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Comparative Effects of NaCl, PEG and Mannitol Iso-osmotic Stress on Solute Accumulation and Antioxidant Enzyme System in Potato (*Solanum tuberosum* L.)

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ABSTRACT

Osmotic and oxidative stress responses to iso-osmotic (-0.4 Mpa) NaCl, PEG-6000 and mannitol stress were studied in 15-day old plants of three potato cultivars *viz.* 'Kufri Kufri Bahar' 'Jyoti' and 'Chandramukhi'. After 2-weeks of treatment, plants were analyzed for biochemical and physiological determinants of stress. Relative water content (RWC) and membrane damage rate (MDR) were significantly affected in all the cultivars in all the treatments. Decrease in RWC was significant in plants subjected to PEG while significant increase in MDR was observed in NaCl-treated plants. Among the treatments, NaCl treatment showed significant MDR (78.57%) over PEG (69.29%) and mannitol (62.90%) treatments. Proline, glycine betaine and total soluble sugar accumulation increased in the stressed plants than controls. Increased SOD activity was observed under NaCl stress compared to PEG/mannitol stress in all the cultivars. Comparison of different iso-osmotic stresses indicates a positive relationship between proline accumulation and tissue hydration.

Keywords: osmotic stress, osmolytes proline, salinity stress, *Solanum* spp.
Abbreviations: APX, ascorbate peroxidase; **CAT**, catalase; **EDTA**, ethylenediamine tetracetic acid; **GB**, glycine betaine; **GPX**, guaiacol peroxidase; **KI-I_2**, potassium iodide-iodine reagent; **NBT**, nitroblue tetrazolium; **Pro**, proline; **PVP**, polyvinylpyrrolidone; **QAC**, quarternary ammonium compound; **ROS**, reactive oxygen species; **RWC**, relative water content; **SOD**, superoxide dismutase

INTRODUCTION

Drought and salinity are two of the most important abiotic stresses that alter plant water status and severely limit plant growth and development. The stress decreases photosynthetic rate and increases photorespiration rate which then lead to an increased reactive oxygen species (ROS) production (Pardo 2010). The comparison of intracellular changes occurring in response to salt (ionic) or mannitol (non-ionic) iso-osmotic treatments can be a useful to isolate specific effects of salinity or drought, and to identify specific physiological traits associated with tolerance (Lefèvre *et al*. 2001).

During abiotic stress, overproduction of ROS damages macromolecules in cells such as proteins, nucleic acids and lipids (Imlay 2003). In order to prevent excessive accumulation of ROS, plant species are endowed with enzymatic as well as non-enzymatic antioxidative systems. Key enzymes involved in detoxification are superoxide dismutase (SOD EC 1.15.1.1.), catalase (CAT EC 1.11.1.6.) and peroxidases i.e. ascorbate peroxidase (APX EC 1.11.1.11.), Guaiacol peroxidase and glutathione peroxidase (GSH-Px EC 1.11.1.9.) while non-enzymatic antioxidants include small molecules such as ascorbate, glutathione as well as tocopherol, flavonids and carotenoids (Appel and Hirt 2004).

Biochemical response of plant cells to osmotic stress is the accumulation of compatible osmolytes such as soluble sugars (Yamada *et al*. 2010), proline (Pro; Szabados and Savouré 2009) and betaines (Chen and Murata 2008). Generally, plant species accumulate low level of Pro under well watered and non-saline soils, while increased accumulation is observed upon imposition of drought or salt stresses. This increased Pro accumulation may be the result of an enhanced biosynthesis, of a net reduction of its use or from a decreased retro-inhibition of its biosynthesis (Stewart and Hanson 1980). It also acts as a free radical scavenger and may be more important in overcoming stress than in acting as a simple osmolyte (Ramachandra *et al*. 2004; Verslues and Bray 2006).

Glycine betaine (GB), a quaternary ammonium compound, is a very effective compatible solute (Rathinasabapathi 2000; Chen and Murata 2002). In plants, this compatible solute accumulates in leaves in response to a water deficit and salt stress (Rhodes and Hanson 1993). Moreover, GB has been shown *in vitro* to stabilize membranes of the oxygen-evolving photosystem II complex (Papageorgiou and Murata 1995).

Osmotic stress causes alteration in membrane lipid composition and properties at cellular level. At least part of the membrane damage is caused by lipid peroxidation resulting from uncontrolled ROS (Rodriquez-Rosales *et al*. 1999). Osmotic stress effects can be investigated by using either ionic and penetrating (e.g. NaCl, KCl), non-ionic and penetrating (e.g. mannitol and sorbitol) and non-ionic and non-penetrating (e.g. polyethylene glycol) stress agents (Gangopadhyay *et al*. 1997a, 1997b). Comparative studies on the use of mannitol and PEG as osmotica are still scarce (Pandey *et al*. 2004). According to some studies (Hohl and Schopfer 1991; Pandey and Agarwal 1998), PEG gives more consistent results than mannitol as an external osmotica, to study water relationships in stressed plants. However, in other studies, PEG 6000 treatments had more dramatic inhibitory effect on leaf elongation than iso-osmotic NaCl treatments (Perez-Alfocea *et al*. 1993).

Potato corresponds to the fourth most cultivated crop in the world and a significant source of food world-wide (Poehlman and Sleper 1995). *Solanum tuberosum* L. has

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been classified as moderately salt-tolerant to moderately salt-sensitive (Mass 1985). Potato plants exposed to salinity showed reduced fresh weight and size with smaller leaf area (Levi 1992). The potato plants manifest higher sensitivity to salinity and drought during tuber initiation, which leads to decreased tuber size and number and starch content. Although studies related to biochemical and physiological characterization of salt-tolerance are abundantly available in diverse plant species using whole plants, limited data is available on the comparison of plant responses under iso-osmotic salt and water stress, using NaCl, PEG and mannitol in *Solanum tuberosum*. In this study, we analyzed the physiological and biochemical changes of potato cultivars in response to iso-osmotic salt or PEG stress.

MATERIALS AND METHODS

Plant material

Tubers of *Solanum tuberosum* cv. 'Kufri Kufri Bahar', 'Jyoti' and 'Chandramukhi' were rinsed in water and pretreated with 0.1% Bavistin fungicides (BASF India Ltd., Mumbai, India) for 20 min. These were then sown in plastic pots filled with fine sand and kept in cage house under natural sunlight. The plastic pots were then kept in plastic bowls and daily watered with irrigation water for 15 days.

Osmotic treatments

Fifteen days post planting, potato plants were treated with iso-osmotic solutions of salt-NaCl (Chemofine chemicals, Mumbai, India) (0.1 $molL^{-1}$), PEG-6000 (Prabhat chemicals, Gujarat, India) (12%) and mannitol (BDH chemical Ltd, Poole, England) (0.1 $molL^{-1}$) creating -0.4 Mpa osmotic potential along with irrigation water. The control plantlets received equal volume of irrigation water (0.0 Mpa). For each treatment, respective solution was added to the bowl. The evapo-transpirational losses were replenished with irrigation water twice a day. The solutions were changed after every three days. The second leaf from each plant was harvested 15 days of the stress treatments.

Relative water content

The leaf relative water content (RWC) was determined from physiologically active second leaf of each treated and control plants. Immediately after harvesting the leaf from the main stem, fresh weight (FW) was recorded. The turgid weight (TW) was measured after 24 h saturation (when leaf weight reached a plateau) on deionized water at 4°C in the dark. The dry weight (DW) was determined after drying the leaves for 48 h in hot air oven at 70°C. RWC was calculated as [(FW-DW)/(TW-DW)]×100.

Membrane damage rate

Leaf discs (1 cm × 1 cm) of treated and controlled plants leaf were washed with DW to remove surface contaminants. Discs were placed in culture tube containing 10 ml of DW. Samples were incubated at RT (25°C) on shaker (100 rpm) for 24 hrs. At the end of incubation, EC (EC1) of the bathing solution from the tubes was recorded. Samples were autoclaved after capping the tubes at 121°C for 20 min to completely kill the tissues and release all electrolytes. Second EC reading (EC2) was recorded after cooling the solution to the RT. Leaf membrane damage rate or relative electrical conductivity (REC) was calculated using the formula. MDR or REC (%) = (EC1/EC2) × 100.

Estimation of proline content

Free Pro content was measured spectrophotometrically according to the method of Bates *et al*. (1973). About 500 mg of fresh leaf pieces were homogenized in 3% (w/v) aqueous sulfosalicylic acid. The filtered homogenate (2.0 ml) was reacted with 2.0 ml each of acid ninhydrin and glacial acetic acid by incubating at 100°C for 1 h. The reaction was terminated in an ice bath and allowed to cool at room temperature. The reaction mixture was extracted with 4.0 ml toluene and mixed vigorously with a stirrer for 10-15 sec. 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. Pro content was determined from a standard curve using L-Pro as standard and expressed as µg of Pro g^{-1} FW.

Glycine betaine analysis

The accumulation of GB was estimated according to Grieve and Grattan (1983). The leaf pieces (500 mg) were ground in liquid nitrogen, and the finely ground powder was mechanically shaken with 20.0 ml of deionized water at 25°C for 16 h. The samples were filtered, and the thawed extract was diluted (1: 1) with 2 *N* H_2SO_4. The extract (500 µl) was cooled in ice water for 1 h, and then mixed with 200 µl of KI-I2 reagent. The tubes were stored at 0-4°C for 16 h, followed by centrifugation at 10,000 rpm for 15 min at 0°C. The per-iodide crystals were dissolved in 9.0 ml of 1,2- dichloroethane, and after 2 h the absorbance was measured at 365 nm using a spectrophotometer (EVOLUTION 300 UV-Visible Spectrophotometer, Thermo Electron Corp., England).

Assessment of total soluble sugars

Total soluble sugars (TSS) were estimated as per Anthrone method (Watanabe *et al*. 2000) with slight modification. 200 mg sample was homogenized in 10.0 ml of 80% ethanol. Then the extract was prepared by centrifugation at 6000 rpm for 10 min at 4°C. 1.0 ml of supernatant-extract was reacted with 3.0 ml of freshly prepared anthrone reagent 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 FW) were quantified using glucose as standard.

Antioxidant enzyme assays

1. Extraction

The leaf (200 mg) samples after 15 days of treatment were used for enzyme analysis. All steps in the preparation of the enzyme extract were carried out at 4°C. The samples were homogenized in 3.0 ml ice cold 50 mM sodium phosphate buffer (pH 7.0) including 0.1 mM EDTA and 1% (w/v) PVP in pre chilled mortar and pestle. The homogenate was centrifuged for 20 min at 15,000 rpm at 4°C. An appropriate aliquot/dilution of the supernatant was used as a crude enzyme(s) 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 (EVOLUTION 300 UV-Visible Spectrophotometer).

2. SOD (EC 1.15.1.1.) assay

The total SOD activity was assayed in terms of inhibition of the photochemical reduction of NBT-nitroblue tetrazolium (Becana *et al*. 1998). The reaction mixture (3.0 ml) containing 50 mM phosphate buffer (pH 7.8) and 0.1 mM EDTA to which an oxygen generating system containing 14.3 mM methionine, 82.5 µM NBT and 2.2 µM riboflavin, prepared freshly *in situ* was added. The reaction was initiated by adding 100 µl of crude enzyme. The entire system was kept 30 cm below the light source (6 15 W fluorescent tubes) for 30 min. The reaction was stopped by switching off the light. For light blank, all the reactants without enzyme extract were incubated in light as for the samples, whereas, all the reactants along with 100 µl enzyme extract were incubated in dark for dark blank. The reduction in nitroblue tetrazolium was measured by monitoring the change in absorbance at 560 nm. The readings of light blank were used in calculation of enzyme units. 1U SOD enzyme was defined as the amount that produces a 50% inhibition of nitroblue tetrazolium reduction under the assay condition.

3. GPX (EC 1.11.1.9.) assay

The total GPX activity was assayed in terms of oxidation of guaiacol (Chance and Maehly 1995). One ml of assay system contained 50 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 10 mM guaiacol and 10 mM H_2O_2 in 1 ml experimental cuvette. Oxidation of guaiacol was monitored by following the increase in absorbance at 470 nm (E=26.6 $mM^{-1}cm^{-1}$) for 1 min at interval of 15 sec after addition of 25 µl of crude enzyme. Guaiacol peroxidase activity was measured as mmol of tetraguaiacol formed mg $protein^{-1}$ min^{-1} (mmol.mg $protein^{-1}$ min^{-1}).

Statistical analysis

All the treatments were replicated thrice and experiments were laid out in a completely randomized design (CRD). The data was analyzed using two-way ANOVA (Tukey's test; $P < 0.01$) (Origin version 8). The treatment means were compared by using the least significant difference (LSD) test at a significance level of $P \leq 0.01$.

RESULTS

Effect of iso-osmotic stress on relative water content (RWC) and membrane damage rate (MDR)

Leaf relative water content was significantly low ($P < 0.01$) in all the three cultivars ('Kufri Bahar', 'Jyoti', 'Chandramukhi') in NaCl, PEG and mannitol stress as compared to control. Among treatments, there was a significant difference between PEG, NaCl and mannitol treated samples with respect to RWC (**Fig. 1**). Among the cultivars, decrease in RWC was observed (7.7, 10.79 and 6.83% under NaCl, PEG and mannitol stress treatment, respectively) compared to control implying that osmotic stress caused significant reduction in shoot water supply. With respect to single stress treatment (NaCl, PEG or mannitol) the three cultivars did not differ much (**Fig. 1**).

Membrane damage rate was significantly pronounced ($P < 0.01$) in all the cultivars under iso-osmotic treatments (NaCl, PEG and mannitol). The difference in the increase was insignificant among cultivars, whereas among treatments, NaCl treatment showed significant MDR (78.57%) over PEG (69.29%) and mannitol (62.90%) treatments (**Fig. 2**).

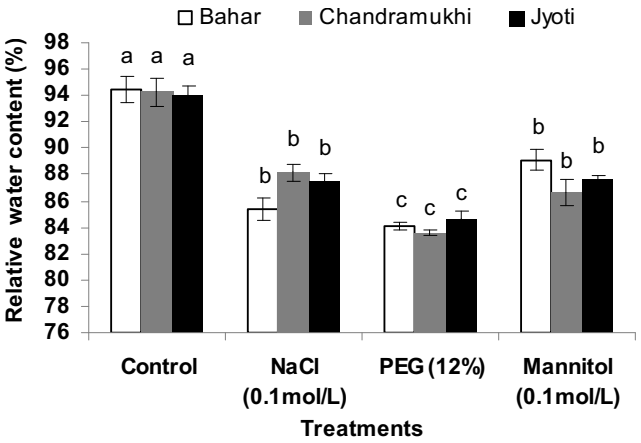

Fig. 1 The effect of isoosmotic treatment of NaCl (0.1 mol/L), PEG (12%) and mannitol (0.1 mol/L), on relative water content (RWC) in potato cultivars 'Bahar', 'Chandramukhi' and 'Jyoti'. Data represents the average of three replicates. Vertical bars indicate ± S.E. Two-way ANOVA was significant ($P < 0.01$). Different letters indicate significant differences between treatments for a particular variety (Tukey; $P < 0.01$).

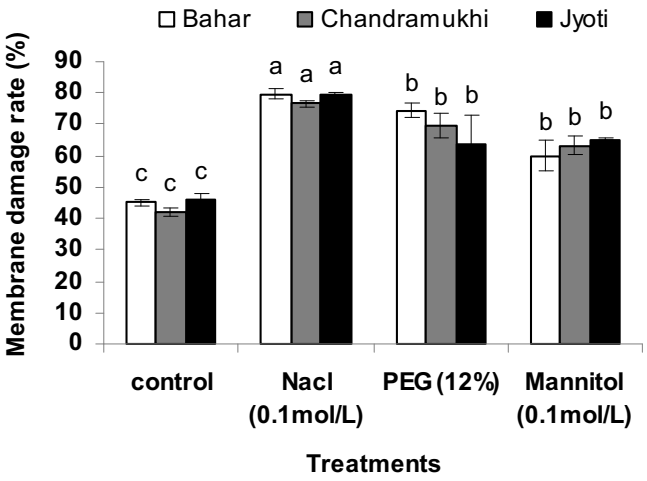

Fig. 2 The effect of isoosmotic treatment of NaCl (0.1 mol/L), PEG (12%) and mannitol (0.1 mol/L), on membrane damage rate (MDR) in potato cultivars 'Bahar', 'Chandramukhi' and 'Jyoti'. Data represents the average of three replicates. Vertical bars indicate ± S.E. Two-way ANOVA was significant ($P < 0.01$). Different letters indicate significant differences between treatments for a particular variety (Tukey; $P < 0.01$).

Accumulation of solutes

1. Proline

Pro levels remained low in the control treatment whereas Pro accumulation was significantly increased in the three iso-osmotic treatments and cultivars (**Fig. 3A**). Highest increase in Pro was observed under NaCl treatment, significantly different from the increase under PEG and mannitol treatments. Among the three cultivars, difference in increase was significant ($P < 0.01$) between 'Kufri Bahar' and 'Jyoti' (**Fig. 3A**). The percentage increase in Pro was 133.38, 106.71 and 75.11%, respectively in the NaCl-, PEG- and mannitol-treated plants.

2. Total soluble sugar

The three isoosmotic (NaCl, PEG and mannitol) stress treatments resulted in a significant increase in total soluble sugar ($P < 0.01$) in the three cultivars compared to control (**Fig. 3B**). In 'Chandramukhi', the increase was significantly different from 'Kufri Bahar' and 'Jyoti'. However, among treatments there was not much difference in TSS (**Fig. 3B**).The percentage increase in NaCl, PEG and mannitol treated plants, over control, was 145.78, 130.93 and 134.92%, respectively.

3. Glycine betaine content

All three cultivars showed significant accumulation ($P < 0.01$) of GB under the iso-osmotic treatments (NaCl, PEG and mannitol) as compared to control (**Fig. 3C**). The highest increase in GB was observed in the PEG treatment than other treatments and control (**Fig. 3C**). Among the cultivars, GB accumulation was significantly higher in 'Chandramukhi' than in 'Kufri Bahar' and 'Jyoti'. The percentage increase in GB was 32, 38.55 and 13.38%, respectively under NaCl, PEG and mannitol treatments.

Antioxidant enzymes

The responses of potato to iso-osmotic salt and drought stress were measured through the enzymatic activity of SOD and GPX in shoots of three cultivars treated with iso-osmotic levels of NaCl, PEG or mannitol (**Fig. 4A, 4B**).

1. Superoxide dismutase

Compared to control, NaCl, PEG and mannitol induced an increase in SOD activity ($P < 0.01$) in the three cultivars (**Fig. 4A**). Higher SOD activity was recorded in 'Chandramukhi' followed by 'Kufri Bahar' and 'Jyoti'. NaCl-treated plants (65.49%) displayed significantly higher SOD activity than PEG (50.81%) and mannitol (46.72) treatments (**Fig. 4A**).

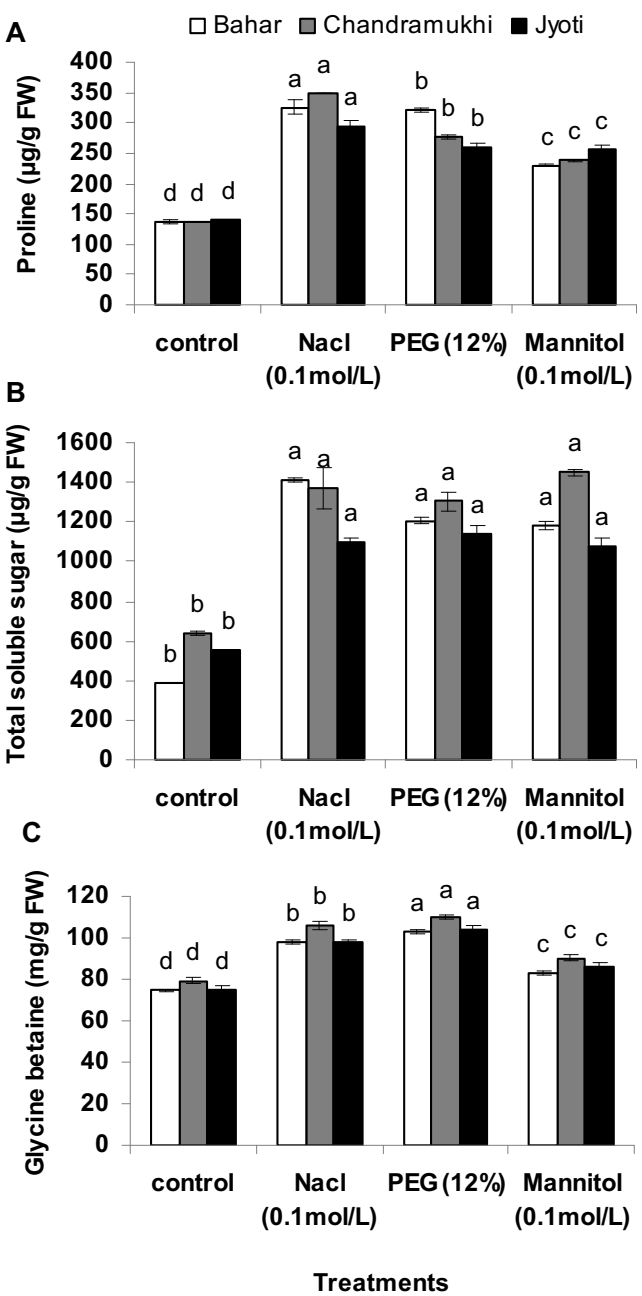

Fig. 3 The effect of isoosmotic treatment of NaCl (0.1 mol/L), PEG (12%) and mannitol (0.1 mol/L), on proline (A), total soluble sugar (B) and glycine betaine (C) in potato cultivars 'Bahar', 'Chandramukhi' and 'Jyoti'. Data represents the average of three replicates. Vertical bars indicate ± S.E. Two-way ANOVA was significant ($P < 0.01$). Different letters indicate significant differences between treatments for a particular variety (Tukey; $P < 0.01$).

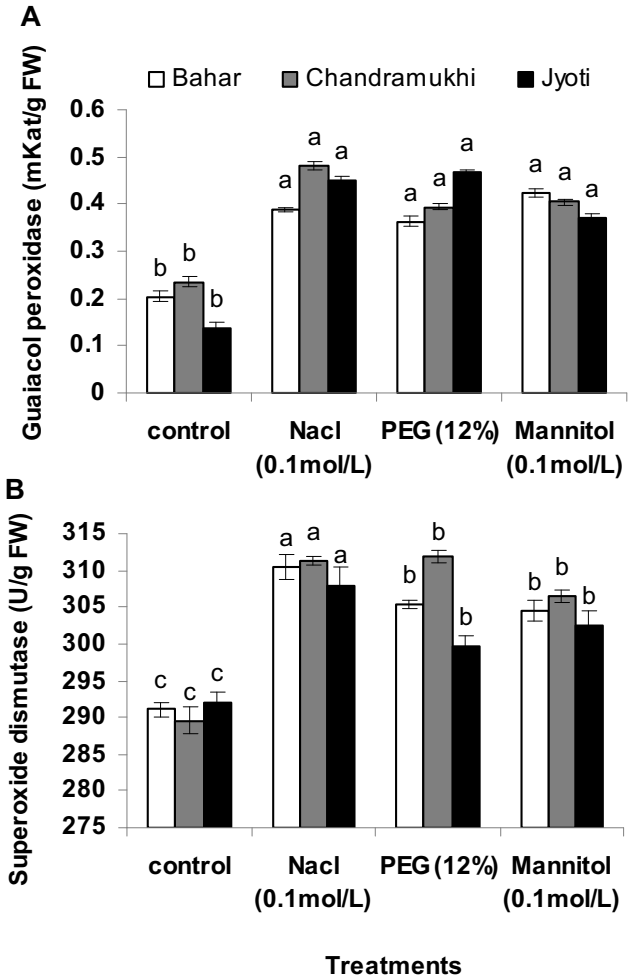

Fig. 4 The effect of isoosmotic treatment of NaCl (0.1 mol/L), PEG (12%) and mannitol (0.1 mol/L), on Superoxide dismutase (SOD) (A) and Guaiacol peroxidase (B) in potato cultivars 'Bahar', 'Chandramukhi' and 'Jyoti'. Data represents the average of three replicates. Vertical bars indicate ± S.E. Two-way ANOVA was significant ($P < 0.01$). Different letters indicate significant differences between treatments for a particular variety (Tukey; $P < 0.01$).

2. Guaiacol peroxidase

In general, there was a significant increase in GPX activity in all the treatments and cultivars, over control (**Fig. 4B**). However, among different treatments there was no significant difference in GPX activity. Among cultivars, 'Chandramukhi' showed a higher increase in GPX activity than other cultivars (**Fig. 4B**). The increase in percentage activity of GPX induced in NaCl, PEG and mannitol treatments was 121.05, 114.73 and 117.36%, respectively.

DISCUSSION

Potato is classified as a moderately salt-sensitive crop and variability in salt sensitivity exists among various cultivars (Ochat *et al*. 1999). The importance of physiological and biochemical analysis of crop response to a given salinity or drought stress condition assumes significance in view of finding suitable indicators for these stresses (Parvaiz and Satyawati 2008). In our study, the effect of osmotic stress on water relations and accumulation of some solutes (Pro, GB and soluble sugars) was studied in three potato cultivars. PEG and mannitol mediated drought stress, and salinity stress disrupt the water status of plants in different ways. PEG and mannitol decrease water availability in the soil, while salt reduces water uptake by the plant. Though the immediate effects of both stresses are osmotic, their long term effects are known to differ. PEG- and mannitol-mediated stress continues to exert osmotic effects on plants, while salt stress effects are largely due to ion toxicity and imbalance (Munns and Tester 2008). Using iso-osmotic concentrations of mannitol, PEG and salt, we attempted to study the induced responses in potato plants to tolerate the stress conditions.

In the case of potato, parameters of plant growth are not always in good agreement with parameters of plant water status (Heuer and Nadler 1998). On the contrary, physiological parameters reflect or may predict plant response to certain stress conditions. In the present study, the RWC was significantly reduced by the stress treatments. PEG stress resulted in highest percentage reduction (10.79%) in RWC because of its greater viscosity that might contribute to the inhibition of water flow through roots (Plaut and Federman 1985). Greater inhibitory effects of PEG compared to iso-

osmotic NaCl stress have been reported in maize by Chazen *et al*. (1995). In agreement with results obtained in the present investigation, Badawi (2000) showed that, water contents of both roots and shoots of *Hyoscyamus muticus* plants were significantly reduced following treatment with salinity and PEG as compared with reduced control plants. Conversely, mannitol is known to penetrate the apoplast and to be taken up by cells (Hohl and Schopfer 1991) which may attenuate the effective osmotic gradient between the medium and the symplast.

In general, the ionic toxicity of salt stress treatment causes more damage to plant cells than that in mannitol drought stress conditions, and plays a major role in membrane injury, organelle damage and pigment degradation prior to cell death, which is well documented in many plant species such as sugarcane (Errabii *et al*. 2007; Patade *et al*. 2008) durum wheat (Lutts *et al*. 2004) and tobacco (Gangopadhyay *et al*. 1997). In this study, MDR was more affected under NaCl stress treatment as compared to other iso-osmotic stress. Membrane damage in plants is induced by ROS, whose maximum production has been observed in salt treatment compared to other iso-osmotic stress treatment.

Osmotic adjustment in response to salt, PEG and mannitol stress was estimated by measuring the levels of accumulation of soluble sugars, Pro and GB. Pro accumulation is the first response of plants exposed to salt stress and water deficit stress in order to reduce injury to cells (Ashraf and Foolad 2007). Additionally, Pro serves as a storage sink for carbon and nitrogen and free-radical scavengers, therefore, it contributes to ameliorate the deleterious effect of salt stress in different ways. In the present study, Pro accumulation was dependent on the type of stress i.e. NaCl salt stress or PEG, mannitol water deficit stress. The highest levels of Pro in osmotically stressed plants corresponded to NaCl treatment (**Fig. 3A**). Slama *et al*. (2006) and Lokhande *et al*. (2010) observed the induction of Pro when *S. portulacastrum* plants or *in vitro* cultures were challenged by salt stress and water deficit. The higher accumulation of Pro in plants treated with PEG as compared to mannitol has also been observed in rice (Pandey *et al*. 2004) and sugarcane (Patade *et al*. 2008).

In general, a positive correlation has been shown between abiotic stress tolerance and Pro levels (Ashraf and Foolad 2007). The correlation between Pro accumulation and relative water content (**Fig. 5**) suggests that the Pro accumulation in leaves, induced by osmotic stresses, might be associated with some protective mechanism in potato. The mean Pro content remained far too low to act as an osmoticum for maintaining the tissue hydration. However, Pro could be concentrated in specific cell sub-compartments (Büssis *et al* 1998), and/or exert some non-osmotic beneficial effect. Several functions have been attributed to Pro accumulation in response to stress. Besides the effects of Pro as an osmo-compatible solute (Larher *et al*. 1998), its biosynthesis can contribute to reduced cellular acidification allowing the regeneration of NADP needed for the maintenance of the respiratory and photosynthetic processes, it may serve also as a nitrogen and carbon source needed in stress recovery (Chiang and Dandeker 1995; Hare and Cress 1997; Aziz *et al*. 1999) and can also act as a scavenger of hydroxyl radicals avoiding cellular damage provoked by osmotic or salt-induced oxidative stress (Polle 1997; Borsani *et al*. 1999).

In addition to Pro, other osmolytes such as GB and soluble sugars also play a major role in osmotic adjustment, for coping with salt and water deficit stress (Gandonou *et al*. 2006). In this study, TSS increased in all the treatments than control in all cultivars. TSS accumulation was also found to be varied among the cultivars. Higher accumulation was observed in 'Chandramukhi' the other two cultivars. In some plant species, the accumulation of sugars in response to water stress is considered to play an important role in osmotic adjustment (Bajji *et al*. 2001). Accumulation of GB increased under PEG stress in the three cultivars used, which might indicate that though PEG solution could not have

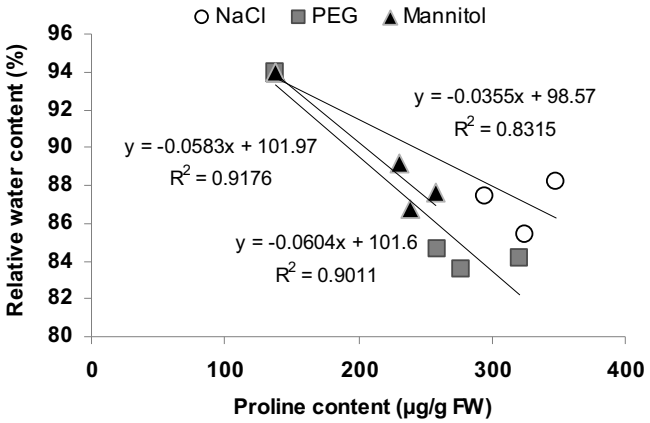

Fig. 5 Relationship between relative water content (%) and proline content (µg/g FW) in leaves of potato cultivars after NaCl, PEG and mannitol treatment. Data are means of three replicates.

entered cells, it would have permeated the apoplast of the tissues leading to the accumulation of GB. The magnitude of GB accumulation was more in 'Chandramukhi' than other cultivars used in the study. Wyn Jones and Storey (1978) also found increased in and GB in Barley in response to PEG and NaCl. Accumulation of these osmolytes such as Pro, GB and sugars are known to occur under the osmotic stress (Hasegava *et al*. 2000; Munns 2005). However, the synthesis of these compatible solutes demands metabolic energy and thus occurs at the expense of plant growth, but may be vital for the plant to survive and recover from the stress (Munns and Tester 2008).

Both salt and dehydration stress are known to cause oxidative damage to cells and tissues. Plants resistant to different abiotic stresses have shown enhanced activities of antioxidative enzymes involved in the detoxification of ROS (Rabinowitch and Fridovich 1983; Lin and Kao 2000). SOD is considered a key enzyme for maintaining normal physiological conditions and coping with oxidative stress in the regulation of intracellular levels of ROS (Lee *et al*. 2001). In this study, increased SOD activity was induced under NaCl stress than PEG and mannitol stress in all the cultivars used which may be due to more production of ROS under salinity stress. Although activity of GPX varied according to cultivar used, there was no significant difference among the treatments.

In conclusion, our results suggest that osmotic stress caused significant decrease in RWC and increase in MDR in potato. Decreased RWC was significant in plants subjected to PEG while significant increase in MDR was observed in NaCl treated plants. Comparison of the two iso-osmotic stresses indicated that Pro accumulation and tissue hydration had a positive relationship (**Fig. 5**). The results also indicate that salt-tolerant potato cultivars have a better defense against ROS by enhanced activity of antioxidant enzymes (especially SOD) under salt stress. The basic knowledge gained should be applicable as indicators for salt and water-deficit tolerance in potato.

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