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RESEARCH ARTICLE

Seed Halopriming Outdo Hydropriming in Enhancing Seedling Vigor and Osmotic Stress Tolerance Potential of Rice Varieties

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Abstract

Seed priming improves the seed performance and also helps the seedlings to alleviate the detrimental effects of various stresses. Seed priming is believed to bring about some biochemical changes in the metabolism within the seed, which ultimately favors germination and the further growth stages of the seedlings even under stressed conditions. The present investigation was carried out to study the effect of hydropriming and halopriming in three rice varieties (Neeraja, Vaisakh, and Vytilla 6), with varied abiotic stress tolerance potential under NaCl and PEG stress. In general, the application of both stresses, NaCl and PEG induced retardation of growth and metabolism of the seedlings. However, seed priming treatments could reduce the extent of decrease in these biological attributes. Both hydro- and halopriming resulted in the enhancement of protein, carbohydrate, and photosynthetic pigment content, modulated antioxidant enzyme activities, reduced the lipid peroxidation of biomembranes, and enhanced the photochemistry and mitochondrial activities in rice seedlings subjected to NaCl and PEG stress as compared to non-primed ones. According to the various morphological, physiological, and biochemical characteristics studied in the rice seedlings raised from primed and non-primed seeds, we confirmed that both hydropriming and halopriming had a positive influence on stimulating metabolism in rice seeds, which ultimately resulted in improved seedling vigor and tolerance under NaCl and PEG stress. Halopriming was found to be more efficient than hydropriming in enhancing the seedling vigor, overall growth, and stress tolerance potential of rice varieties.

Key words : Abiotic stress, halopriming, hydropriming, mitochondrial activity, photochemical activity, seed priming

Introduction

Crop productivity is adversely influenced by various biotic and abiotic stresses of which drought and salinity are the most common abiotic stresses to which plants are frequently exposed and in many regions, these stresses form the bottleneck of agricultural development. The inhibitory affects of salinity include lowering of soil water potential, ion toxicity, and ionic imbalance within the tissues (Dubey 1997; Hasegawa et al. 2000; Rontein et al. 2002). Similarly, drought induces the reduction of water content, loss of turgidity, reduction in total water potential, wilting, closure of stomata, and decrease in cell enlargement and growth. Severe water stress even results in the inhibition of photosynthesis, disturbance of metabolism, and finally death of plants (Amarjit et al. 2005; Berry et al. 1988). According to Tavilli et al. (2011), the physiological mechanisms through which plants respond to salinity and drought show high similarity, thus suggesting that both these stresses must be perceived primarily by the plant cell as deprivation of water.

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world and forms the staple food of more than 50% of the world's population and is the most important food crop in India (Khush 2005). The traditional agricultural practices are not sufficient to produce rice grains according to the needs of an ever-increasing world population. It has been postulated that the world's annual paddy production must have to increase up to 781 million tons by 2020 and over a billion tons by the next century (Sass and Cicerone 2002). In India, due to the irregularity of rainfall distribution, the rice-growing areas

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frequently experience severe drought, which directly influences rice productivity (Dey and Upadhyay 1996). In rainfed ecosystems, drought is the leading environmental stress in rice and furthermore, rice is also highly sensitive to salinity and its tolerance varies with growth stages. In rice, seed germination and seedling growth stages are very sensitive to abiotic stresses, especially salinity (Deivanai et al. 2011).

Various strategies are employed from time to time to generate plants which can withstand abiotic stresses. Among the different methods used to overcome the problem of poor seed germination and seedling establishment in crop plants under abiotic stresses, seed treatment with different agents (seed priming) has gained much importance recently because of it is effective and cheap (Basra et al. 2005; Harris et al. 1999; Iqbal and Ashraf 2005, 2007). Seed priming is the induction of a particular physiological state in plants by the treatment of natural and/or synthetic compounds to the seeds before germination. In seed priming, the seeds are either soaked in water (hydropriming), PEG (osmopriming), or salt ($CaCl_2$, $CaSO_4$, or NaCl, etc.), or any other chemical prior to germination (Patade et al. 2009).

The beneficial effects of seed priming include faster emergence, better stands, and lower incidence of re-sowing, more vigorous plants, better drought tolerance, earlier flowering, earlier harvest, and higher grain yield (Harris et al. 1999). These beneficial effects of seed priming are due to several reasons such as activation of enzymes associated with endosperm utilization and seed germination (Habib et al. 2010), mobilization of storage proteins, changes in hormonal balance (Iqbal and Ashraf 2005, 2007, 2010, 2013), and synthesis of proteins that play an important role during seed germination (Gallardo et al. 2001). Priming for enhanced resistance to abiotic stress is obviously operating via various pathways involved in different metabolic processes (Jisha et al. 2013). There are many reports on the different seed priming methods in rice (Anwar et al. 2013; Basra et al. 2006; Birendra and Shambhoo 2011; Farooq et al. 2005, 2006a, b), but there are very less work done to elucidate the physiological and biochemical changes occurring in rice seedlings after the seed priming treatment.

The present investigation was carried out to compare the effect of hydropriming and halopriming on osmotic stress tolerance potential of rice varieties with varied tolerance potential to NaCl and drought, by analyzing the biochemical changes and primary metabolism in seedlings emerging from primed and non-primed seeds subjected to the above stress conditions.

Materials and Methods

Materials

The three rice varieties selected for the present study were based on their varied levels of drought/NaCl tolerance potential. 'Neeraja' is a abiotic stress-sensitive variety whereas 'Vaisakh' is drought tolerant and 'Vytilla 6' is a NaCl-tolerant variety. The seeds of rice varieties: Neeraja and Vaisakh were procured from the regional rice research station, Pattambi, Kerala, India and the seeds of Vytilla 6 variety were procured from the rice research station, Vytilla, Kerala, India.

Methods

Seed priming techniques **:** Uniform-sized seeds of rice were selected for the seed priming and were immersed in distilled water for hydropriming. For halopriming treatments, the seeds were immersed in different concentrations of NaCl solution (0, 25, 50, 75, 100 mM). The seeds were immersed in respective solutions, the volume of which was three times more than that of the total seed weight. After 12 h priming treatment in a screw-cap bottle, the seeds were washed with distilled water for 2 min and surface dried on absorbent paper. Later, seeds were placed on a piece of clean filter paper, allowing dehydration under shade at 25°C for 48 h to retrieve the original seed moisture before priming treatment. The primed seeds were allowed to germinate immediately after the drying process. The unprimed seeds were used as the control. Primed as well as non-primed seeds were germinated in light transparent plastic bottles (19 × 11 cm). The bottom of the bottles were lined with absorbent cotton and soaked with distilled water (control) or different concentrations of NaCl/PEG solution as the case may be. To select stress imparting concentrations of NaCl and PEG, the rice seeds were germinated in various concentrations of NaCl (0, 25, 50, 75, 100, 125 mM) / PEG (0, 5, 10, 15, 20, 25%).

The bottles were kept under continuous light (120 mmol m^{-2} s^{-1}) at 25 + 2 °C. All the seed materials used for investigation were pre-washed for 1 min with 0.25% Triton X-100 (Boehringer Mannheim Gmbh). The growth and biochemical attributes of primed, non-primed, as well as control seedlings were recorded on 9 days after germination.

Morphological, physiological, and biochemical studies **:** Shoot length was measured with the help of a student scale. For fresh and dry weight measurements, the seedlings were blotted and wrapped separately in pre-weighed labeled aluminium foil. Fresh weights of the samples were determined by weighing them immediately after wrapping. For dry weight measurements, the samples were kept in an oven maintained at 80°C. After 48 h, the samples were transferred to a desiccator, allowed to cool, and then weighed.

Chlorophyll estimation was carried out by the method of Arnon (1949). Total carbohydrate was estimated according to Dubois et al. (1956). Protein content was estimated using Folin-Ciocalteau reagent according to the method of Lowry et al. (1951). Proline content in the seedlings was estimated as per Bates et al. (1973). The malondialdehyde (MDA) content estimation was done according to Heath and Packer (1968). Nitrate reductase activity was measured according to the method suggested by Hageman and Reed (1980). For the esti-

Table 1. Shoot length, fresh weight and dry weight of the seedlings of three rice varieties under hydro- and halopriming exposed to 0, NaCl, and PEG-6000 stress. The data is an average of recordings from three independent experiments each with three replicates (i.e. n = 9). The data represent mean ± standard error. Analysis of variance revealed a significant difference in SL and FW (P = 0.01) and DW (P = 0.05) due to the interaction effects of priming, variety, and treatment.

VARIETIES	SHOOT LENGTH/PLANT (cm)			FRESH WEIGHT/PLANT (g)			DRY WEIGHT/PLANT (g)		
	0 stress	NaCl stress	PEG stress	0 stress	NaCl stress	PEG stress	0 stress	NaCl stress	PEG stress
NEERAJA *Hydropriming*	13.90 ± 0.51 (10.47 ± 0.43)	13.00 ± 0.60 (7.90 ± 0.31)	7.11 ± 0.56 (5.30 ± 0.32)	0.0998 ± 0.01 (0.0928 ± 0.01)	0.0997 ± 0.01 (0.0750 ± 0.01)	0.0700 ± 0.01 (0.0548 ± 0.01)	0.0221 ± 0.01 (0.0181 ± 0.01)	0.0156 ± 0.01 (0.0109 ± 0.01)	0.0168 ± 0.01 (0.0111 ± 0.01)
Halo priming	9.60 ± 0.51 (08.03 ± 0.43)	6.01 ± 0.43 (4.63 ± 0.21)	6.70 ± 0.46 (6.13 ± 0.32)	0.0603 ± 0.01 (0.0547 ± 0.01)	0.0534 ± 0.01 (0.0505 ± 0.01)	0.0489 ± 0.01 (0.0465 ± 0.01)	0.0168 ± 0.01 (0.0158 ± 0.01)	0.0184 ± 0.01 (0.0135 ± 0.01)	0.0150 ± 0.01 (0.0108 ± 0.01)
VAISAKH *Hydropriming*	12.43 ± 0.75 (11.80 ± 0.64)	12.40 ± 0.43 (9.93 ± 0.32)	5.95 ± 0.21 (4.18 ± 0.28)	0.0640 ± 0.01 (0.0637 ± 0.01)	0.0638 ± 0.01 (0.0557 ± 0.01)	0.0465 ± 0.01 (0.0413 ± 0.01)	0.0131 ± 0.01 (0.0113 ± 0.01)	0.0091 ± 0.01 (0.0061 ± 0.01)	0.0092 ± 0.01 (0.0078 ± 0.01)
Halo priming	11.33 ± 0.75 (09.40 ± 0.64)	8.73 ± 0.43 (7.90 ± 0.32)	8.67 ± 0.31 (4.97 ± 0.28)	0.0710 ± 0.01 (0.0684 ± 0.01)	0.0669 ± 0.01 (0.0545 ± 0.01)	0.0520 ± 0.01 (0.0459 ± 0.01)	0.0212 ± 0.01 (0.0208 ± 0.01)	0.0248 ± 0.01 (0.0197 ± 0.01)	0.0260 ± 0.01 (0.0206 ± 0.01)
VYTILLA 6 *Hydropriming*	13.13 ± 0.75 (10.62 ± 0.58)	12.45 ± 0.51 (7.37 ± 0.38)	6.22 ± 0.25 (4.50 ± 0.22)	0.0833 ± 0.01 (0.0810 ± 0.01)	0.0905 ± 0.01 (0.0747 ± 0.01)	0.0615 ± 0.01 (0.0535 ± 0.01)	0.0156 ± 0.01 (0.0145 ± 0.01)	0.0120 ± 0.01 (0.0097 ± 0.01)	0.0140 ± 0.01 (0.0098 ± 0.01)
Halopriming	12.43 ± 0.75 (9.23 ± 0.58)	9.13 ± 0.51 (4.37 ± 0.28)	7.53 ± 0.45 (4.77 ± 0.22)	0.0838 ± 0.01 (0.0820 ± 0.01)	0.0727 ± 0.01 (0.0558 ± 0.01)	0.0623 ± 0.01 (0.0535 ± 0.01)	0.0224 ± 0.01 (0.0214 ± 0.01)	0.0241 ± 0.01 (0.0172 ± 0.01)	0.0262 ± 0.01 (0.0198 ± 0.01)

*The values in the parenthesis denote the value of respective parameters in the seedlings of three rice varieties raised from non-primed seeds exposed to 0, NaCl, and PEG-6000 stress

mation of superoxide dismutase (SOD) activity, the protocol of Giannoplitis and Reis (1977) was adopted. The peroxidase (PER) assay was done as per the protocol of Gaspar et al. (1975).

Thylakoids from leaves were isolated according to the method of Puthur (2000). The photochemical activities of thylakoids were assayed polarographically with a Clark-type oxygen electrode (DW1/AD, Hansatech, Norflok, UK) at 4°C. The light-dependent O_2 uptake/evolution was measured by irradiating the sample with saturating intensity of white light (~1800 mmol photons m^{-2} s^{-1}), provided by a 100 W halogen lamp (LS2, Hansatech). The activity of PS I and PS II was calculated in terms of nmol of O_2 consumed/evolved mg chlorophyll/min.

Mitochondrial isolation from the seedlings was carried out according to Kolloffel (1967). Oxygen consumption by mitochondria was measured polarographically with a Clark-type oxygen electrode (DW1/AD, Hansatech) which was connected to a digital control box (OXYG1, Hansatech) at 25°C as per the protocol of Schmitt and Dizengremel (1989). The mitochondrial activity was calculated in terms of nmol O_2 consumed/mg protein/min.

Statistical analysis **:** Univariate analysis of variance (ANOVA) was conducted with SPSS 18 by taking priming, varieties and treatments as factors and the treatment means were compared with LSD (least significant difference) wherever necessary in the various morphological, physiological, and biochemical parameters studied.

Results and Discussion

Standardization of halopriming concentration and stress imparting concentration

After halopriming treatments, the seeds were germinated as described in Materials and Methods. The growth parameters data (shoot length, fresh and dry weight) revealed that the most effective halopriming concentrations were 50 mM for Neeraja and Vaisakh, 75 mM for Vytilla 6 (Supplementary Table 1).

The concentration of NaCl and PEG which imparted 40-50% retardation in various growth parameters (shoot length, fresh and dry weight) of each variety was selected as stress imparting concentrations. Neeraja showed 50% growth retardation at 75 mM NaCl and 15% PEG; whereas, it was at 75 mM NaCl and 20% PEG for Vaisakh and 100 mM NaCl and 20% PEG for Vytilla 6 (Supplementary Figs. 1 and 2).

Seedling growth parameters

In general, the application of stress (NaCl/PEG) induces retardation of growth (measured in terms of shoot length, fresh and dry weight) in seedlings raised from primed and non-primed seeds. However, seed priming could decrease the extent of reduction in growth of the seedlings as compared to the seedlings raised from non-primed seeds. The results with respect to various parameters recorded in seedlings raised from primed seeds were described in comparison to the values recorded for each parameter of the seedlings raised from non-primed seeds.

The varieties Neeraja and Vytilla 6 recorded a prominent increase in the shoot length of seedlings raised from hydroprimed seeds and subjected to NaCl stress (65 and 69%, respectively), while the drought tolerant variety Vaisakh on hydropriming, showed an increase in shoot length of seedlings which were subjected to PEG stress (42%). Halopriming was found to be effective in bringing about a prominent increase in shoot length of seedlings particularly in the tolerant varieties subjected to the particular stressed conditions, for which the variety is known to be tolerant. Haloprimed Vytilla 6 showed the maximum increase in the shoot length of seedlings subject-

Table 2. Photosynthetic pigment content of leaves of seedlings of three rice varieties under hydro- and halopriming exposed to 0, NaCl and PEG-6000 stress. The data is an average of recordings from three independent experiments each with three replicates (i.e. n = 9). The data represent mean ± standard error. Analysis of variance revealed significant differences inchlorophyll a, chlorophyll b, total chlorophyll, and carotenoids (P = 0.01) due to the interaction effects of priming, variety, and treatment

VARIETIES	Chlorophyll a (mg/g dw)			Chlorophyll b (mg/g dw)			Total chlorophyll (mg/g dw)			Carotenoids (mg/g dw)		
	0 stress	NaCl stress	PEG stress	0 stress	NaCl stress	PEG stress	0 stress	NaCl stress	PEG stress	0 stress	NaCl stress	PEG stress
NEERAJA Hydropriming	2.21 ± 0.36 (1.55 ± 0.25)	1.74 ± 0.26 (1.54 ± 0.23)	1.34 ± 0.36 (1.19 ± 0.25)	0.64 ± 0.05 (0.43 ± 0.03)	0.46 ± 0.04 (0.41 ± 0.03)	0.37 ± 0.04 (0.32 ± 0.03)	2.84 ± 0.56 (2.00 ± 0.64)	2.19 ± 0.35 (1.95 ± 0.33)	1.71 ± 0.45 (1.51 ± 0.21)	0.81 ± 0.12 (0.61 ± 0.11)	0.65 ± 0.05 (0.58 ± 0.06)	0.49 ± 0.10 (0.46 ± 0.06)
Halo priming	1.80 ± 0.06 (1.64 ± 0.05)	2.55 ± 0.76 (1.56 ± 0.83)	1.54 ± 0.36 (1.17 ± 0.45)	0.52 ± 0.01 (0.47 ± 0.01)	0.79 ± 0.03 (0.48 ± 0.03)	0.47 ± 0.02 (0.37 ± 0.01)	2.32 ± 0.11 (2.11 ± 0.14)	3.34 ± 0.07 (2.04 ± 0.04)	2.01 ± 0.12 (1.53 ± 0.06)	0.74 ± 0.03 (0.71 ± 0.03)	1.05 ± 0.09 (0.62 ± 0.06)	0.65 ± 0.03 (0.51 ± 0.02)
VAISAKH Hydropriming	2.12 ± 0.16 (2.09 ± 0.05)	2.26 ± 0.15 (2.05 ± 0.14)	1.35 ± 0.07 (1.02 ± 0.06)	0.58 ± 0.03 (0.58 ± 0.01)	0.61 ± 0.05 (0.57 ± 0.04)	0.40 ± 0.03 (0.28 ± 0.02)	2.69 ± 0.15 (2.65 ± 0.16)	2.86 ± 0.25 (2.61 ± 0.31)	1.76 ± 0.14 (1.29 ± 0.12)	1.31 ± 0.05 (1.02 ± 0.04)	1.05 ± 0.04 (0.98 ± 0.05)	0.71 ± 0.05 (0.54 ± 0.06)
Halo priming	2.22 ± 0.16 (2.13 ± 0.13)	2.59 ± 0.15 (2.04 ± 0.11)	1.69 ± 0.03 (1.04 ± 0.02)	0.68 ± 0.03 (0.48 ± 0.02)	0.69 ± 0.04 (0.54 ± 0.03)	0.63 ± 0.01 (0.29 ± 0.01)	2.89 ± 0.15 (2.61 ± 0.14)	3.28 ± 0.15 (2.58 ± 0.15)	2.32 ± 0.04 (1.33 ± 0.02)	1.28 ± 0.06 (1.26 ± 0.05)	1.06 ± 0.03 (0.87 ± 0.06)	1.06 ± 0.02 (0.50 ± 0.01)
VYTILLA 6 Hydropriming	3.99 ± 0.26 (2.90 ± 0.15)	2.95 ± 0.11 (2.41 ± 0.10)	2.95 ± 0.13 (2.72 ± 0.18)	1.28 ± 0.05 (0.97 ± 0.04)	0.91 ± 0.04 (0.79 ± 0.03)	0.95 ± 0.06 (0.83 ± 0.06)	5.96 ± 0.32 (3.86 ± 0.21)	3.85 ± 0.17 (3.20 ± 0.18)	3.89 ± 0.25 (3.56 ± 0.24)	1.67 ± 0.04 (1.34 ± 0.07)	1.19 ± 0.04 (1.08 ± 0.02)	1.25 ± 0.06 (1.17 ± 0.05)
Halopriming	4.10 ± 0.21 (2.84 ± 0.18)	3.16 ± 0.13 (2.45 ± 0.15)	2.80 ± 0.04 (2.54 ± 0.05)	1.15 ± 0.05 (0.80 ± 0.06)	0.95 ± 0.02 (0.73 ± 0.01)	1.50 ± 0.01 (0.84 ± 0.02)	5.24 ± 0.24 (3.64 ± 0.14)	4.12 ± 0.14 (3.17 ± 0.23)	4.42 ± 0.05 (3.37 ± 0.04)	1.66 ± 0.05 (1.14 ± 0.05)	1.31 ± 0.06 (1.07 ± 0.07)	1.30 ± 0.04 (1.04 a 0.03)

*The values in the parenthesis denote the value of photosynthetic pigments in leaves of the seedlings of three rice varieties raised from non-primed seeds exposed to 0, NaCl, and PEG-6000 stress.

ed to NaCl stress (109%) and haloprimed Vaisakh showed a prominent enhancement in the shoot length under PEG stress (74%) when compared to non-primed ones of respective varieties. But in the abiotic stress sensitive variety Neeraja, halopriming was not able to bring about such an increase in shoot length of the seedlings (Table 1).

In general, seedling fresh weight was found to increase under hydropriming and it was higher in the stressed conditions than in unstressed condition in all three varieties. Hydropriming resulted in enhancement of the fresh weight in the case of Neeraja (28 - 32%) seedlings which were subjected to stressed (NaCl/PEG) conditions, while in other two varieties hydropriming could only bring about a lesser increase in fresh weight of the seedlings (an increase of only 1 - 21%). Although, halopriming of tolerant *O. sativa* varieties (Vaisakh and Vytilla 6) caused an increase up to 30% in the fresh weight of seedlings subjected to unstressed and stressed (NaCl/PEG) conditions, it was found to be less effective in bringing about prominent enhancement of seedling fresh weight in the abiotic stress sensitive variety Neeraja (Table 1).

The variety Neeraja on hydropriming recorded an almost equal rate of enhancement (43 and 51%) in the dry weight of seedlings which were subjected to stressed (NaCl/PEG) conditions, whereas in Vaisakh, hydropriming resulted in an enhancement of the dry weight under NaCl stress (49%) conditions and in Vytilla 6, under PEG stress (43%) conditions. Halopriming also led to a prominent increase in dry weight (up to 40%) under stressed (NaCl/PEG) conditions while in the unstressed conditions all varieties showed a lower percentage of increase in dry weight (up to 6%) of the seedlings (Table 1)

The functional mechanism of seed priming is to initiate the repairing system for damaged membranes and also to facilitate the metabolic preparation for germination through controlling the water absorption rate of seeds. Because of these physiological and biochemical changes associated with seed priming, the germination rate and seedling vigor of wild rye (*Leymus chinensis*) plants increases (Liu et al. 2002). It has already been reported that seed priming treatments significantly improved the germination and seedling growth in sunflower and ajowan plants (Kaya et al. 2006; Mahdavi and Rahimi 2013).

From the growth parameters studied in rice varieties, Vaisakh and Vytilla 6 showed a high percentage of increase in shoot length and fresh weight when the seeds were haloprimed while the abiotic stress sensitive variety Neeraja showed maximum increase in the above parameters when the seeds were hydroprimed. The increase in growth parameters of the seedlings raised from primed seeds is due to the increased cell division or cell enlargement. The increase in size of the cell is a result of increased water uptake of seeds during the seed priming treatments, which in turn results in increased turgidity and finally results in enhanced growth of the embryo/seedlings. In rice, it was reported that seed priming treatments reduced the time taken to initiate the germination process, improves the rate of germination and synchronization, moreover enhances the lengths of shoots and roots and thus increases the fresh and dry mass of the seedlings (Farooq et al. 2006a; Mathew and Mohanasarida 2005). According to Yagmur and Kaydan (2008), in triticale, the increase in the seedling growth can be attributed to the higher water uptake by primed seeds as compared to non-primed ones, which further supports the faster and enhanced growth of the seedling.

Biochemical and physiological changes under priming

Photosynthetic pigments and activity of photosystems **:** Although both hydropriming and halopriming caused enhancement in photosynthetic pigment content of the seedlings in all

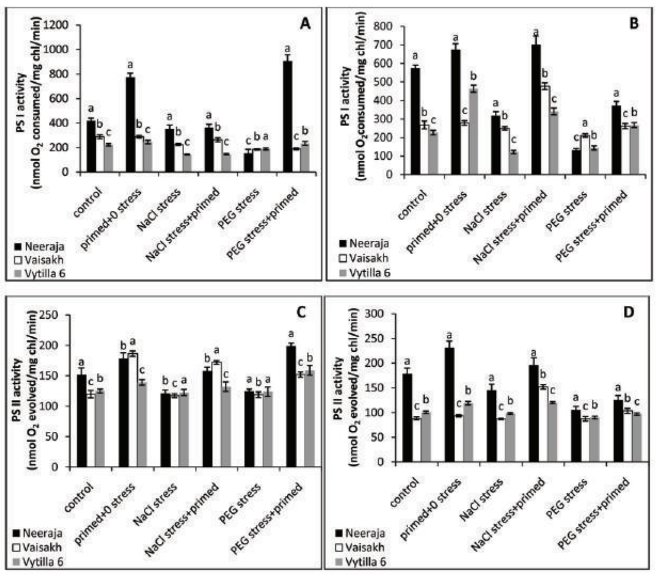

Fig. 1. Effect of hydropriming and halopriming on PS I activity (A, B); on PS II activity (C, D) of three rice varieties grown under 0, NaCl, and PEG-stressed conditions. The vertical bars represent SE of the mean value of recordings from three independent experiments each with a minimum of three replicates. Values with different letters are significantly different at 1% level ($P < 0.01$; ANOVA).

the three varieties studied, it was the halopriming treatment which resulted in the maximum increase. The exception to this was observed in the variety Neeraja subjected to unstressed condition, wherein hydropriming resulted in the maximum increase of photosynthetic pigment content (43 and 49% increase in chlorophyll *a* and *b* content, respectively). The haloprimed Vaisakh showed a prominent enhancement in chlorophyll *a* and *b* content (63 and 117%, respectively) under PEG stress, and halopriming in Vytilla 6 resulted in an increase of 44% in chlorophyll *a* content of the seedlings which were subjected to unstressed condition and 79% increase in chlorophyll *b* under PEG stress. Maximum enhancement in the carotenoid content was observed in the haloprimed Vaisakh subjected to PEG stress (111%) and was followed by haloprimed Neeraja subjected to NaCl stress (70%) (Table 2).

The activity of the photosystem I (PS I) increased significantly in the leaves of rice seedlings raised from haloprimed seeds under 0, NaCl, and PEG stresses. In Neeraja, halopriming highly enhanced the PS I activity of the seedlings which were raised under stressed conditions, but the increase in PS I activity was much lower and insignificant in seedlings raised from hydroprimed seeds except under 0 stress where a significant increase in PS I activity was observed (Figs. 1A and B). In general, the PS II activity of rice seedlings raised from hydro- and haloprimed seeds under non-stressed and stressed (NaCl/PEG) conditions showed increase as compared to their non-primed controls. The seedlings raised from hydroprimed seeds of Neeraja under PEG stress and that of haloprimed Vaisakh under NaCl stress showed significant increase in PS II activity (Figs. 1C and D).

As a result of NaCl/PEG stress, the photosynthetic pigment content and the activity of photosystems reduced in all the varieties studied. This reduction may be due to the degradation of chlorophyll pigments or degradation of complexes involved in photosynthetic machinery. According to some authors, the reduction in chlorophyll content under osmotic stress is due to the suppression of enzymes required for chlorophyll synthesis or may be due to the destruction of chloroplast and instability of pigment protein complex (Ashraf and Rasul 1988; El-Samad et al. 2011). The enhancement of photosynthetic pigments under hydro- and halopriming in all the three rice varieties points out towards the role of seed priming in positively influencing the synthesis of cholorophylls and carotenoids in the seedlings raised from primed seeds. Earlier it was reported that seed priming in rice caused increase in chlorophyll and carotenoid contents under NaCl stress (Jamil et al. 2013).

There are previous reports on the decline of PS I and PS II activities in plants under abiotic stresses. In wheat leaves, the number of PS II inactive centers increases upon NaCl treatment which ultimately leads to reduced PS II activity (Sing-Tomar et al. 2012). A reduced activity of C3 cycle under drought stress is reported to result in an increased production of superoxide on the acceptor side of PS I, ultimately reducing the PS I activity (Dat et al. 2000; Mittler 2002; Oukarroum et al. 2009). In the present study, seed priming significantly ameliorated the reduction in PS I and PS II activities under NaCl and PEG stress. Halopriming brought about significant increase in PS I activity of the rice seedlings as compared to PS II activity. The enhancement in PS I activity of haloprimed rice seedlings under conditions of NaCl and PEG stress could be explained as a means of meeting the demand for the additional requirement of ATP by the plants to counter the stress situation. It was already reported that the PS I activity in tolerant plants enhances under stress conditions to cope with stress and helps to meet the additional demand of ATP for countering stress (Sudhir et al. 2005). The increased PS I and PS II activities as a result of seed priming treatments might be due to an increase in the number of photosystem reaction centers or due to an increase in the efficiency of existing reaction centers.

Mitochondrial activity **:** Maximum increase in the mitochondrial activity was obtained in the seedlings of Neeraja which were raised from haloprimed seeds and grown under stressed conditions (59 and 61% increase under NaCl and PEG stress, respectively) as compared to the seedlings which were grown under unstressed conditions. In Vaisakh, it was the hydropriming treatment which resulted in more enhancement of the mitochondrial activity under both unstressed and stressed (NaCl/PEG) conditions. In Vytilla 6, hydropriming resulted in more enhancement in the mitochondrial activity of seedlings under NaCl stress when compared to that of seedlings raised from haloprimed seeds (Figs. 2A and B).

The reduction in mitochondrial activity under osmotic stress may be due to the closure of stomata which results in the non-availability of O_2. Moreover, abiotic stresses like salinity and drought usually result in the generation of reactive oxygen species (ROS) which damage the biomembranes

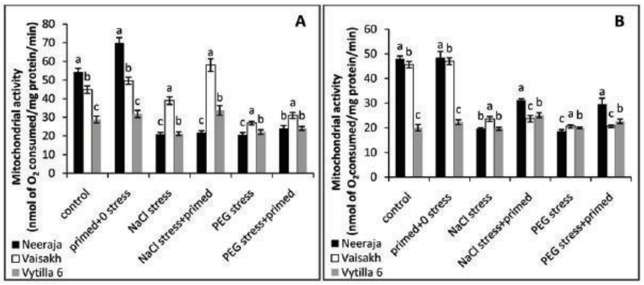

Fig. 2. Effect of hydropriming (A) and halopriming (B) on mitochondrial activity of three rice varieties grown under 0, NaCl, and PEG-stressed conditions. The vertical bars represent SE of the mean value of recordings from three independent experiments each with a minimum of three replicates. Values with different letters are significantly different at 1% level ($P < 0.01$; ANOVA).

especially that of mitochondria and chloroplast. This damage leads to loss of organelle intactness, finally affecting the activity of the mitochondria and also affects the carbon-fixing ability of the chloroplast (Scandalios 1993). There are earlier reports that salt stress has negative impacts on mitochondria causing decreased electron transport activities and increased lipid peroxidation due to the formation of reactive oxygen species (Chen et al. 2009; Mittova et al. 2004), but the seedlings raised from hydroprimed as well as haloprimed seeds showed a lower reduction in the mitochondrial activity. In the variety Neeraja, halopriming was found to be superior to hydropriming in preventing reduction in mitochondrial activities. Seed priming probably improved the mitochondrial intactness by maintaining the membrane integrity and/or resulted in an increase in the distribution of mitochondria in unit area. Seed priming is known to improve the integrity of the outer membrane of mitochondria and even increase the number of mitochondria in various plant species (Benamar et al. 2003; Varier et al. 2010).

***Total protein and total carbohydrate* :** The percentage of increase in total protein content in all three rice varieties which were hydro- and haloprimed and grown in unstressed and stressed (NaCl/PEG) conditions was to the extent of 1 to 43% (Figs. 3A and B). Total carbohydrate content was also increased in the rice seedlings which were raised from hydro- and haloprimed seeds. While haloprimed Neeraja and Vytilla 6 recorded a significant increase in total carbohydrate, hydropriming was unable to show such an increase in these varieties. But in the variety Vaisakh, hydropriming showed a significant increase in total carbohydrate content under unstressed and NaCl stressed conditions (Figs. 3C and D).

The increase of total protein content in the seedlings raised from primed (hydro- and haloprimed) seeds and that exposed to unstressed and stressed conditions (NaCl/PEG) indicate that seed priming resulted in the accumulation of certain additional proteins which might have a role in stress alleviation when the seedlings were exposed to NaCl/PEG stress. Under osmotic stresses, heat-shock proteins (HSPs), molecular chaperones and LEA protein families are known to get involved in conferring abiotic stress tolerances in plants (Wang et al. 2003; 2004). Moreover, Conrath (2006) reported the accumulation of stress proteins and transcription factors under the influence of priming. In amaranth, it was reported that seed priming increases total protein content in the seedlings (Moosavi et al. 2009). Halopriming was found to be superior to hydropriming in increasing the total carbohydrate content in Neeraja and Vytilla 6 varieties. The significant enhancement in the carbohydrates in plants raised from primed seeds, on exposure to stress could be to counter the stress by increasing the osmoticum in the seedlings. It was earlier found that in rice, total soluble sugar content increased in the seedlings raised from primed seeds (Mondal 2011; Nawas et al. 2013). Moreover, there are earlier reports that accumulations of soluble carbohydrates increase the tolerance to drought in plants (Kameil and Losel 1993).

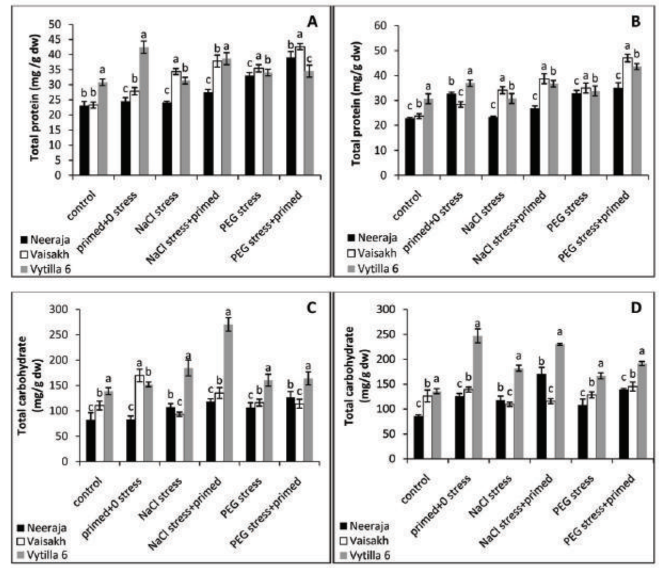

Fig. 3. Effect of hydropriming and halopriming on total protein (A, B); on total carbohydrate (C, D) of three rice varieties grown under 0, NaCl, and PEG-stressed conditions. The vertical bars represent SE of the mean value of recordings from three independent experiments each with a minimum of three replicates. Values with different letters are significantly different at 1% level ($P < 0.01$; ANOVA).

***Proline and MDA content* :** Proline accumulation occurred in the seedlings of all the three *O. sativa* varieties which were raised from hydroprimed seeds and grown under unstressed conditions. But under stressed (NaCl/PEG) conditions, reduction of proline content occurred in the *O. sativa* seedlings raised from hydroprimed seeds of all the three *O. sativa* varieties as compared to non-primed ones. Maximum accumulation of proline occurred in the seedlings of hydroprimed Vaisakh under unstressed (32%) conditions. On halopriming the seeds, proline content was found to reduce the variety Neeraja under PEG stress (56%), whereas in the variety Vaisakh, halopriming resulted in an increase of proline content under PEG stress (70%) as compared to non-primed ones (Figs. 4A and B).

The MDA content was found to decrease (7 - 63%) in the rice seedlings raised from hydroprimed and haloprimed seeds as compared to seedlings from non-primed seeds, either in the absence or presence of stress (NaCl/PEG). Maximum reduc-

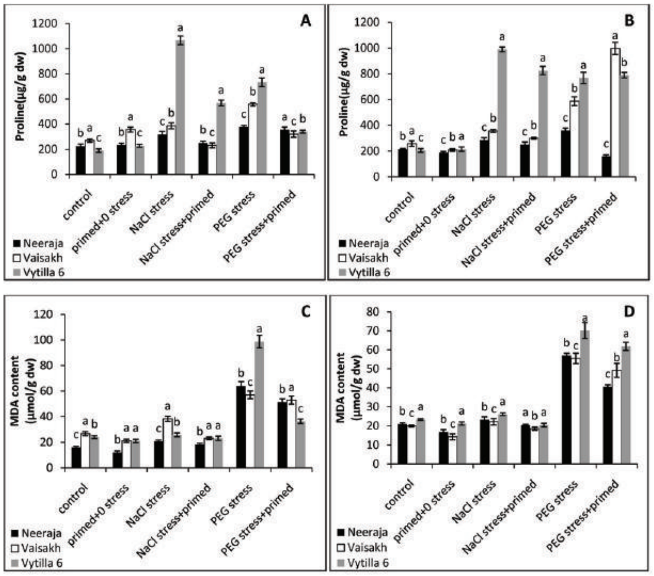

Fig. 4. Effect of hydropriming and halopriming on proline (A, B); on MDA (C, D) content of three rice varieties grown under 0, NaCl, and PEG-stressed conditions. The vertical bars represent SE of the mean value of recordings from three independent experiments each with a minimum of three replicates. Values with different letters are significantly different at 1% level ($P < 0.01$; ANOVA).

tion in the MDA content was observed in the Vytilla 6 seedlings which were raised from hydroprimed seeds and subjected to PEG stress (63%) (Figs. 4C and D).

Increase in the synthesis of proline is known as a common metabolic reaction of plants under stress (Behairy et al. 2012). Proline is strongly hydrophilic and it alleviates stress damage in plant cells by reducing the water potential. Proline, in addition to its major role as compatible solute, provides a carbon and nitrogen source for post-stress recovery growth, stabilizes membranes and protein machinery, scavenges free radicals, etc. (Hsu et al. 2003). In our study, seed priming differentially influenced the proline biosynthesis in rice varieties. The accumulation of proline mainly occurred in the haloprimed Vaisakh seedlings under PEG stress. As Vaisakh is a drought-tolerant variety, halopriming improved its tolerance by enhancing the proline metabolism to cope with PEG stress. Proline accumulation in hydro- and haloprimed rice seedlings has already been reported by Mondal (2011). NaCl and PEG stress caused lipid peroxidation of biomembranes which was evident from the increased MDA content in seedlings raised from both primed and non-primed seeds under NaCl and PEG stress. The lipid peroxidation reactions could impair various metabolic functions by changing the physicochemical properties of cell membranes through disruption of lipid bilayers which further promote leakage of solutes, leading to cell death (Scandalios 1993). Both hydro- and halopriming equally reduced MDA content in all three varieties. The reduction in MDA content under seed priming was already reported in maize (Randhir and Shetty 2005), bitter gourd (Yeh et al. 2005), lucerne (Zhang et al. 2007), mung bean (Saha et al. 2010), and rice (Ella et al. 2011).

Enzyme activities **:** Nitrate reductase (NR) activity

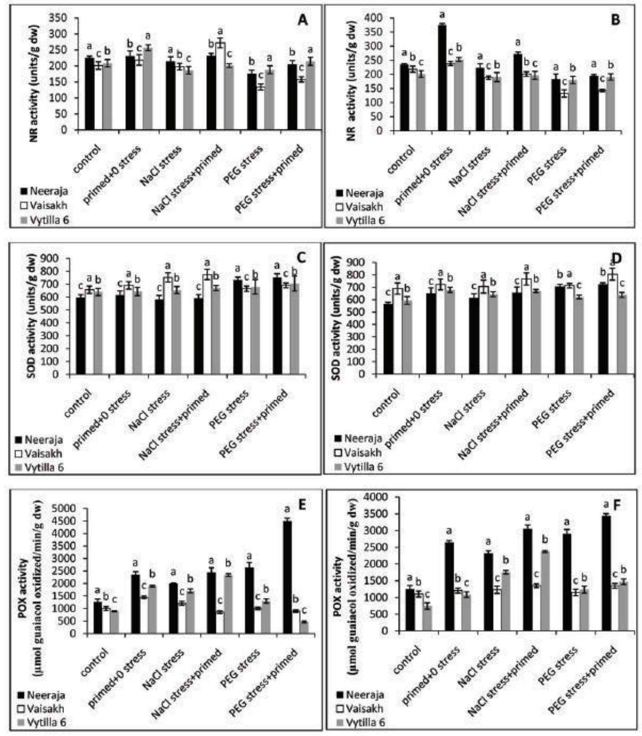

Fig. 5. Effect of hydropriming and halopriming on nitrate reductase (NR) activity (A, B); on superoxide dismutase (SOD) activity (C, D); on guaiacol peroxidase (PER) activity (E, F) of three rice varieties grown under 0, NaCl, and PEG-stressed conditions. The vertical bars represent SE of the mean value of recordings from three independent experiments each with a minimum of three replicates. Values with different letters are significantly different at 1% level ($P < 0.01$; ANOVA).

increased in rice seedlings which were raised from hydro- and haloprimed seeds under unstressed and stressed (NaCl/PEG) conditions when compared to their respective controls (Figs. 5A and B). Seed priming treatments also caused an increase in superoxide dismutase (SOD) activity in rice seedlings and was significantly higher in the seedlings raised from haloprimed seeds (Figs. 5C and D). In the present study, enhancement in the activity of guaiacol peroxidase (PER) occurred especially in the abiotic stress sensitive variety Neeraja under 0 stress, when the seeds were haloprimed and hydroprimed (Figs. 5E and F).

It has been reported in rice seedlings that NR activity itself can be used as a measure of seedling vigor (Yang and Sung 1980). Our results are in accordance with the findings of Mondal (2011) and Anwar et al. (2013) wherein rice seed priming caused increase in the activity of NR. A positive correlation between the antioxidant enzyme activities and osmotic stress tolerance were already reported in many plants (Ashraf and Ali 2008; Munns 2002). Seed priming caused the increased activities of PER and SOD which are key enzymes for scavenging the free radicals. These two enzymes may play a major role in scavenging the free radicals generated as a result of NaCl and PEG stress as indicated by the low levels of MDA in the rice seedlings raised from hydro- and haloprimed seeds. There are previous reports of accumulation of PER and SOD under hydropriming and osmopriming in rice cultivars (Yuan-Yuan et al. 2010). It was also reported that antioxidant enzyme activities of catalase and peroxidase improves salt tolerance of canola seedlings (Ashraf and Ali 2008). Rouhi et al. (2012) reported the accumulation of catalase and SOD in the berseem clover seedlings raised from primed seeds where as in amaranth PER activity gets increased on seed priming (Moosavi et al. 2009).

Conclusion

The results obtained from the present study suggest that seed priming significantly ameliorated the adverse effects of osmotic stresses by altering the various metabolic pathways. The beneficial effect of these priming treatments can be attributed to increased accumulation of primary metabolites, increased activity of photosystems and mitochondria, and by the activation of antioxidant systems in the rice seedlings. Although both halo- and hydropriming of seeds was found to improve the performance of rice under osmotic stresses, the latter was found to be more efficient. Moreover, in the current scenario of increasing abiotic stresses, our findings have immense importance as both priming techniques impart osmotic stress tolerance to sensitive varieties and additional stress tolerance potential to tolerant varieties of rice.

Acknowledgments

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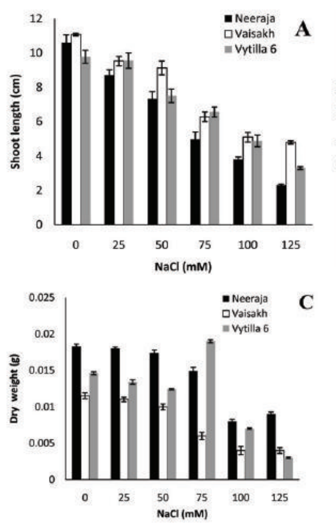
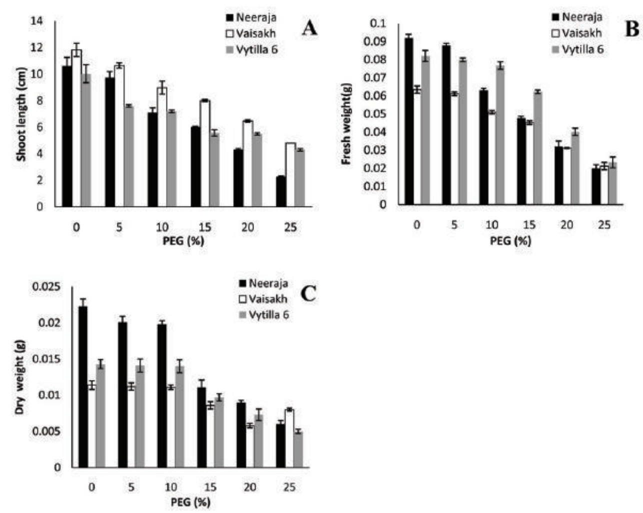
Supplementary Table 1. Shoot length, fresh weight, and dry weight of the seedlings of three rice varieties raised from non-primed and haloprimed (with different concentrations of NaCl) seeds. The data is an average of recordings from three independent experiments each with three replicates (i.e. n = 9). The data represent mean ± standard error

NaCl (mM)	Shoot length (cm)			Fresh weight (g)			Dry weight (g)		
	Neeraja	Vaisakh	Vytilla 6	Neeraja	Vaisakh	Vytilla 6	Neeraja	Vaisakh	Vytilla 6
0	10.48 ± 0.80	11.80 ± 0.38	9.98 ± 0.60	0.0921 ± 0.01	0.0637 ± 0.01	0.0820 ± 0.01	0.0181 ± 0.01	0.0113 ± 0.01	0.0145 ± 0.01
25	11.76 ± 0.77	11.99 ± 1.40	10.34 ± 0.13	0.0954 ± 0.01	0.0638 ± 0.01	0.0822 ± 0.01	0.0193 ± 0.01	0.0121 ± 0.01	0.0147 ± 0.01
50	13.87 ± 0.63	12.51 ± 1.61	12.07 ± 0.46	0.0994 ± 0.01	0.0643 ± 0.01	0.0832 ± 0.01	0.0222 ± 0.01	0.0132 ± 0.01	0.0150 ± 0.01
75	13.07 ± 0.69	12.34 ± 0.65	13.17 ± 0.42	0.0986 ± 0.01	0.0640 ± 0.01	0.0838 ± 0.01	0.0220 ± 0.01	0.0129 ± 0.01	0.0157 ± 0.01
100	13.00 ± 1.23	12.28 ± 0.74	13.00 ± 0.29	0.0987 ± 0.01	0.0638 ± 0.01	0.0835 ± 0.01	0.0199 ± 0.01	0.0130 ± 0.01	0.0154 ± 0.01

*The values in the parenthesis denote the value of respective parameters in the seedlings of three rice varieties raised from non-primed seeds exposed to 0, NaCl, and PEG-6000 stress



Supplementary Fig. 1. Shoot length (A), fresh weight (B), and dry weight (C) of rice seedlings grown in unstressed (control) and various concentrations of NaCl solutions. The vertical bars represent SE of the mean value of recordings from three independent experiments each with a minimum of three replicates.



Supplementary Fig. 2. Shoot length (A), fresh weight (B), and dry weight (C) of rice seedlings grown in unstressed (control) and various concentrations of PEG solutions. The vertical bars represent SE of the mean value of recordings from three independent experiments each with a minimum of three replicates.