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ORIGINAL ARTICLE

Salt and drought tolerance of sugarcane under iso-osmotic salt and water stress: growth, osmolytes accumulation, and antioxidant defense

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In order to discriminate between the ionic and osmotic components of salt stress, sugarcane (*Saccharum officinarum* L. cv. Co 86032) plants were treated with salt-NaCl or polyethylene glycol-PEG 8000 solutions (-0.7 MPa) for 15 days. Both the salt and PEG treatments significantly reduced leaf width, number of green leaves, and chlorophyll stability index. Osmotic adjustment (OA) indicated that both the stresses led to significant accumulation of osmolytes and sugars. Salt stressed plants appeared to use salt as an osmoticum while the PEG stressed plants showed an accumulation of sugars. Oxidative damage to membranes was not severe in plants subjected to salt or PEG stress. The salt stressed plants showed an increase in the activities of superoxide dismutase (SOD) and ascorbate peroxidase (APX), while PEG stress led to an increase in SOD but not APX activity as compared to the control. Thus, results indicate that the iso-osmotic salt or PEG stress led to differential responses in plants especially with respect to growth, OA, and antioxidant enzyme activities.

Keywords: sugarcane; iso-osmotic; salt; PEG; osmotic adjustment; oxidative damage; antioxidant enzymes

Introduction

Salinity and drought are important environmental factors that limit crop productivity mainly due to alterations in water relations, ionic, and metabolic perturbations; generation of reactive oxygen species (ROS); and tissue damage. Plant growth arrest is the first symptom observed in plants exposed to salt stress and can be considered as a mechanism to preserve carbohydrates for sustained metabolism, prolonged energy supply, and for better recovery after stress relief (Bartels and Sunkar 2005). Shoot growth is more affected than root growth, and continuation of root growth under stress is an adaptive mechanism that facilitates water uptake from deeper soil layers. In addition, emergence of new leaves is slower and the older leaves show early senescence. These symptoms arise due to the osmotic effect of salt stress and are similar to those observed on exposure of plants to dehydration stress imposed by withholding water or by treatment with PEG. The leaves of plants subjected to salt stress also show succulence, which is mainly attributed to increased vacuole size in leaves that accumulate salt. This feature is not seen in plants subjected to dehydration stress (Munns and Tester 2008).

Plants have evolved complex mechanisms for avoiding the osmotic effects of salt and drought stress, one of them being osmotic adjustment (OA; lowering of osmotic potential, ψP, in plant tissues through accumulation of osmolytes that maintain flow of water into cells). Two types of osmolytes, organic solutes and inorganic ions, play a key role in osmotic adjustment. Organic solutes – known as compatible solutes include sugars, proline, polyols, quarternary ammonium compounds like glycine betaine, and other low molecular weight metabolites – serve a function in cells to lower or balance the osmotic potential of intracellular and extracellular ions to tolerate osmotic stresses. Inorganic ions mainly Na^+, K^+, Ca^{2+}, and Cl^- also make great contribution in osmotic adjustment (Chen and Jiang 2010). The organic compatible solutes besides OA, play a role in stabilization of enzymes/proteins and in protection of membrane integrity (Yancey et al. 1982; Bohnert and Jensen 1996; Yeo 1998); however, its synthesis occurs at an energy cost (Raven 1985) and may be one of the causes of decrease in plant growth. The accumulated ions as an osmoticum, needs to be stored in the vacuoles, to prevent ion toxicity. This leads to water inflow into the vacuoles (succulence) from the cytoplasm, which is compensated by accumulation of compatible solutes in the cytoplasm, thereby permitting water uptake from the apoplast into the cytoplasm. Thus, plants exposed to salt stress may use saline ions as an osmoticum; however, synthesis of

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compatible solute is also required to prevent ion toxicity, while plants exposed to dehydration stress solely rely on synthesis of compatible solutes for maintenance of cell turgor (Munns and Tester 2008; Lokhande et al. 2010).

Generation of ROS is observed as an outcome of the metabolic perturbations caused by osmotic effects of salt or dehydration stress as well as ionic toxicity of salt stress (Neill et al. 2002; Imlay 2003). Overproduction of ROS leads to oxidative damage to the biomolecules in cells (Imlay 2003) or even cell death (Jones 2000). At least part of the membrane damage is caused by lipid peroxidation resulting from the uncontrolled ROS production (Rodriquez-Rosales et al. 1999). Measurement of the concentration of thiobarbituric acid reactive substances (TBARS) such as malondialdehyde (MDA) is routinely used as an index of lipid peroxidation under stress conditions (Lokhande et al. 2011). The harmful effects of ROS are prevented by the presence of lipid soluble antioxidants (α-tocopherol and carotenoids), water-soluble reductants (glutathione and ascorbate), and antioxidant enzymes such as catalase (CAT, EC 1.11.1.6), ascorbate peroxidase (APX, EC 1.11.1.11), and superoxide dismutase (SOD, EC 1.15.1.1) present in plant cells (Desikan et al. 2004). Some of the osmolytes that accumulate in plant cells in response to stress also play a role in scavenging of free radicals and protecting enzymes (Sharma and Dubey 2005).

Sugarcane (*Saccharum officinarum* L.) is a major sugar producing plant. It is also a high biomass producer and consumes large amounts of water and nutrients from the soil for achieving maximum productivity. Sugarcane is a glycophyte; reported to be salt sensitive; and exhibits toxicity symptoms, low sprout emergence, nutritional imbalance, and overall growth reduction leading to low biomass (Wahid 2004). Salinity effects on sugarcane have been studied at the physiological level (Kumar et al. 1994; Wahid et al. 1997; Wahid 2004; Patade et al. 2008). Errabii et al. (2007) has analyzed effects of salt and mannitol stress on sugarcane calli in relation to growth, ions, and proline concentrations. Patade et al. (2008) reported growth reduction in sugarcane calli exposed to lethal salt concentrations, which might either be due to additive or individualistic effects of osmotic and toxic components of salinity. However, which of the two is more causative is not yet clear. In addition, a distinction between osmotic or ionic effects has not been made at the plant or cellular level. In order to distinguish the osmotic and ionic effects arising due to salt stress, sugarcane plants at the formative growth stage were subjected to iso-osmotic levels of PEG (which would impose only osmotic stress), and a comparative analysis of the changes in the growth, ions, osmolytes, and activities of antioxidant enzymes was carried out.

Materials and methods

Plant materials

Single eye buds of 11-month-old sugarcane cv. Co 86032 were sown keeping the buds facing upward in plastic pots (20 cm diameter; 40 cm height) filled with fine sand. The pots were kept in cage house under natural sunlight and watered daily with distilled water. Four weeks post-planting, the plantlets were irrigated with ½ MS (Murashige and Skoog 1962) salts instead of distilled water.

Plant stress treatment

Treatment of salt (NaCl, 150 mM) and PEG-8000 (20% w/v) in ½ MS liquid medium was initiated 150 days after sowing. The control plantlets received equal volume of ½ MS liquid medium. For each treatment, the respective solutions were added to trays in which the plastic pots were placed. The evapo-transpirational losses were replenished with distilled water twice a day. The solutions were changed every three days. The second leaf from each plant was harvested after 15 days of the stress treatments and all the analyses were performed in quadruplet.

The morphological observations were recorded on four plants ($n = 4$) before and at the end of each treatment, in terms of plant height (cm), number of green leaves, and leaf width (mm). The fresh weight (FW) and length of shoot as well as root was measured at the end of the stress treatment. The plants were removed from the pots and the roots were first cleaned of the sand and then washed with deionized water. Fresh weight was measured after absorbing the water on the roots with tissue paper. Dry weight (DW) was determined after drying the samples for 48 hrs in the hot air oven at 70°C and water content (WC) was calculated.

At the end of 15 days, the stress treatment was stopped and the plantlets were grown in the same way as the controls, to study the extent of recovery after the iso-osmotic stress exposure.

Total chlorophyll and carotenoid content

Chlorophyll (a, b, and total) and total carotenoids (xanthophylls + β-carotene) concentrations were determined from leaf material (200 mg FW) ground in a pre-chilled mortar in acetone (80% v/v). After complete extraction, the mixture was filtered and the volume was adjusted to 10 mL with cold acetone. The absorbance of the extract was measured at 664, 647, and 470 nm using a spectrophotometer (UV-1700 PharmaSpec, Shimadzu, Japan) and the pigment concentrations were calculated according to Lichtenthaler (1987). The chlorophyll stability indices (CSI) were measured using the formula:

Total chlorophyll content in stressed leaves/total chlorophyll content in control leaves × 100.

Plant water relations

The leaf relative water content (RWC) was determined from the second leaf of four independent plants per treatment. Fresh weight was recorded immediately after harvesting the leaf from the main stem. The turgid weight (TW) was measured after 24 hrs saturation (when leaf weight reached a plateau) with deionized water in the dark. Dry weight was determined after drying the leaves for 48 hrs in the hot air oven at 70°C. The RWC was calculated as:

$$[(FW - DW)/(TW - DW)] \times 100.$$

Desiccated weight (DSW) was recorded after incubating the leaf and root samples at 25°C; 65% relative humidity (RH) for 24 hrs. Leaf and root water retention ability (WRA) was calculated by using formula:

$$WRA\ (\%) = [(DSW - DRW)/(FW - DRW)] \times 100.$$

Ionic concentration and total dissolved solutes (TDS)

The ionic concentration in the sap was determined by measuring the electrolytic conductivity of diluted sap. For this, the sap collected as supernatant after centrifuging the homogenized leaf samples. The sap was diluted to 10 mL with MilliQ water and electrical conductivity (EC, $mScm^{-1}$) was measured with a portable EC/TDS meter (HANNA portable pH/conductivity/TDS meter; HI-9812; Sigma-Aldrich, US). The TDS were measured with the diluted sap using the same instrument.

Estimation of total soluble sugars

Total soluble sugars (TSS) were estimated using Anthrone reagent (Watanabe et al. 2000) with slight modification. Samples (200 mg) were homogenized in 10 mL of ethanol (80% v/v). The extract was centrifuged at 6000 rpm for 10 min at 4°C. Supernatant (1 mL) was mixed with freshly prepared Anthrone reagent (3 mL) and the tubes heated at 100°C for 10 min. The reaction was terminated by quick cooling on ice. The absorbance was measured at 620 nm. The total soluble sugars (mg g^{-1} FW) were quantified using glucose as standard.

Estimation of reducing sugars

The sample (100 mg) was homogenized in ethanol (80% v/v, 3 mL). The homogenate was centrifuged at 6000 rpm for 10 min at 4°C and the supernatant was mixed with equal volume of 3, 5-dinitro-salicylic acid (DNSA) reagent. For blank, distilled water was used instead of the supernatant. The reactants were mixed by vortexing and the tubes were placed in a boiling water bath for 10 min after which they were cooled on ice. The absorbance was measured at 540 nm and the reducing sugars content (mg g^{-1}FW) was calculated based on standard curve with glucose.

Proline estimation

Free proline content was determined according to Bates et al. (1973). Leaf samples (200 mg) were homogenized in aqueous sulfosalicylic acid (3% w/v, 12 mL). The filtered homogenate (2 mL) was reacted with equal volume each of acid ninhydrin and acetic acid at 100°C for 1 hr and the reaction was terminated in an ice bath. The reaction mixture was extracted with 4 mL toluene and mixed vigorously with a stirrer for 10–15 s. The chromophore containing toluene was aspirated from the aqueous phase and warmed to room temperature. The absorbance was recorded at 520 nm using toluene as a blank. Proline concentration (μg g^{-1} FW) was determined from a standard curve using L-proline.

Glycine betaine analysis

Glycine betaine content was estimated, according to the method of Grieve and Grattan (1983). The leaf samples (200 mg) were ground in liquid nitrogen, and the finely ground powder was mechanically shaken with 3 mL of deionized water at 25°C for 16 hrs. The samples were filtered, and the thawed extract was diluted (1:1) with 2N H_2SO_4. The extract (500 μl) was cooled on ice for 1 hr and then mixed with 200 μl of potassium iodide-iodine ($KI\text{-}I_2$) reagent. At the end of incubation at 4°C for 16 hrs, the tubes were centrifuged at 10,000 rpm for 15 min at 0°C. The periodide crystals formed were dissolved in 9 mL of 1, 2-dichloroethane, and after 2 hrs the absorbance was measured at 365 nm. Glycine betaine content (μg g^{-1} FW) was quantified from standard curve.

Estimation of lipid peroxidation

Lipid peroxidation was estimated by measuring the concentration of thiobarbituric acid reactive substances (TBARS) as per the method of Heath and Packer (1968) with few modifications. Each sample (400 mg) was ground in liquid nitrogen in a pre-chilled mortar and homogenized in trichloro acetic acid-TCA (5% w/v, 10 mL). Then the extract was centrifuged at 10,000 g for 10 min. To the supernatant, equal volume of 2-thiobarbituric acid (TBA, 0.67% w/v) was added and the mixture was heated to 100°C for 30 min. The reaction was terminated by quickly cooling on ice. Absorbance was read at 532 nm keeping TBA (0.25% w/v) in TCA (10% w/v) as blank, after centrifugation at 5000 g for 1 min. A correction of non-specific turbidity was made by subtracting the absorbance value taken at 600 nm. The MDA content was

expressed as μmol TBARS g^{-1} FW by using an extinction coefficient ($\varepsilon = 155\ mM^{-1}\ cm^{-1}$).

Membrane damage rate

Leaf pieces (1 cm^2) after washing with distilled water were transferred to glass culture tubes containing 10 mL distilled water and incubated for 24 hrs with intermittent shaking. At the end of incubation, initial EC reading (EC_1) of the bathing solution was recorded. Tubes were capped and then autoclaved at 121°C for 20 min to completely kill the tissues and release all electrolytes. EC reading (EC_2) was recorded after cooling the solution to the RT. Leaf membrane damage rate (MDR) was calculated using the formula (Lutts et al. 1995):

$$MDR\ (\%) = (EC_1/EC_2) \times 100.$$

Antioxidant enzyme assays

Extraction

The leaf (200 mg) samples were used for enzyme analysis. All the steps in the preparation of the enzyme extract were carried out at 4°C. The samples were homogenized in 3 mL ice cold 50 mM sodium phosphate buffer (pH 7.0) including 0.1 mM EDTA and polyvinyl pyrrolidone (PVP 1% w/v) in pre-chilled mortar and pestle. The homogenate was centrifuged at 15,000 rpm for 20 min at 4°C. The supernatant was used as a crude enzyme extract for the antioxidant enzyme assays. An aliquot of the extract was used to determine its protein content by the method of Bradford (1976) utilizing bovine serum albumin as the standard. All the enzyme assays were performed at room temperature and the activities of the enzymes were determined with a spectrophotometer.

Superoxide dismutase (SOD) assay

The specific SOD activity was assayed in terms of inhibition of the photochemical reduction of NBT-nitroblue tetrazolium (Beyer and Fridovich 1987). The reaction mixture (3 mL) consisted of 50 mM phosphate buffer (pH 7.8) and 0.1 mM EDTA to which a superoxide generating system containing 14.3 mM methionine, 82.5 μM NBT, and 2.2 μM riboflavin was added. The reaction was initiated by adding 100 μl of crude enzyme. Free radical induced NBT reduction was measured in the reaction medium containing all the ingredients except enzyme. The tubes were kept 30 cm below a light source (six 15 W fluorescent tubes) for 30 min. The reaction was stopped by switching off the light. All the reactants along with 100 μl enzyme extract were incubated in dark as the dark blank. The reduction of NBT was measured by monitoring the change in absorbance at 560 nm. The readings of dark blank were used in calculation of enzyme units. One U SOD enzyme was defined as the amount of enzyme that brings about 50% inhibition of NBT reduction under the assay conditions. Enzyme activity was expressed as units mg^{-1} protein.

Catalase (CAT) assay

CAT activity was measured by following the decomposition of hydrogen peroxide as described by Cakmak and Marschner (1992) with some modifications. The activity was measured in a reaction mixture (1 mL) containing 50 mM phosphate buffer (pH 7.0) and 15 mM H_2O_2. The reaction was initiated by adding 50 μl enzyme extract and the activity was determined by monitoring decrease in absorbance at 240 nm ($\varepsilon = 36\ mM^{-1}\ cm^{-1}$) for 2 min at intervals of 15 s. The slope of the rate assay (ΔA) was used to determine the enzyme activity, which was expressed as mKat mg^{-1} protein.

Ascorbate peroxidase (APX) assay

APX activity was determined according to Nakano and Asada (1981). The reaction mixture (a total volume of 2 mL) consisted of 50 mM (pH 7.0) sodium phosphate buffer, 0.1 mM EDTA, 0.25 mM ascorbate, 1 mM H_2O_2 (hydrogen peroxide), and 100 μl enzyme extract. The H_2O_2-dependent oxidation of ascorbate was followed by a decrease in the absorbance at 290 nm ($\varepsilon = 2.8\ mM^{-1}\ cm^{-1}$). APX activity was measured in terms of mKat mg^{-1} protein.

Statistical analyses

The treatments and controls of the experiments were replicated four times. CropStat for Windows (7.2.2007.2 module), developed by the Biometrics unit, IRRI, Philippines was used for analysis of variance (ANOVA) for experiments laid out in Completely Randomized Design (CRD). The treatment means were compared by Least Significant Difference (LSD) Test at a significance level of $P \leq 0.05$.

Results

Growth responses of NaCl or iso-osmotic PEG stress

Shoot dry weight was significantly lower (by 40%) in salt stressed sugarcane plants as compared to the control plants; however, it did not differ significantly in plants stressed with 20% w/v PEG (Table 1). Significant differences for root dry weight and shoot and root lengths were not observed in the stressed plants as compared to the control. However, the salt or PEG treatments decreased significantly the leaf width and number of green leaves (Table 1).

Chlorophyll content was significantly reduced in salt (by 10%) or PEG (by 40%) stressed plants as compared to control plants (Table 1). The total carotenoids content also decreased significantly in

Table 1. Effect of 15 days iso-osmotic (–0.70 MPa), NaCl (150 mM), or PEG-8000 (20% w/v) stress on growth responses in sugarcane plants. Different letters in each column indicate significant differences at $P \leq 0.05$, as determined using Least Significant Difference (LSD) test. Standard error of the three treatment means, SE (M) and LSD values for each parameter are given in the last row.

Treatment	Shoot dry weight (g)	Root dry weight (g)	Shoot length (cm)	No. of green leaves	Leaf width (mm)	Root length (cm)	Total chlorophyll (μg g^{-1} FW)	Carotenoids content (μg g^{-1} FW)	Chlorophyll stability index	Protein content (mg g^{-1} FW)
Control	5.77	0.52	152.83	5.00	19.67	2.52	1049.74	166.21	100.0	4.62
NaCl (150 mM)	3.44	0.29	144.33	4.00	14.67	1.93	947.77	157.82	90.3	3.34
PEG (20% w/v)	4.67	0.62	137.83	3.33	15.67	2.12	633.28	116.4	60.3	3.47
SE (M)	0.35	0.11	6.13	0.19	0.82	0.20	3.95	1.02	0.4	0.03
LSD	1.37	0.44	24.04	0.75	3.2	0.78	15.5	3.98	1.81	0.13

salt (158 μg g^{-1} FW) and PEG (116 μg g^{-1} FW) treatments as compared to the control (166 μg g^{-1} FW) plants. The salt or PEG stress treatments resulted in significant reduction in chlorophyll stability index by 10 and 40% of the control, respectively (Table 1). Significant reduction in total soluble protein content was also observed in salt or PEG stressed plants as compared to the control (Table 1).

Osmotic adjustment in response to the NaCl or iso-osmotic PEG stress

The leaf RWC was significantly low in salt (89%) or PEG (90%) treated plants as compared to the control (93%). There were insignificant differences for shoot and root WRA in the salt or PEG treated plants as compared to the control (Table 2). Electrolytic conductivity of the leaf sap was significantly more in salt (by 33%) or PEG (by 17%) stressed plants than the control. The salt stressed plants accumulated significantly more total dissolved solutes as compared to the PEG stressed and control plants (Table 2). The PEG stressed plants accumulated significantly more total soluble and reducing sugars as compared to the salt stressed and control plants (Table 2). The salt or PEG stressed plants accumulated more than four-fold free proline as compared to the control. Similarly, the glycine betaine accumulation was also significantly more in salt (by 23%) or PEG (by 16%) stressed plants as compared to the control plants (Table 2).

Antioxidant metabolism in response to the NaCl or iso-osmotic PEG stress

Lipid peroxidation was significantly higher in salt (by 13%) or PEG (by 9%) stressed plants as compared to the control plants (Table 3). However, there were insignificant differences for the membrane damage rate in the stressed plants as compared to the control (Table 3). Statistically significant higher SOD activity was observed in salt (by 32%) or PEG (by 27%) stressed plants over the control. CAT activity did not differ significantly in stressed and control plants (Table 3). The salt stressed plants have significantly more APX activity as compared to the control plants (Table 3).

Discussion

The immediate effects of salt and PEG stresses are osmotic, but their long-term effects (in days) differ. PEG-mediated stress continues to exert osmotic effects on plants, while salt stress effects are largely due to ion toxicity and metabolic imbalance (Munns and Tester 2008). Using iso-osmotic concentrations of PEG and salt, the mechanisms used by plants to avoid or tolerate the respective stress conditions were studied. Sugarcane plants treated with 150 mM NaCl for 15 days showed significant reduction in shoot dry weight, leaf width, and protein content, as compared to the control plants. Plants treated with iso-osmotic PEG concentration for the same period

Table 2. Effect of 15 days iso-osmotic (–0.70 MPa), NaCl (150 mM), or PEG-8000 (20% w/v) stress on osmotic adjustment in sugarcane plants. Different letters in each column indicate significant differences at $P \leq 0.05$, according to Least Significant Difference (LSD) test. Standard error of the three treatment means, SE (M) and LSD values for each parameter are given in the last row.

Treatment	Relative water content (%)	Water retention ability (%)		Electrical conductivity ($mS\ cm^{-1}$)	Total dissolved solutes (ppm)	Total soluble sugars ($mg\ g^{-1}$ FW)	Reducing sugars ($mg\ g^{-1}$ FW)	Proline ($\mu g\ g^{-1}$ FW)	Glycine betaine ($\mu g\ g^{-1}$ FW)
		Shoot	Root						
Control	92.86	92.55	92.5	1500	750	4.06	3.52	8.02	259.75
NaCl (150 mM)	88.96	85.75	95.8	2000	1000	4.38	3.37	36.19	320.64
PEG (20% w/v)	90.01	87.29	96.9	1750	750	7.95	4.05	36.61	302.44
SE (M)	3.40	4.08	1.29	0.00	0.04	0.12	0.11	0.51	6.60
LSD	1.34	15.98	5.07	0.00	0.14	0.46	0.45	2.01	25.87

exhibited severe reduction in chlorophyll stability index as compared to the control and iso-osmotic salt treated plants. Greater inhibitory effects of PEG compared to iso-osmotic NaCl stress have been reported in maize by Chazen et al. (1995). Besides, PEG is also known to cause greater inhibitory effects on water flow through roots, thus leading to the inhibition of leaf growth in whole plants. The greater viscosity (more than four-fold) of PEG 8000 as compared to iso-osmotic NaCl could also explain the long-term inhibitory effect of PEG than NaCl on growth of sugarcane plants. As reported earlier (van der Weele et al. 2000), the low ψ_w causes a large inhibition of shoot growth while root growth is relatively unaffected or even slightly increased. The relative maintenance of root growth at low ψ_w has been reported in several plants (Hsiao and Xu 2000). Growth reduction under salinity stress in terms of leaf area and length, and dry weight due to accumulation of the toxic saline ions at formative stage has been earlier reported in sugarcane (Wahid and Ghazanfar 2006). Decreased growth rate possibly helps the plant in reducing water use and, hence, can be considered as a stress avoidance mechanism. Reduction in shoot growth and the number of green leaves observed in salt or PEG stressed sugarcane plants could be a possible water conservation mechanism. Severe chlorophyll loss (40%) was also observed in plants exposed to PEG stress, indicating an early onset of senescence, which could be attributed to the redistribution of water to younger leaves. While growth was inhibited in salt stressed plants, they did not show significant senescence as the PEG stressed plants, though the two stresses exerted equivalent osmotic pressure. Hence, growth inhibition in salt stressed plants probably occurred due to ion toxicity rather than dehydration.

At the physiological level, OA is an important aspect of osmotic stress avoidance. The RWC in sugarcane plants subjected to salt or PEG stress was only marginally reduced, indicating that plants could osmotically adjust to the high salt or PEG concentration in the soil and could maintain turgor. Osmotic adjustment in response to salt or PEG stress was estimated by measuring the levels of accumulation of soluble sugars, proline, and glycine betaine as well as the ionic concentration in leaf tissues. Salt or PEG stress led to significant accumulation of proline (four-fold higher) and glycine betaine (15 and 23% higher, respectively) in the sampled leaf. Errabii et al. (2007) also reported accumulation of free proline in sugarcane cultured cells in response to salt and iso-osmotic mannitol stress. There was 33% higher accumulation of ionic species due to salt stress (measured in terms of changes in electrolytic conductivity of sap), which was not observed in PEG-stressed plants. On the other hand, PEG-stressed plants accumulated sugars (two-fold higher), which were not observed in the salt stressed plants. Hence, in the absence of salt as an osmoticum, the plants appeared to use sugars for OA,

Table 3. Effect of 15 days iso-osmotic (–0.70 MPa), NaCl (150 mM), or PEG-8000 (20% w/v) stress on oxidative damage in sugarcane plants. Different letters in each column indicate significant differences at $P \leq 0.05$, according to Least Significant Difference (LSD) test. Standard error of the three treatment means, SE (M) and LSD values for each parameter are given in the last row.

Treatment	MDA content (μmol g^{-1} FW)	Membrane damage rate	SOD activity (U mg^{-1} protein)	CAT activity (mKat mg^{-1} protein)	APX activity (μKat mg^{-1} protein)
Control	11.00	22.73	51.27	1.74	5.15
NaCl (150 mM)	12.39	35.52	67.44	2.58	10.43
PEG (20% w/v)	11.99	21.97	64.92	1.65	9.13
SE (M)	0.08	3.70	0.13	0.38	1.01
LSD	0.3	14.49	1.5	4.00	0.49

besides the stress-induced osmolytes like proline and glycine betaine. Accumulation of osmolytes such as proline, glycine betaine, and sugars are known to occur under osmotic stress (Hasegawa et al. 2000; Garg et al. 2002; Munns 2005). However, the synthesis of these compatible solutes demands metabolic energy and thus occurs at the expense of plant growth; hence, this trade-off may be vital for the plant to survive and recover from the stress (Munns and Tester 2008). As accumulation of saline ions is the cheapest form of osmotic adaptation (Raven 1985), the growth penalty was probably lesser in salt stressed sugarcane plants than in PEG stressed plants, enabling better growth under salt but not in PEG stress. Lokhande et al. (2010) attributed higher relative growth rate in NaCl stressed calli of *Sesuvium portulacastrum* than iso-osmotic (–0.7 MPa) PEG stressed calli to osmotic adjustment mainly through accumulation of saline ions. However, the PEG-stressed calli solely relied on synthesis of organic osmolytes (proline, glycine betaine, and soluble sugars). The findings of the present study also support the results.

When stress avoidance mechanisms are insufficient, stress tolerance mechanisms are required to prevent cellular damage arising from dehydration or ion toxicity (Verslues et al. 2006). The extent of damage was compared in leaves of sugarcane subjected to salt or PEG stress. The oxidative damage measured in terms of MDA content and membrane damage rate was relatively more in leaves exposed to salt stress than in PEG stressed plants. This could therefore be attributed to the ion toxicity of salt rather than its dehydration effects. The oxidative damage of membranes was not very significant in salt or PEG stressed plants, indicating an efficient antioxidant system. Salt stressed plants showed an increase in the levels of antioxidant enzymes SOD and APX while PEG stress led to an increase in SOD activities but not APX activity. The SOD dismutates superoxide radicals but generates H_2O_2, which needs to be scavenged through APX (in chloroplasts) and CAT (in cytoplasm) activity. Hence, coordinated activities of these enzymes are required for effective scavenging of ROS (Chagas et al. 2008).

Conclusion

To discriminate between the ionic and osmotic stress impact on plants, short-term effects of iso-osmotic concentrations of salt and PEG were studied in sugarcane. Both the stresses affected growth, however, plants exposed to NaCl appeared to use salt as an osmoticum while those under PEG stress, showed an accumulation of sugars. The antioxidant system was more pronounced under salt stress situation, suggesting that the elevated level of antioxidant defense at least in part, could contribute to salinity tolerance, thus protecting the photosynthetic machinery without affecting plant growth significantly. The metabolic pathways of osmolyte synthesis and antioxidant protection probably contribute to the tolerance response in a coordinated manner. Thus, results of the study suggest that differential responses to the salt and iso-osmotic PEG stress in terms of osmotic adjustment and antioxidant system appears to be the prime defense mechanism for tolerance that occur at the expense of growth. Understanding the specific and differential responses of sugarcane to the ionic and/or osmotic components of salt stress would be an important step in devising strategies for improving salinity tolerance of crop plants.

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