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ORIGINAL PAPER

Comparative evaluation of hydro-, chemo- , and hormonal-priming methods for imparting salt and PEG stress tolerance in Indian mustard (*Brassica juncea* L.)

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Abstract The present study was aimed to evaluate the effect of different seed priming methods to enhance the sodium chloride (NaCl) and polyethylene glycol-8000 (PEG-8000) stress tolerance in Indian mustard (*Brassica juncea* L.). Seeds subjected to different priming treatments such as water (hydro-priming), calcium chloride ($CaCl_2$) (chemo-priming), and abscisic acid (ABA) (hormonal-priming) showed increased rate of germination as compared to non-primed seeds. The primed and non-primed seeds were grown for 15 days and then the seedlings were independently subjected to iso-osmotic salt (150 mM NaCl) or PEG-8000 (20%) stress. The different biochemical responses were studied 10 days after treatment. Under NaCl and PEG stress, the dry weight and total chlorophyll content were higher in primed sets as compared to non-primed treatment which was also evident by the phenotype of the seedlings. In general, the higher activities of superoxide dismutase and glutathione reductase resulted in lower oxidative damage, in terms of malondialdehyde content, under NaCl and PEG stress in hydro-primed set as compared to non-primed, ABA-, and $CaCl_2$-primed treatments. Besides, the level of total phenolics and accumulation of osmolytes such as free proline, glycine betaine, and total soluble sugars was also lower in hydro-primed set as compared to other primed and non-primed treatments. The study thus suggests the use of hydro-priming as a simple and cost-effective strategy to alleviate the NaCl and PEG induced stress in *B. juncea*.

Keywords *Brassica juncea* · Seed priming · Hydro-priming · Stress imprints · Antioxidant enzymes · Osmolytes accumulation

Abbreviations

ABA	Abscisic acid
ANOVA	Analysis of variance
$CaCl_2$	Calcium chloride
CP	Seedlings derived from the $CaCl_2$-primed seeds
CRD	Completely randomized design
DMRT	Duncan's multiple range test
EDTA	Ethylenediaminetetraacetic acid
GR	Glutathione reductase
HP	Seedlings derived from the ABA-primed seeds
MDA	Malondialdehyde
MS	Murashige and Skoog basal medium
NaCl	Sodium chloride

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NP	Seedlings derived from the non-primed seeds
PEG	Polyethylene glycol-8000
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive substances
WP	Seedlings derived from the hydro-primed seeds

Introduction

Rapeseed is a cosmopolitan oil bearing plant. India is the third largest producer of mustard oil in the world. According to an estimate, India produces about 5 million tones of mustard annually that contributes to about 11% of the world's total mustard production (Gunstone 2001). However, one of the major obstacles to high yield and production for Indian mustard (*Brassica juncea* L.) is the lack of synchronized crop establishment due to poor weather and soil conditions caused by salinity and drought stresses (Purty et al. 2008). The primary effect of these stresses is that they render water less available to plants due to an increase in the osmotic pressure of the soil solution as compared to that of roots. In addition, the excessive concentration and absorption of individual ions may prove toxic to the plants and/or may retard the absorption and homeostasis of other essential nutrients (Zhu 2001). To tackle such problems, research efforts are being made to seek effective and low-cost methods to enhance stress tolerance of the plants under field conditions. In this direction, seed priming has been demonstrated as a successful cost-effective strategy for improving the seed vigour and seedling growth under stress conditions (Foti et al. 2008; Yagmur and Kaydan 2008; Beckers and Conrath 2007).

Seed occupies a central position in the life cycle of a plant. Dry mature seeds are the resting organs, having low moisture content (5–15%) with metabolic activities almost at standstill. For germination to occur, they need to be hydrated under favorable conditions, e.g., suitable temperature and the presence of oxygen. The process of seed germination is a triphasic phenomenon which includes an initial phase of rapid water uptake, followed by a plateau phase with little change in water content, and subsequently, the last phase which coincides with radicle emergence and resumption of growth (Bewley 1997). Seed priming is an important method associated with the process of seed germination and is widely used to synchronize the germination of individual seeds (Heydecker et al. 1973; Taylor and Harman 1990). During priming, the germination-related processes are initiated but the emergence of the radicle is prevented since hydration is followed by drying of seeds. Seed priming generally increases the rate of germination and emergence, which have practical agronomic implications, notably under adverse germination conditions (McDonald 2000). Therefore, there is a strong interest in the seed industry to find a suitable priming agent that might be used to increase the tolerance of plants under adverse field conditions (Job et al. 2000). The beneficial effects of seed priming with different priming agents have already been successfully demonstrated in many crop plants, such as mustard (Srinivasan et al. 1999), chickpea (Kaur et al. 2002), sunflower (Kaya et al. 2006), wheat (Iqbal and Ashraf 2007), cotton (Casenave and Toselli 2007), and sugarcane (Patade et al. 2009). Although priming-induced salt tolerance has been shown, the underlying mechanisms including physiological and biochemical basis of priming-induced beneficial effects under the stressed environments are poorly understood. In addition, the differential efficiency of various priming agents, such as chemicals, hormones, and hydropriming in increasing the tolerance against different stresses has not been comparatively investigated. Therefore, the present study was aimed to examine the comparative potential of water (hydro-priming), $CaCl_2$ (chemo-priming), and ABA (hormonal-priming) to overcome the negative effects of NaCl and PEG stress in *B. juncea*. The experimental results showed that hydropriming treatment imparts the maximal effect, and hence, it can be adopted as a cost effective and user-friendly method for alleviating the NaCl and PEG stress.

Materials and methods

Plant material and seed priming treatment

The study was performed on Indian mustard (*B. juncea* L.) var. TM-2. Three different agents viz., distilled water (hydro-primed), $CaCl_2$ (100 μM; chemo-primed), and ABA (100 μM; hormonal-primed) were used for the purpose of seed priming. Seeds were first surface sterilized with 30% ethanol for 3 min, washed thoroughly to remove any traces of ethanol, and were distributed in four different sets. Three sets were separately immersed in different priming solutions (ca. 150 seeds/200 ml solution) for 18 h at 25°C under dark conditions and slow agitation. The remaining one set was unsoaked and treated as non-primed control. After the priming period, seeds were rinsed three times with distilled water and then dried to their original moisture content in shed (at room temperature; 25 ± 2°C and 45% relative humidity). Moisture content of non-primed and primed seeds was equilibrated at room temperature for 2 days.

Germination tests

For assessing the germination ability, six replicates (each with 25 seeds) of both primed and non-primed seeds were independently sown in plastic pots (10 × 15 cm) containing thoroughly washed and sterilized fine sand saturated with distilled water. The seeds were allowed to germinate under natural daylight conditions and germination percentage was recorded at every 12 h for 2 days. The emergence of 2-mm-long radicle is considered as a marker for germination. The rate of germination was estimated using a modified Timson index (Khan and Ungar 1984; Patade et al. 2009) of germination velocity as follows:

$$\text{Timson index} = \Sigma(G/t)$$

where G is total germination percentage at the interval of 12 h for 2 days (i.e. % of germinated seeds of all seeds that germinated finally); t, total period of germination (days). The higher value of Timson's index indicated the rapid rate of germination.

Iso-osmotic NaCl or PEG stress treatments

Four different sets (three primed and one non-primed) were grown independently under distilled water for 6 days and then water was changed with half-strength MS medium (Murashige and Skoog 1962). After 15 days of germination, each set was distributed into three different sets. Two sets were subjected to iso-osmotic (−0.7 MPa) salt (NaCl, 150 mM) or polyethylene glycol (PEG-8000; 20% w/v) stress, and the remaining one set was maintained as control. All the solutions were prepared in half-strength MS. After 10 days of treatment, shoots from all the four sets, given seed priming treatment with water (WP; hydro-primed); $CaCl_2$ (CP; chemo-primed); ABA (HP; hormonal-primed), and unsoaked control (NP; non-primed), were harvested for the analysis of various biochemical parameters.

Growth parameters

The differential growth of the seedlings given various seed priming treatments was monitored by observing their differential phenotype and measuring the dry weight and total chlorophyll content. For each treatment, 25 seedlings (with three replicates) were harvested and dry weight was recorded after drying them to constant weight at 60°C in the hot air oven for 48 h. For the measurement of chlorophyll content, the photosynthetic pigments were extracted in 80% chilled acetone in dark and the total chlorophyll content was estimated by the method of Arnon (1949).

Lipid peroxidation

Oxidative damage to the membrane lipids was estimated by analyzing the content of total thiobarbituric acid-reactive substances (TBARS), expressed as equivalents of malondialdehyde (MDA). The amount of MDA was calculated as described by Hichem et al. (2009).

Determination of compatible solutes

The accumulation of compatible solutes such as free proline and glycine betaine was estimated following Bates et al. (1973) and Grieve and Grattan (1983), respectively, as described previously (Patade et al. 2008). The level of total soluble sugars was calculated described previously by Watanabe et al. (2000).

Assay of antioxidant enzymes

For the assay of antioxidant enzymes, plant samples (500 mg) were homogenized in 100 mM chilled potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 1% polyvinyl pyrrolidone (w/v) at 4°C. Homogenate was squeezed through four layers of cheese cloth and the extract thus obtained was centrifuged at 15,000*g* for 15 min at 4°C. The supernatant was used to measure the activities of superoxide dismutase (SOD; EC 1.15.1.1) and glutathione reductase (GR; EC 1.6.4.2) following the methods of Beauchamp and Fridovich (1971) and Smith et al. (1988), respectively, as detailed previously (Srivastava et al. 2006). The protein content in the supernatant was measured according to Lowry et al. (1951).

Total phenolic content

For the estimation of total phenolics, leaf samples were extracted with 95% methanol, and the phenolic content was estimated using Folin–Ciocalteu method as described by Ainsworth and Gillespie (2007).

Statistical analysis

The experiment was laid out in a completely randomized design (CRD) with two factorials and four replications. The first factor was the priming agent and the second was the stress treatment (NaCl or PEG). One-way analysis of variance (ANOVA) was done with all the data to confirm the variability of data and validity of results, and Duncan's multiple range test (DMRT) was performed to determine the significant difference between treatments using SPSS 10.0.

Results

Differential seed germination ability in response to various priming treatments

The seed germination response was varied among the different priming treatments. In general, the germination percentage of the primed seeds was higher than that of non-primed. At 24 h after sowing, the germination percentage was increased by 25% in water- and ABA-primed and 67% in $CaCl_2$-primed seeds as compared to that of non-primed seeds (Fig. 1a). The rate of germination measured in terms of Timson index was also higher in response to the priming treatments. Among the different priming sets, the Timson index was maximum in $CaCl_2$- followed by water- and ABA-primed seeds (Fig. 1b).

Effect of seed priming on the phenotype and growth of the seedling in response to NaCl or PEG stress

Under control condition, the phenotype of the seedlings in primed sets (WP, CP and HP) resembled that of NP (Fig. 2); however, a slight increase and decrease in the dry weight (Fig. 3a) and chlorophyll content (Fig. 3b), respectively, was observed in response to the priming treatments. Under NaCl and PEG stress, NP demonstrated diminutive growth of the seedlings along with a significant decrease in dry weight and chlorophyll contents (Fig. 3a, b). On the contrary, under similar treatment, the phenotype of the primed sets was comparable to their respective controls and their dry weight and chlorophyll content were significantly higher than that of NP (Fig. 3a, b).

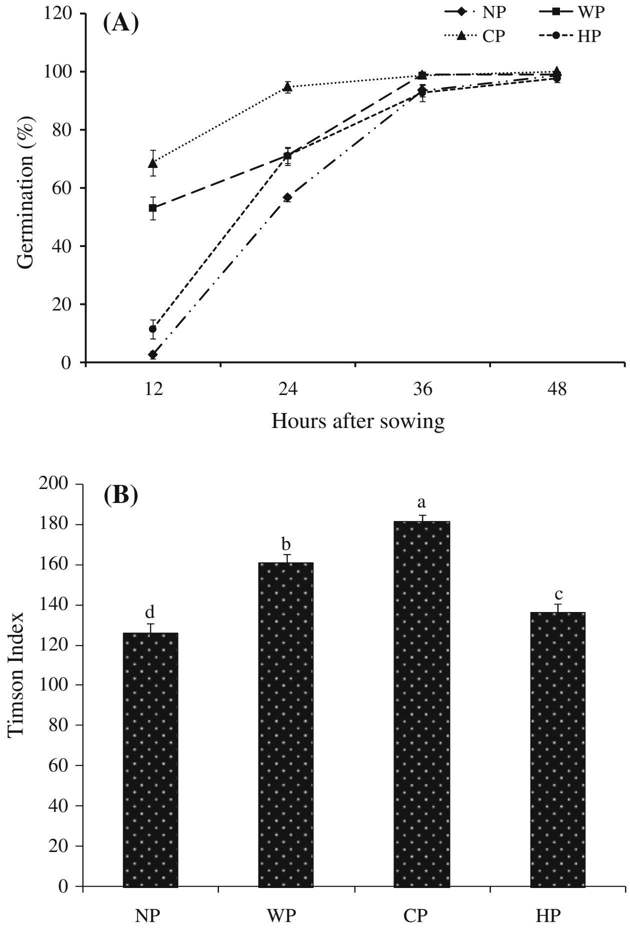

Fig. 1 Effect of different seed priming treatments on seed germination response. The seeds were given different priming treatments and then the germination percentage (**a**) was recorded at the interval of 12 h for 2 days. The rate of germination, in terms of Timson index, was also calculated (**b**). The WP, CP, and HP represent different priming treatments such as water-, $CaCl_2$-, and ABA, respectively; while, NP represents the non-primed control. *Different letters* indicate significantly different values (DMRT, $P < 0.05$)

Effect of seed priming on the lipid peroxidation of the seedlings subjected to NaCl or PEG stress

The extent of oxidative stress-induced lipid peroxidation was found to be dependent upon stress treatments. Under control condition, the level of MDA was almost equal in all the seedlings, though it was slightly lower in WP and HP treatment, as compared to NP. Upon exposure to either salt or PEG stress, the MDA content increased in all treatments; however, in WP treatment, the increase was either comparable or lower than that of NP, whereas in CP and HP treatment the increase was either comparable or higher as compared to NP (Fig. 4).

Differential responses of antioxidant enzymes and total phenolics

Under control condition, in primed treatments, both the GR and SOD activities were either similar or higher, as compared to NP (Fig. 5a, b). Under NaCl stress, the GR activity was lower in primed sets, as compared to NP, whereas under PEG stress, the GR activity of primed set was almost equal to that of NP, except for the WP in which the GR activity was 2.2-fold higher than that of NP (Fig. 5a). Under NaCl stress, the SOD activity was increased in CP and HP by 15 and 56%, respectively, while, in WP, 54% decreased in the activity was observed, as compared to NP. However, under PEG stress, as compared to NP, the SOD activity was significantly higher in WP and CP by 27 and 32%, respectively, while 51% decrease was observed in HP treatment (Fig. 5b). Under control condition, the level of total phenolics was found comparable in the non-primed and primed sets, except for WP in which the phenolics level was 28% lower than that of NP. Under NaCl stress, phenolics content increased by 40% in CP and HP, as compared to NP, while in WP the level was almost equal to that of NP. In contrast, under PEG stress, the phenolic content was decreased in all the primed set, with the maximum decrease (35%) being in WP, as compared to that of NP (Fig. 5c).

Accumulation of different osmolytes

The accumulation of proline was negligible under control condition; however, significant accumulation was observed in response to NaCl and PEG stress. The level of proline

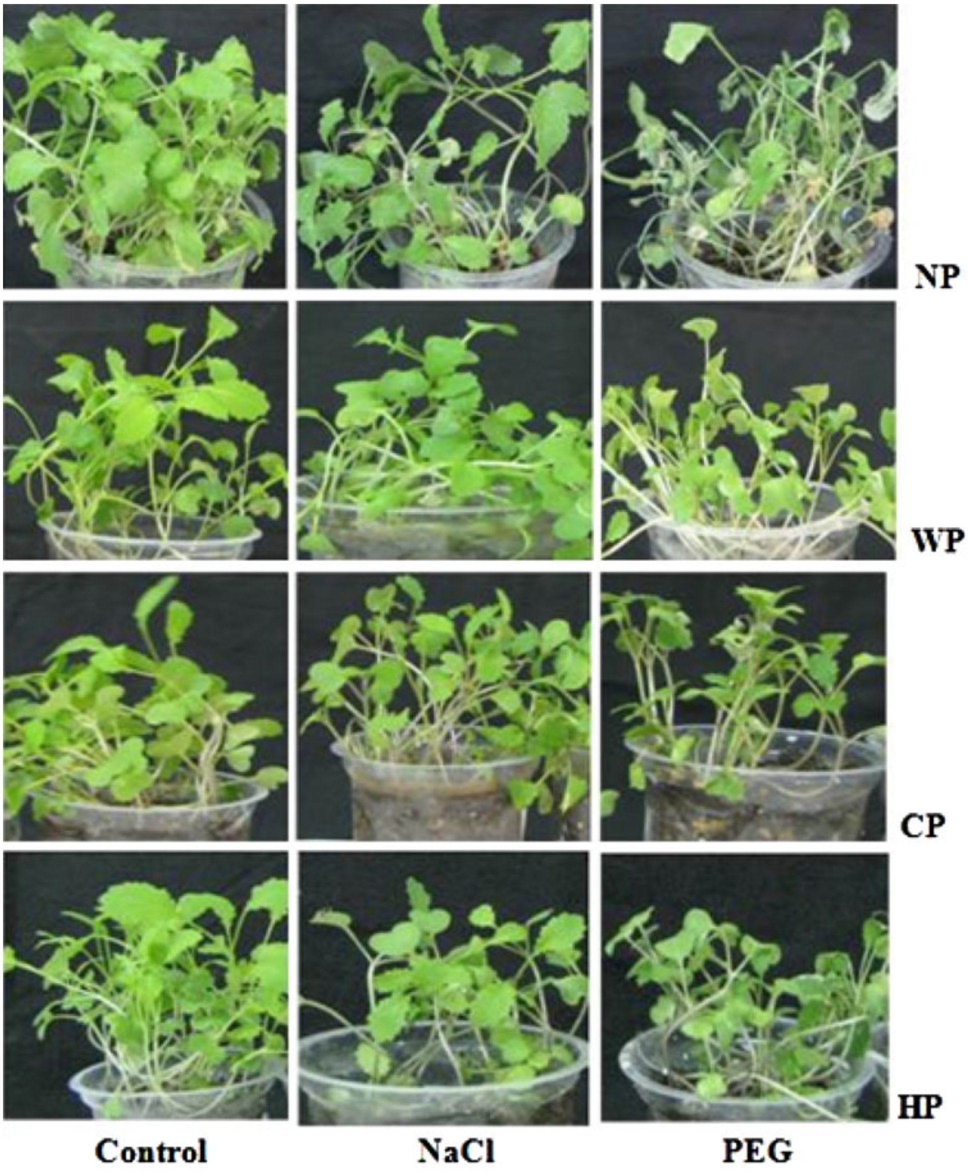

Fig. 2 Differential phenotype of the seedlings derived from the seeds given different priming treatments. Seeds given different priming treatments such as water (WP)-, $CaCl_2$ (CP)-, and ABA (HP) along with the non-primed control (NP) were grown independently and then subjected to the iso-osmotic NaCl/PEG stress 15 days after germination. After 10 days of treatment, the differential phenotype was recorded

under NaCl stress was at par in WP, 53% lower in HP, and 23% higher in CP as compared to NP. On the other hand, under PEG stress the level of proline was equal or higher in the primed sets, except for WP in which 60% decrease in the proline level was observed as compared to NP (Fig. 6a). The accumulation of glycine betaine was observed mainly under PEG stress, where all the priming treatments led to a decrease in the level of glycine betaine, with the maximum being in HP, as compared to NP (Fig. 6b). Under control condition, the accumulation of total soluble sugars was at par in all the treatments, except for HP, which showed 24% lower level as compared to NP. Under both NaCl and PEG stress, the priming treatments led to an increase in the level of soluble sugars, except for WP under PEG stress, in which the accumulation of soluble sugars was 42% lower than NP (Fig. 6c).

Discussion

The present study was aimed to evaluate the stress alleviation potential of different seed priming agents (water, $CaCl_2$ and ABA) for increasing the stress tolerance of Indian mustard (*B. juncea*) under NaCl and PEG stress. In order to have a comparative evaluation, the concentrations of $CaCl_2$ and ABA were first optimized to find their optimal dose. Among the different concentrations tested, the seeds primed with 100 µM, $CaCl_2$, and ABA showed maximum seed germination percentage (data not shown), and hence, the same concentration was selected for the present study. Significantly higher germination percentage and rate of germination observed in hydro- (water), chemo- ($CaCl_2$), and hormonal-(ABA) primed seeds as compared to non-primed seeds indicated a positive effect of seed

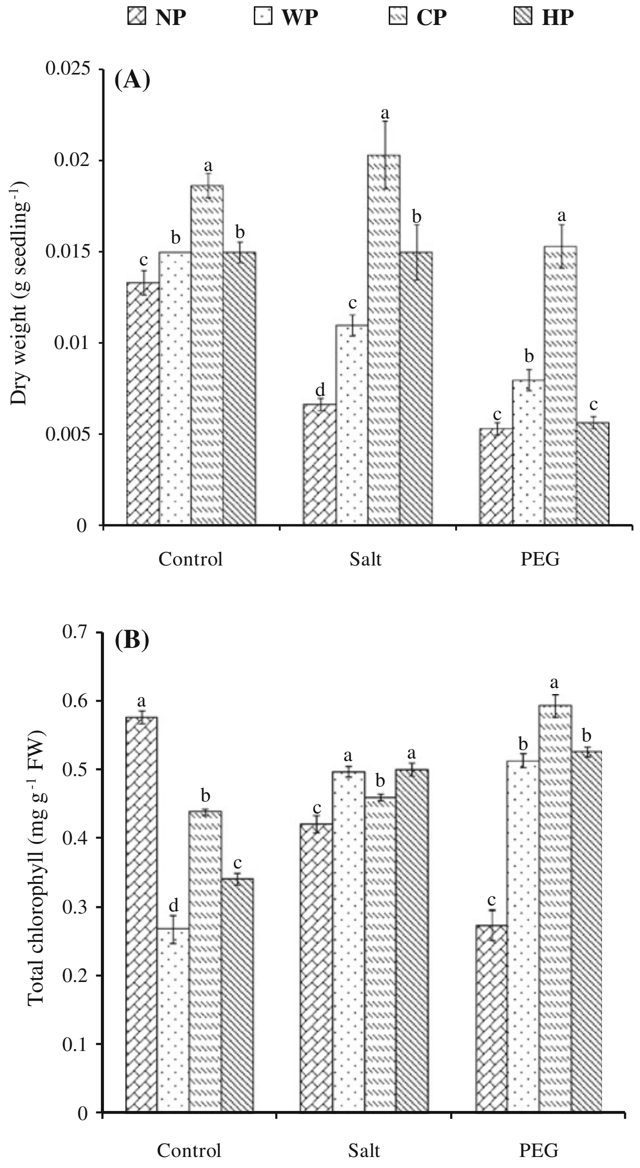

Fig. 3 Measurement of dry-weight and total chlorophyll content in seedlings derived from the seeds given different priming treatments. Seeds given different priming treatments such as water (WP)-, $CaCl_2$ (CP)-, and ABA (HP) along with the non-primed control (NP) were grown independently and then subjected to the iso-osmotic NaCl/PEG stress 15 days after germination. After 10 days of treatment, the dry-weight (**a**) and total chlorophyll content (**b**) were measured. The values represent the mean (± S.E.) of three replicates repeated thrice. *Different letters* indicate significantly different values at a particular duration (DMRT, $P < 0.05$)

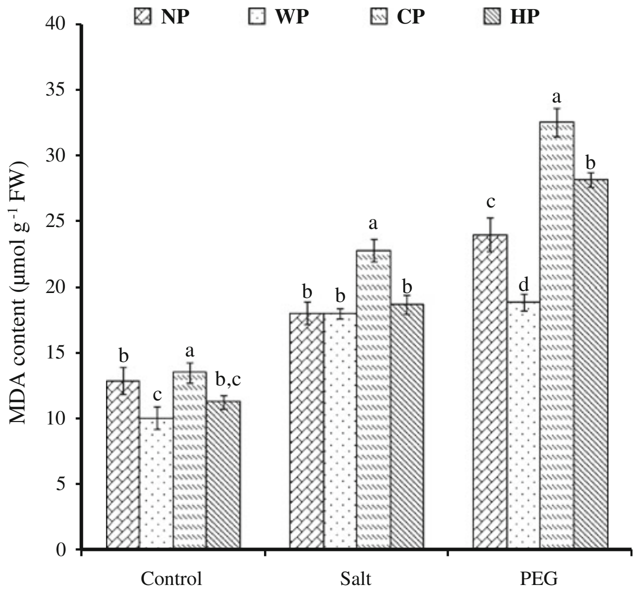

Fig. 4 Measurement of MDA content in seedlings derived from the seeds given different priming treatments. Seeds given different priming treatments such as water (WP)-, $CaCl_2$ (CP)-, and ABA (HP) along with the non-primed control (NP) were grown independently and then subjected to the iso-osmotic NaCl/PEG stress 15 days after germination. After 10 days of treatment, the MDA content was measured. The values represent the mean (± S.E.) of three replicates repeated thrice. *Different letters* indicate significantly different values at a particular duration (DMRT, $P < 0.05$)

priming in synchronizing the seed germination process (Fig. 1a, b). The increased rate of germination in primed seeds was found to be associated with an increased water uptake potential (data not shown). However, detailed investigation is required to reveal the mechanism of priming-mediated increase in the seed germination process.

After establishing the positive effect of priming at the seed germination stage, the next objective was to evaluate its potential for ameliorating the effects of NaCl and PEG stress. In order to achieve this, the differential phenotype of the seedlings derived from the primed and non-primed seeds were compared (Fig. 2) and their growth was measured in terms of dry weight and chlorophyll content (Fig. 3). In general, under NaCl and PEG stress, the dry weight and total chlorophyll content, an important parameter for the measurement of growth (Brougham 1960), decreased in NP as compared to that of primed treatments. The decrease in chlorophyll content might be attributed to the increased degradation of chlorophyll pigments due to stress-induced metabolic imbalance (Ashraf et al. 1994). In addition, high osmotic pressure generated under stress has been suggested to create an interference with the de novo synthesis of the structural components of chlorophyll (Kaiser et al. 1983). Although, at present, the underlying mechanisms of priming-mediated response is not clear, the results certainly demonstrated the positive effect of seed priming in improving the growth of seedlings under NaCl/PEG stress.

The NaCl and PEG stress also led to an excessive generation of reactive oxygen species (ROS), such as superoxide radicals, which cause various damages including the peroxidation of membrane lipids (Bartels and Sunkar 2005). The NaCl and PEG stress led to the significant increase in the level of MDA in all the treatments

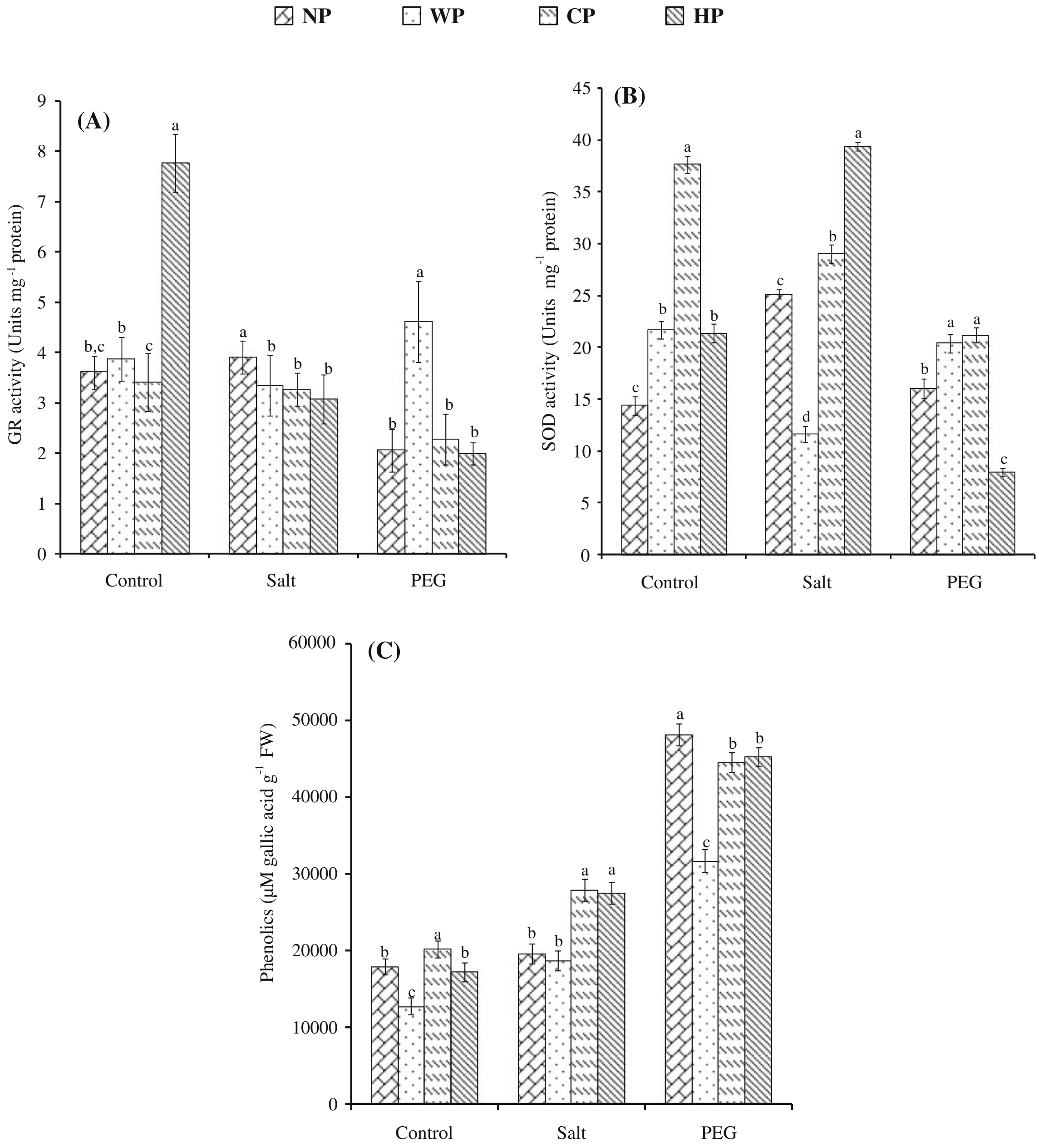

Fig. 5 Measurement of activities of GR, SOD, and phenolic content in seedlings derived from the seeds given different priming treatments. Seeds given different priming treatments such as water (WP)-, $CaCl_2$ (CP)-, and ABA (HP) along with the non-primed control (NP) were grown independently and then subjected to the iso-osmotic NaCl/PEG stress 15 days after germination. After 10 days of treatment, the GR (**a**) and SOD (**b**) activities along with the phenolic content (**c**) were measured. The values represent the mean (± S.E.) of three replicates repeated thrice. *Different letters* indicate significantly different values at a particular duration (DMRT, $P < 0.05$)

which indicated the induction of oxidative stress. However, the least increase in the MDA content in WP treatment suggested that the extent of oxidative stress was comparatively lower in WP than that of other treatments (Fig. 4). Among NaCl and PEG stress, the PEG was found to impose more damage as compared to iso-osmotic NaCl in terms of MDA accumulation. This could be attributed to the impermeable nature of the PEG, which probably maintained the high osmotic pressure in the external solution throughout the period of stress, whereas, under NaCl stress, the ions might get sequestered in different compartments leading to time-dependent decrease in the effective stress Ahmad et al. (2007). On the basis of chlorophyll and MDA contents, WP treatment was found most effective in ameliorating the effects of NaCl and PEG stress.

The protection against the ROS is achieved through the stimulation of different antioxidant mechanisms, which

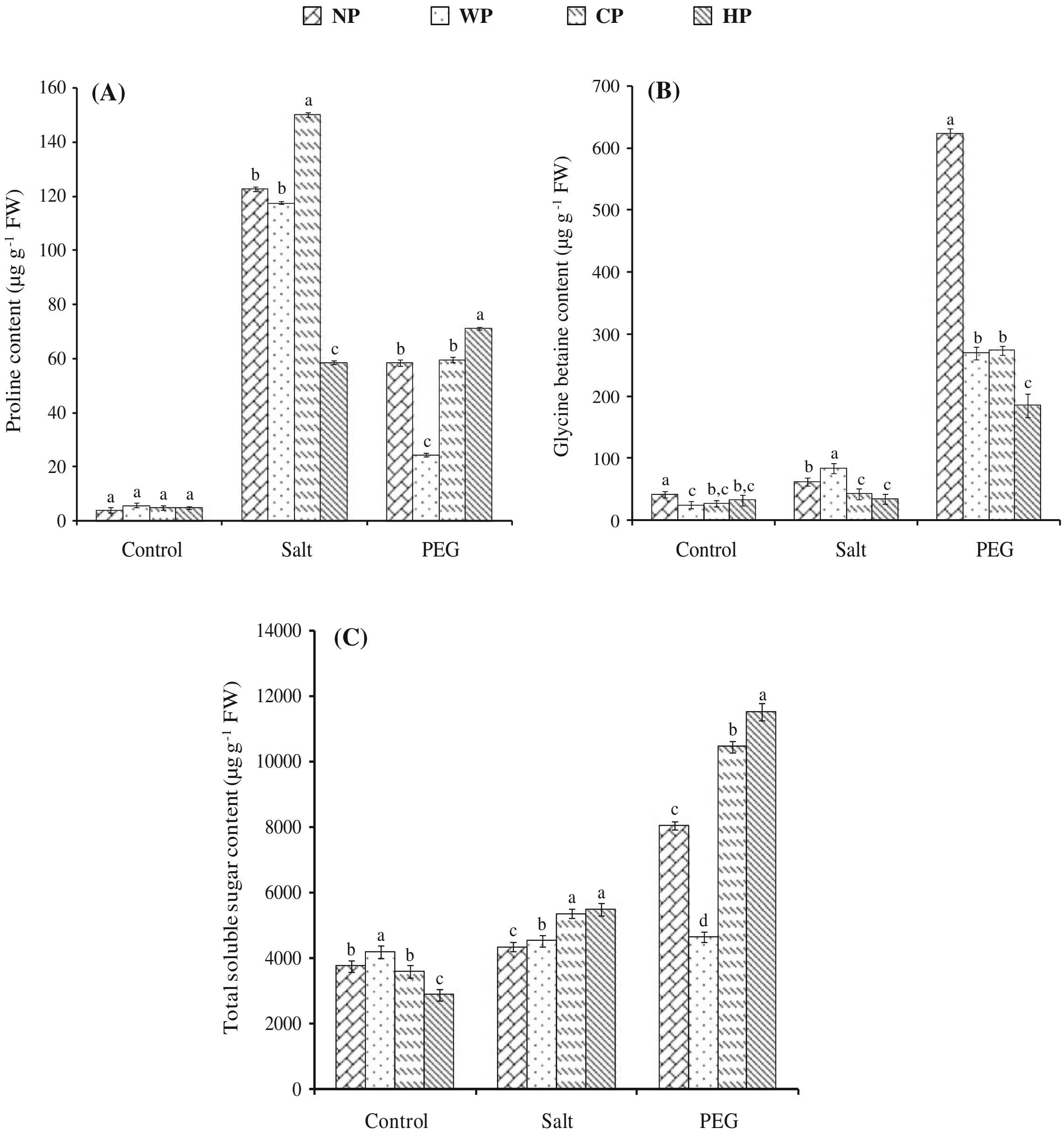

Fig. 6 Measurement of the level of free proline, glycine betaine, and total soluble sugars in seedlings derived from the seeds given different priming treatments. Seeds given different priming treatments such as water (WP)-, $CaCl_2$ (CP)-, and ABA (HP) along with the non-primed control (NP) were grown independently and then subjected to the iso-osmotic NaCl/PEG stress 15 days after germination. After 10 days of treatment, the levels of proline (**a**), glycine betaine (**b**), and total soluble sugars (**c**) were measured. The values represent the mean (± S.E.) of three replicates repeated thrice. *Different letters* indicate significantly different values at a particular duration (DMRT, $P < 0.05$)

include enzymatic (such as SOD, GR etc.) and non-enzymatic (reduced glutathione, tocopherol, carotenoids, phenolics etc.) constituents (Mittler 2002). Among the antioxidant enzymes, SOD constitutes the primary line of defense as it dismutates the superoxide radicals to hydrogen peroxide (Fatima and Ahmad 2004). Subsequent detoxification of hydrogen peroxide may be achieved by the action of catalase or ascorbate–glutathione cycle. In this connection, GR constitutes an important component catalyzing the conversion of oxidized glutathione (GSSG) to reduced form (GSH) and thus maintaining the proper functioning of ascorbate–glutathione cycle. In addition, GSH itself is an important non-enzymatic antioxidant, which may directly quench ROS species (Alscher 1989). Therefore, the activity of SOD and GR was measured under different treatments to understand the mechanism of priming-mediated detoxification of ROS. In WP treatment, the SOD activity was not increased in response to NaCl and

PEG stress as compared to control (Fig. 5b), which signified the reduction in the extent of ROS production and thus the effective stress (Badawi et al. 2004). Unlike SOD, no significant change in the activity of GR was observed under salt stress but an increase under PEG stress in WP (Fig. 5a). This suggested stimulation of the enzyme activities as per the need since PEG was found to impose a greater stress as compared to salt in the present study. Significantly higher GR activity under PEG stress in WP as compared to other treatments would lead to a greater availability of GSH and thus better ROS detoxification. This is also supported by a previous study which demonstrated that overexpression of GR in wheat protoplast led to an increase in GSH content and GSH/GSSG ratio, which resulted in the maintenance of cellular redox state under drought stress (Melchiorre et al. 2009). A better ROS detoxification was also obvious from the decrease in MDA content in WP treatment as compared to other treatments, particularly under PEG stress (Fig. 3). In contrast to WP, CP and HP treatments decreased the SOD and GR activities under both NaCl and PEG stress as compared to control (except for SOD activity in HP under NaCl stress) which indicated the lack of co-ordination in modulating the activities of antioxidant enzymes as per the requirements of the plant. Apart from antioxidant enzymes, the phenolics are also known for their ability to scavenge free radicals (Hichem et al. 2009). In both primed and non-primed treatments, the level of total phenolics was increased in response to NaCl and PEG stress as compared to control; however, the WP treatment demonstrated least increase as compared to other treatments (Fig. 5c). This further supports the hypothesis that WP treatment effectively decreased the NaCl- and PEG-stress-induced oxidative damage.

Apart from oxidative stress, the NaCl and PEG treatments also led to the accumulation of different osmolytes inside the cell required to maintain a continuous flow of water under stress situations (Cushman 2001). Proline is the most important among different osmolytes, and is known to interact with crucial macromolecules of the cell to maintain their biological activity under stressed conditions (Gangopadhyay and Basu 2000). In addition, under in vitro system, it is established as an osmotic stress marker and its accumulation increases manyfold upon exposure to salt (Camara et al. 2000) and drought (He and Yu 1995). Apart from proline, glycine betaine and total soluble sugars also play important roles in maintaining the osmotic balance under stress. Our results indicated that proline was accumulated to a higher level under NaCl stress (Fig. 6a) while glycine betaine (Fig. 6b) and total soluble sugars (Fig. 6c) accumulated under PEG stress in NP treatment, indicating the plant's response to maintain the osmotic homeostasis. Among the different priming treatments, WP, in general, showed lower accumulation of different osmolytes under both NaCl and PEG stress, as compared to other treatments, which signified the better maintenance of water balance in WP treatment that delimited the need for the synthesis of osmolytes.

Therefore, antioxidant and osmolyte responses together indicated that in WP treatment the effective stress is somehow reduced, and hence, the need for increased antioxidant metabolism and osmolyte accumulation is reduced. Such an effect might be attributed to the positive changes at the epigenetic level at an early stage of seed treatment in the form of ‘‘stress imprint’’ that presumably played an important role in enabling the long-term modulation in the gene expression (Bruce et al. 2007). We propose that the phase of re-drying of seeds during priming leads to a sort of water stress that might cause the epigenetic changes to occur and activate set of genes to combat the stress. This sort of water stress might be optimum (neither low nor too high) in case of WP treatment that allowed the epigenetic changes to occur with maximum efficiency. However, this proposed hypothesis needs further investigation. Nevertheless, we demonstrate that seedlings given WP treatment perform better under both salt and PEG stress as compared to NP, CP, and HP treatments. This is in accordance with the previous reports substantiating the importance of hydropriming for the better germination and growth of plants under both saline and non-saline conditions viz., *Acacia tortilis* (Rehman et al. 1998), wheat (Kamboh et al. 2000) and maize, rice, and chickpea (Harris et al. 2001). In conclusion, our study is an important step in establishing WP (hydro-priming) as an economical tool for imparting the stress tolerance in crop plants. Further exploration under field conditions would provide more insights into the yield components.

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