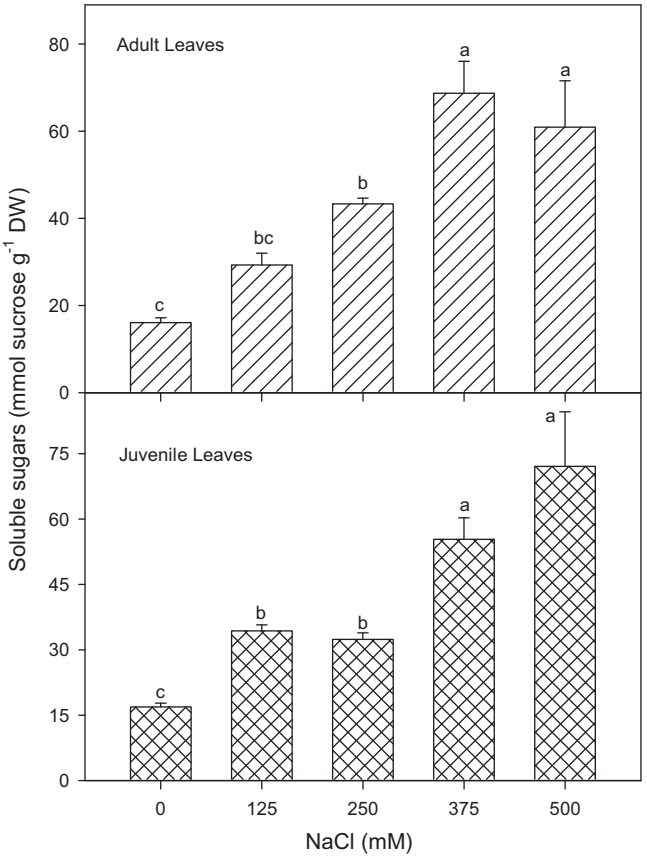


**Fig. 4.** Changes in soluble sugars in adult and juvenile leaves of *Panicum antidotale* induced by various NaCl concentrations. Other details as for Fig. 1.

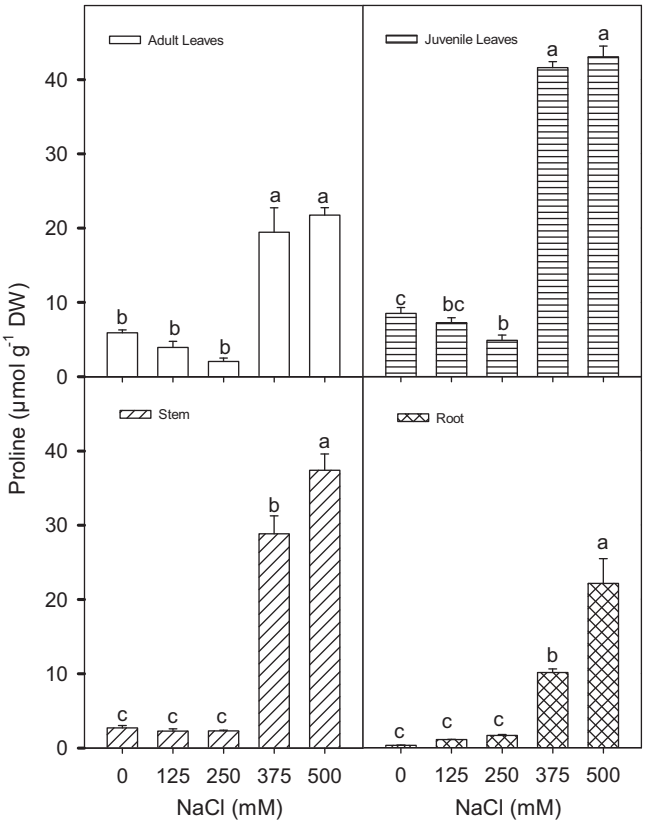


**Fig. 5.** Proline in leaves (adult and juvenile), stem and roots of *Panicum antidotale* under various NaCl concentrations. Other details as for Fig. 1.

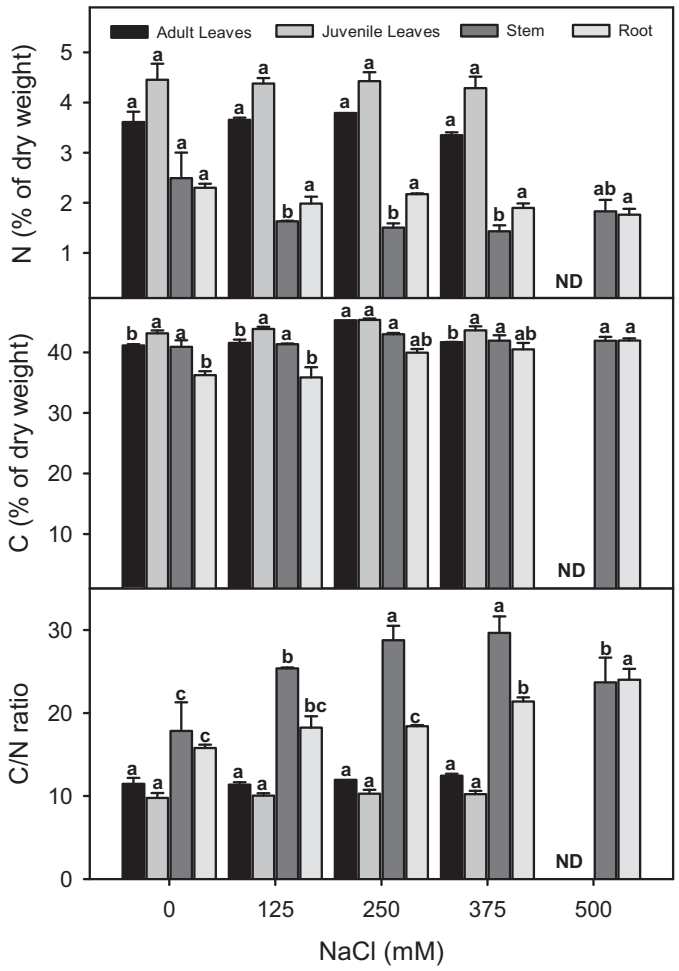


**Fig. 6.** Nitrogen (N), Carbon (C) and C/N ratio in leaves (adult and juvenile), stem and roots of *Panicum antidotale* in response to various NaCl concentrations. Other details as for Fig. 1.

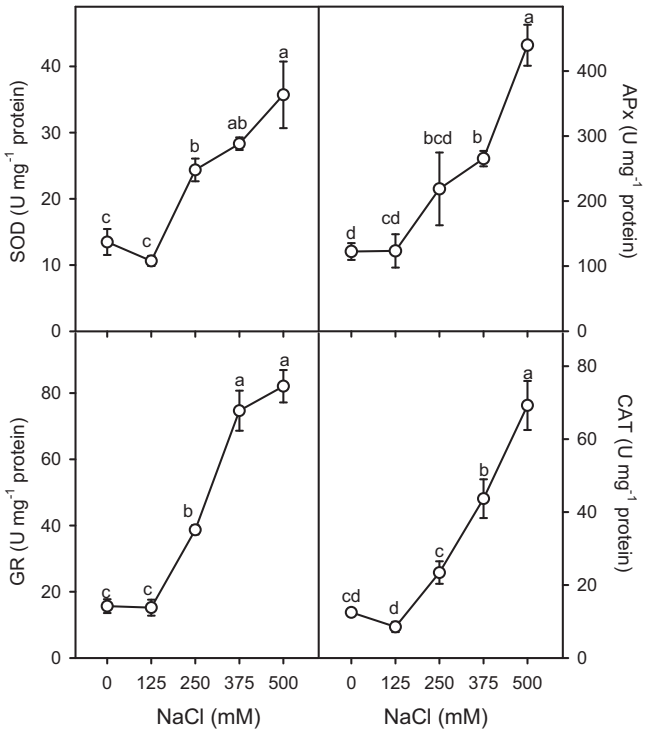


**Fig. 7.** Antioxidant enzymes of *Panicum antidotale* in response to various NaCl concentrations. Superoxide dismutase (SOD), ascorbate peroxidase (APx), glutathione reductase (GR) and catalase (CAT) were measured on fully expanded (3rd and 4th node) leaves of *P. antidotale*. Other details as for Fig. 1.

salinity. High $Na^+$ concentrations may also lead to imbalances in nutrient uptake and alter the transport of $K^+$ leading to starvation (Guo et al., 2012; Marcum, 2008). However, the opposite was true for *P. antidotale*. $K^+$ concentration increased 7-fold at high salinity in roots of *P. antidotale* concomitant with an increase of $Ca^{++}$. Increased $K^+$ and $Ca^{++}$ concentrations in plants treated with high NaCl concentration are reported as a sign of maintenance of ionic balance (Bafeel, 2013; Guo et al., 2012; Hauser and Horie, 2010).

Compatible organic solutes like proline, sugars and sugar alcohols are produced in response to salinity and drought (Zhou and Yu, 2009). High salinities lead to an increase of proline concentration in *P. antidotale* (Fig. 5). Therefore (see also Lee et al., 2007) it may be concluded for *P. antidotale* that it has various vital functions during hyperosmotic salinity, such as channel regulator (Cuin and Shabala, 2007) or as ROS scavenger (Ashraf et al., 2012; Kaul et al., 2008). Presumably, at higher salinities the increased synthesis of proline may contribute to a reduction of ROS production and maintenance of redox homeostasis (Jedy et al., 2014; Verbruggen and Hermans, 2008).

Besides proline, the concentration of total soluble sugars (equivalent to sucrose) was increased up to 4-fold in leaves of *P. antidotale* in response to salinity (from 0 to 500 mM NaCl). This response is in agreement with other reports (Williamson et al., 2002) where carbohydrate (e.g. sucrose) synthesis was related to reduction of the ionic burden. Synthesis of organic osmolytes is a high energy requiring process (Ashraf and Foolad, 2007), therefore halophyte grasses use, if tolerable, inorganic ions (below toxic level) as cheap osmolytes (Lee et al., 2007). Nevertheless, carbohydrates seem to play an important role in *P. antidotale* as compatible osmolytes at higher salinities. However, sugars may also act as signaling molecules, for example by inducing a micro-RNA (miR398) that is considered to be a sucrose modulated regulator of SOD translation (Couee et al., 2006). This is also attested by the fact that sugar concentration in the present study displayed a high correlation ($R^2 = 0.78$) with increased activity of SOD under saline treatments.

Stress-related changes of the carbon and nitrogen content are often connected to the formation of photosynthetic pigments and proteins (Rubisco), and in this combination are commonly used as an index of oxidative stress (Chapin, 1980; Ignatova et al., 2005). Our data showed that C:N ratios were not influenced by NaCl while growth was reduced and redox status (e.g AsA/DAsA redox couple) remained constant under saline conditions. AsA/DAsA redox couple is a well known modulator of enzymes responsible for carbon assimilation under stress conditions (Kocsy et al., 2013). Hence, in the present study, this redox couple may also protect or even trigger carbon assimilation in *P. antidotale* to some extent under hyperosmotic salinity.

Nevertheless high salinity leads to a reduced fixation of $CO_2$ (Jubany-Marí et al., 2010; Sobhanian et al., 2011) that can change the balance between demand and supply of electrons for photosynthesis. This surplus can lead already at the place of origin to structural damage of both membranes and photosynthetic apparatus due to production of reactive oxygen species (ROS) such as $O_2^-$, $OH^-$ or $H_2O_2$ (Koyro et al., 2013). Our data proved that the antioxidant defense system responded to this salinity-related threat by increasing activity of ROS scavenging enzymes (Fig. 7). SOD activity increased significantly in the leaves of *P. antidotale* with increasing salinity in agreement with other reports (Hu et al., 2011; Sobhanian et al., 2011) but in contrast to others indicating no response to hyperosmotic salinity (Sekmen et al., 2007). This is not

astonishing because different interactions between enzymes can lead to the same result – an antioxidant defense (Blokhina et al., 2003; Demidchik, 2015).

The enhanced SOD activity in *P. antidotale* at high salinity corresponds well with the about 2-fold increase in the concentration of $H_2O_2$ (Koyro et al., 2013). Although $H_2O_2$ is a toxic molecule it plays a very important role in signaling (Maruta et al., 2012) and is an important intermediate step in ROS defense. The production of $H_2O_2$ due to hyperosmotic salinity triggers also the activation of the enzymatic (catalase, ascorbate peroxidase and glutathione reductase) and/or non-enzymatic (ascorbate, glutathione, tocopherol, carotenoids etc.) antioxidant system as shown in the present study.

CAT activity increased significantly with an increase in salinity (Fig. 7) and this response is in agreement with results reported for *Pennisetum clandestinum* (Muscolo et al., 2013), *Spartina alterniflora* (Subudhi and Baisakh, 2011) and *Echinochloa crusgalli* (Abogadallah et al., 2010).

APx and GR are key enzymes in the Halliwell-Asada pathway and play a critical role in removal of indigenous $H_2O_2$ (Duarte et al., 2013). In this study, the APx and GR activities in *P. antidotale* were increased up to 4- and 5-fold, respectively, under NaCl salinity. The increased GR activity in plants under saline conditions maintains a high $NADP^+$/NADPH ratio, thereby ensuring the ability of $NADP^+$ to accept electrons from photosynthetic electron transport chain and minimizing ROS accumulation in chloroplasts (Muscolo et al., 2013).

The increased activities of these keys enzymes can be interpreted as a high demand and capacity to eliminate $H_2O_2$ in the leaves of *P. antidotale* under saline conditions. Besides Halliwell-Asada pathway enzymes, ascorbate (oxidized and reduced) and glutathione (oxidized and reduced) substrate can directly scavenge superoxide, hydroxyl radicals, and singlet oxygen (Foyer and Halliwell, 1976; Foyer and Noctor, 2009). The increased level of ascorbate in *P. antidotale* is likely to participate not only as a substrate for APx but also as a ROS scavenger and signaling molecule to trigger the production of abscisic acid (ABA) (Jubany-Marí et al., 2010) and closing of stomata (Chen and Gallie, 2004). The decline in transpiration and stomatal conductance in *P. antidotale* (Koyro et al., 2013) is also supporting the same role of ascorbate with an increase of NaCl concentration in the present study. Along with all these physiological adaptations due to increase in ascorbate, redox homeostasis (e.g AsA/DAsA) is one of the important factors to save cells from ROS toxicity (Foyer and Noctor, 2009). The AsA/DAsA ratio or the availability of AsA are frequently used as indicators for oxidative stress and also discussed as initiators of the plant defense system (Kocsy et al., 2013). High salinity causes a slight decrease of AsA/DAsA ratio in *P. antidotale* (Table 2). This first sign of an imbalance could be attributed to a reduction in $CO_2$ fixation and the synthesis of organic solutes (proline and sugars) leading to a reduction in available energy, disturbing ROS defense and causing growth reduction.

Our results clearly indicate that *P. antidotale* is capable of managing ROS stress at high salinity. This could be attributed to well-regulated water relations, the high effectiveness of $Na^+$ exclusion, a high selective accumulation of nutrients such as $K^+$ and the consequent maintenance of ion homeostasis. *Panicum antidotale* regulates the plant anti-oxidative response by an increase in anti-oxidative enzyme activity and the accumulation of non-enzymatic antioxidants such as ascorbate.

We learned that salt resistance of *P. antidotale* depends on the avoidance of ionic stress and the maintenance and protection of metabolic activity at low water potential. *Panicum antidotale* therefore is capable of managing hyperosmotic salinity and producing biomass given sufficient energy is available. The above mentioned arguments support the idea that *P. antidotale* can be sustainably grown as a fodder crop in saline arid regions where soil salinity is often too high to grow any conventional crop.

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