



Halopriming of seeds imparts tolerance to NaCl and PEG induced stress in *Vigna radiata* (L.) Wilczek varieties

K. C. Jisha · Jos T. Puthur

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Abstract The investigation was carried out to study the effect of halopriming on NaCl and polyethylene glycol-6000 (PEG-6000) induced stress tolerance potential of three *Vigna radiata* (L.) Wilczek varieties, with varied abiotic stress tolerance potential. Halopriming is a seed priming technique in which the seeds were soaked in various salt solutions (in this study NaCl was used). The results of the study indicated that the application of stresses (both NaCl and PEG) induced retardation of growth attributes (measured in terms of shoot length, fresh weight, dry weight) and decrease in physiological attributes like total chlorophyll content, metabolites, photosynthetic and mitochondrial activity of the seedlings in all three *V. radiata* (L.) varieties. However, halopriming of the seeds could reduce the extent of decrease in these biological attributes. NaCl and PEG stress also caused increase in MDA content (a product of membrane lipid peroxidation) in all the varieties studied and this increase was significantly minimized under halopriming. From the present investigation it was evident that among the green gram varieties studied, Pusa Vishal, a NaCl tolerant variety showed enhanced tolerance to NaCl and PEG induced stress, when the seeds were subjected to halopriming followed by Pusa Ratna (stress sensitive variety). Pusa 9531 (drought tolerant variety) also showed positive halopriming effects but it was less significant when compared to other two varieties. It could be concluded that halopriming improved the drought and salinity stress tolerance potential of all varieties and it was significantly higher in the Pusa Vishal as compared to Pusa 9531 and Pusa Ratna.

Keywords Abiotic stress · Drought · Salinity · Seed priming · Mitochondrial activity · Photochemical activity

Introduction

Plants are exposed to various adverse environmental conditions such as drought, salinity, extreme temperature, etc. These abiotic stresses adversely affect the plant growth and productivity. Among the various abiotic stresses, osmotic stress (drought and salinity) causes a variety of biochemical, physiological and metabolic changes in plants, ultimately reducing the yield (Xiong and Zhu 2002).

Green gram (*Vigna radiata* (L.) Wilczek) is a tropical legume which is an important dietary pulse crop of India enriched with lysine and protein. Green gram being a short duration crop is commonly cultivated as a rotation crop/intercrop in India. Various abiotic stresses, especially drought and salinity are major limitations to its production. Salt stress alone was found to cause reduction in germination percentage, shoot and root lengths, fresh weight and seedling vigour in green gram (Misra et al. 1996; Promila and Kumar 2000; Misra and Dwivedi 2004).

Seed priming has been developed as an indispensable method to produce tolerant plants against various stresses. It is a pre-germination treatment method which improves seed performance and provides faster and synchronized seed germination. In seed priming, the seed is either soaked in water (hydropriming), solutions of PEG (osmopriming), salt (CaCl₂, CaSO₄, NaCl etc.) or some definite chemicals prior to germination (Patade et al. 2009). Priming is believed to bring about some biochemical changes within the seed, which ultimately favours germination and further growth stages. According to Gurusinge et al. (1999), priming enhances the early events of germination. Even though some metabolic changes take place within the seed during priming, are not enough to induce

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K. C. Jisha · J. T. Puthur (✉)
Plant Physiology and Biochemistry Division, Department of Botany,
University of Calicut, C.U. Campus, P.O., Kerala 673635, India
e-mail: jtputhur@yahoo.com

radical protrusion (McDonald 2000). Due to the effect of some of these biochemical changes likely to take place prior to seed germination, plants raised from primed seeds showed various advantages over the non primed ones, such as sturdy and quick cellular defense response against abiotic stresses. The seedlings emerging from primed seeds also showed early and uniform germination. Moreover, the overall growth of plants was enhanced due to the effect of various seed priming treatments. The priming effects depend on the nature of priming agent, priming duration, concentration of priming agent and the plant (Jeong et al. 2000). Priming for enhanced resistance to abiotic stress is operating via various pathways involved in different metabolic processes (Jisha et al. 2013).

In halopriming, the seeds are immersed in different salt solutions (in this study NaCl was used) which facilitate the process of seed germination and subsequent seedling emergence even under adverse environmental conditions. In halopriming with NaCl, the seeds are treated in NaCl concentrations of tolerable limits. Halopriming is a simple and cheap agrotechnique and therefore found suitable to be recommended to the farmers owing to better synchrony of emergence and crop stand under various conditions of environment (Sedghi et al. 2010). Halopriming with NaCl was previously reported to bring about enhancement in germination and seedling establishment in milk thistle (Sedghi et al. 2010), enhancement in the salt tolerance of melon plants grown under salinity (Sivritepe et al. 2003, 2005). In sugarcane, halopriming with NaCl was found to be an effective pre germination practice for overcoming salinity and drought induced negative effects (Patade et al. 2009). Farhoudi and Sharifzadeh (2006) while working with canola reported salt priming-induced improvement in seed germination, seedling emergence and further growth under saline conditions. In green gram, pretreatment of the seeds with sub lethal dose of NaCl was found to ameliorate the injurious effects of NaCl stress (Saha et al. 2010).

The primary metabolism and associated biochemical changes occurring in seedlings emerging from primed and non primed seeds subjected to NaCl and polyethylene glycol-6000 (PEG-6000) stressed conditions was analyzed to study the effect of halopriming on osmotic stress tolerance potential of green gram varieties with varied tolerance potential to NaCl and drought.

Materials and methods

Materials

Green gram belongs to the family papilionaceae and is an important protein rich pulse crop. The seeds of *V. radiata* varieties were obtained from seed science and technology division, IARI, New Delhi. Pusa Ratna is generally considered

as a sensitive variety to all kinds of abiotic stresses, especially to NaCl (Sehrawat et al. 2014). Therefore, in the present study Pusa Ratna is taken as a common sensitive variety to both NaCl and PEG stress. Pusa Vishal is a NaCl tolerant (Nazar et al. 2011) and Pusa 9531 is a drought tolerant (Dutta and Bera 2008) variety.

Methods

Halopriming technique

The seeds which were grown and harvested at uniform growth conditions were used for the present study. The seeds were stored in seed desiccators under vacuum condition for 3–6 months and within this period studies related to halopriming of seeds were carried out. To determine the effective NaCl concentration, which could bring about halopriming effect, the uniform sized seeds of three varieties *V. radiata* were soaked in various concentrations of NaCl (0, 20, 25, 30, 35, 40, 45, 50, 55, 60 mM) for 6 h in a screw cap bottle. The soaked seeds were further placed on a piece of clean filter paper, allowing dehydration under shade at 25 °C till the seeds retrieved the original moisture level as that of pre-priming stage. Seed weights were tested repeatedly at fixed intervals to ensure the seeds have attained the original dry weight. The untreated seeds were used as the control. After ~24 h dehydration, the seeds were germinated in light transparent plastic bottles (19×11 cm) containing absorbent cotton soaked with distilled water. The bottles were kept under a continuous light ($120 \mu\text{mol m}^{-2} \text{s}^{-1}$) at 25 ± 2 °C. The growth and biochemical attributes of seedlings germinated from primed seeds were recorded on 7 d after germination.

To select stress imparting concentrations of NaCl and PEG-6000, the seeds of three *V. radiata* varieties were germinated in various concentrations of NaCl (0, 25, 50, 75, 100, 125 mM) and PEG (0, 5, 10, 15, 20, 25 %). The growth and biochemical attributes of seedlings germinated from primed and non-primed seeds were recorded on 7 d after germination. All the seed materials used for investigation were pre-washed for one min with 0.25 % Triton X-100 (Boehringer Mannheim GmbH) to remove the dirt.

Morphological, physiological and biochemical studies

Shoot length was measured with the help of a meter scale in cm. For fresh weight measurements the seedlings were blotted and wrapped separately in pre-weighed labeled aluminium foils and was immediately weighed. For dry weight measurements, the same samples weighed for fresh weight 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. The samples were reweighed as described above at regular intervals, until the weights became constant.

Total carbohydrate was estimated according to Dubois et al. (1956). Protein content of the plant material 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 MDA content estimation was done according to Heath and Packer (1968). The peroxidase (PER) assay was followed after Abeles and Biles (1991) with some minor modifications. Chlorophyll estimation was carried out by the method of Arnon (1949).

Thylakoids from leaves were isolated according to Puthur (2000) and the photochemical activities of the isolated thylakoids were assayed polarographically with a Clark-type oxygen electrode (DW1/AD, Hansatech, Norflok, UK) which was connected to a digital control box (OXYG1, Hansatech) at 4 °C as per the protocol of Puthur (2000). The light dependent O₂ uptake/evolution was measured by irradiating the sample with saturating intensity of white light (1,800 μmol photons m⁻² s⁻¹), provided by a 100 W halogen lamp (LS2, Hansatech). The activity of Photosystem I (PS I) and Photosystem II (PS II) was expressed in terms of μmol of O₂ consumed/evolved min⁻¹ mg⁻¹ chlorophyll.

Mitochondrial isolation from the seedlings was carried out according to Kolloffel (1967). Oxygen consumption by mitochondria was measured polarographically with the same Clark-type oxygen electrode at 25 °C as per the protocol of Schmitt and Dizengremel (1989). The mitochondrial activity was calculated in terms of μmol O₂ consumed min⁻¹ mg⁻¹ protein.

Statistical analysis

Two way analysis of variance (ANOVA) was conducted taking varieties as one factor and treatments as the second factor and the treatment means were compared with LSD (least significant difference) wherever necessary in the data of Tables 1 and 2. One way ANOVA was conducted to find the variation in percentage increase in treatments of each variety (Figures) by using SPSS 17.0.

Results

Selection of optimal concentration of NaCl for halopriming

After halopriming of three varieties of *V. radiata*, the seeds were germinated as described in materials and methods. The growth attributes (shoot length, fresh weight and dry weight) revealed that the most effective halopriming concentrations was 35 mM for Pusa Ratna, 50 mM for Pusa 9531 and Pusa Vishal respectively (supplementary table).

Table 1 Shoot length, fresh weight and dry weight of three varieties of *V. radiata* seedlings under 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

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
Pusa Ratna	18.47±0.51 (17.83±0.43)	11.07±0.60 (08.93±0.31)	10.73±0.66 (07.30±0.32)	0.2911±0.01 (0.2152±0.01)	0.1907±0.01 (0.1297±0.01)	0.1588±0.01 (0.1240±0.01)	0.0291±0.01 (0.0261±0.01)	0.0192±0.01 (0.0126±0.01)	0.0175±0.01 (0.0155±0.01)
Pusa 9531	17.00±0.75 (13.53±0.64)	09.73±0.43 (09.63±0.32)	03.93±0.21 (03.87±0.18)	0.2180±0.01 (0.1988±0.01)	0.2010±0.01 (0.1660±0.01)	0.0750±0.01 (0.0660±0.01)	0.0130±0.01 (0.0100±0.01)	0.0170±0.01 (0.0160±0.01)	0.0246±0.01 (0.0200±0.01)
Pusa Vishal	18.67±0.75 (17.57±0.78)	06.30±0.51 (04.63±0.38)	06.97±0.65 (05.93±0.42)	0.2921±0.01 (0.2919±0.01)	0.1312±0.01 (0.1107±0.01)	0.1585±0.01 (0.1298±0.01)	0.0194±0.01 (0.0179±0.01)	0.0154±0.01 (0.0110±0.01)	0.0181±0.01 (0.0139±0.01)

*The values in the parenthesis denote the value of respective parameters in the three varieties of *V. radiata* seedlings raised from non-primed seeds exposed to 0, NaCl and PEG-6000 stress. All the values were significantly different as estimated by two way ANOVA test (p<0.01).

Table 2 Photosynthetic pigment content of leaves of three varieties of *V. radiata* seedlings under 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 \pm standard error

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			
Pusa Ratna	18.78 \pm 0.86 (16.51 \pm 0.95)	12.00 \pm 0.76 (12.00 \pm 0.83)	09.00 \pm 0.36 (09.00 \pm 0.45)	05.99 \pm 0.21 (05.15 \pm 0.35)	02.92 \pm 0.11 (03.03 \pm 0.21)	02.81 \pm 0.08 (02.24 \pm 0.01)	24.77 \pm 1.66 (21.69 \pm 1.34)	14.51 \pm 0.55 (15.01 \pm 0.63)	11.12 \pm 0.55 (12.44 \pm 0.31)	08.69 \pm 0.42 (06.79 \pm 0.31)	05.14 \pm 0.32 (05.30 \pm 0.21)	03.72 \pm 0.11 (04.07 \pm 0.13)
Pusa 9531	18.51 \pm 0.56 (15.98 \pm 0.13)	15.17 \pm 0.85 (12.72 \pm 0.65)	05.23 \pm 0.13 (03.23 \pm 0.12)	06.29 \pm 0.42 (05.09 \pm 0.32)	04.50 \pm 0.32 (04.02 \pm 0.34)	01.41 \pm 0.01 (00.94 \pm 0.01)	24.74 \pm 1.57 (21.03 \pm 1.06)	19.64 \pm 0.75 (16.65 \pm 0.65)	08.33 \pm 0.44 (04.17 \pm 0.12)	07.61 \pm 0.31 (06.41 \pm 0.22)	06.02 \pm 0.22 (05.25 \pm 0.08)	02.63 \pm 0.12 (01.48 \pm 0.06)
Pusa Vishal	24.20 \pm 1.06 (20.82 \pm 0.95)	09.96 \pm 0.44 (06.36 \pm 0.83)	11.55 \pm 0.84 (09.16 \pm 0.54)	07.76 \pm 0.31 (06.31 \pm 0.21)	02.64 \pm 0.09 (01.62 \pm 0.01)	03.64 \pm 0.09 (02.46 \pm 0.02)	31.93 \pm 1.24 (27.03 \pm 1.04)	12.96 \pm 0.14 (07.96 \pm 0.23)	18.72 \pm 0.45 (11.59 \pm 0.54)	09.97 \pm 0.42 (07.78 \pm 0.31)	04.46 \pm 0.11 (03.36 \pm 0.09)	04.65 \pm 0.07 (04.14 \pm 0.08)

*The values in the parenthesis denote the value of photosynthetic pigments in leaves of the three varieties of *V. radiata* seedlings raised from non-primed seeds exposed to 0, NaCl and PEG-6000 stress. All the values were significantly different as estimated by two way ANOVA test ($p<0.01$).

Selection of stress imparting concentration of NaCl and PEG

The concentration of NaCl and PEG which imparted 40–50 % retardation in various growth attributes (shoot length, fresh weight and dry weight) was selected as stress imparting concentrations of NaCl and PEG. It was 75 mM NaCl and 15 % PEG for Pusa Ratna; 75 mM NaCl and 20 % PEG for Pusa 9531; and 100 mM NaCl and 15 % PEG for Pusa Vishal (Supplementary Figs. i and ii).

Changes in growth parameters under halopriming

In general, the application of stress (both NaCl and PEG) retarded growth of seedlings raised from primed as well as non primed seeds. However, halopriming of the seeds could decrease extent of reduction in shoot length, fresh and dry weight of the seedlings as compared to the seedlings raised from non primed seeds. The results given below with respect to various parameters recorded in seedlings raised from primed seeds were obtained in comparison to the values recorded for each parameter of the seedlings raised from non primed seeds.

The NaCl primed Pusa Ratna recorded 24 % (NaCl stress) and 47 % increase (PEG stress) in shoot length when compared to respective non primed controls. Whereas NaCl primed Pusa 9531 and Pusa Vishal did not show significant increase in shoot length under stressed and non-stressed conditions (Table 1). Halopriming caused an increase of 35 % (0 stress), 47 % (NaCl stress) and 28 % (PEG stress) in fresh weight of the variety Pusa Ratna but the increase in fresh weight under halopriming was less significant in the other two varieties of *V. radiata* (Table 1). The NaCl primed Pusa Ratna recorded significant increase in dry weight only under NaCl stress (52 %) whereas in Pusa 9531 the dry weight increase on halopriming was observed under 0 stress (30 %). In Pusa Vishal, halopriming resulted in 40 % (NaCl stress) and 30 % (PEG stress) increase in dry weight (Table 1).

Biochemical and physiological changes under halopriming

Primary metabolites

The percentage increase of total protein in the seedlings of all three varieties of *V. radiata* which were haloprimed and grown in non-stressed and stressed (NaCl and PEG) condition was to the extent of 3 to 23 % (Fig. 1a). In general, the accumulation of total carbohydrates in the seedlings of all the three varieties of *V. radiata* was at an increased level as compared to the accumulation of total proteins except in the haloprimed Pusa Ratna under NaCl stress. Haloprimed Pusa Ratna recorded 72 % (0 stress), 8 % (NaCl stress) and 61 % (PEG stress) increase in total carbohydrate. In Pusa 9531 and Pusa Vishal,

the increase in total carbohydrate was less significant when compared to Pusa Ratna (Fig. 1b).

Proline and MDA

Among the three varieties of *V. radiata*, haloprimered Pusa Ratna recorded 50 % increase in proline content under unstressed condition while there was no significant increase in proline content under NaCl stress and PEG stress. At the same time, in Pusa 9531, haloprimering resulted in proline accumulation under NaCl (39 %) and PEG (56 %) stress, but no significant increase of proline was recorded under unstressed condition. In Pusa Vishal, 36 % (0 stress), 22 % (NaCl stress) and 63 % (PEG stress) increase in proline content occurred as a result of haloprimering (Fig. 2a). The MDA content was found to decrease (2–27 %) in the seedlings raised from haloprimered seeds as compared to seedlings from non primed seeds, either in the absence or presence of stress (NaCl and PEG) (Fig. 2b).

Guaiacol peroxidase (PER) activity

Accumulation of PER occurred especially in the NaCl tolerant variety Pusa Vishal, where 108 % (0 stress), 209 % (NaCl stress) and 103 % (PEG stress) increase was recorded. Pusa Ratna also accumulated PER in response to haloprimering at the rate of 35 % (0 stress), 92 % (NaCl stress) and 25 % (PEG stress) respectively. The PER increase on haloprimering was less significant in the drought tolerant variety Pusa 9531, where the increase occurred only under PEG stress (Fig. 3).

Chlorophyll content and activity of photosystems

Haloprimering enhanced the photosynthetic pigment content of Pusa Vishal as compared to Pusa 9531 and Pusa Ratna. Maximum increase in chlorophyll *a* was observed in the haloprimered Pusa Vishal and Pusa 9531 subjected to NaCl stress (57 %) and PEG stress (62 %) respectively. Chlorophyll *b* was found to be maximum in haloprimered Pusa Vishal (63 %) under NaCl stress and 48 % under PEG stress. Total Chlorophyll was also found to be maximum in haloprimered Pusa Vishal (63 %) under NaCl stress and 62 % under PEG stress. On haloprimering, the carotenoids increased up to 78 % in Pusa 9531 under PEG stress and 33 % in Pusa Vishal under NaCl stress (Table 2).

The activity of the PS I increased significantly in the leaves of Pusa Vishal seedlings raised from NaCl primed seeds under NaCl (302 %) and PEG (233 %) stress. While there was no significant increase in PS I activity under unstressed condition. But the increase in PS I activity was much lower and insignificant in Pusa 9531, with the exception of 95 % increase in seedlings exposed to PEG stress. In Pusa Ratna the increase in PS I activity was 161 % (0 stress), 48 % (NaCl stress) and 182 % (PEG stress) in seedlings raised from haloprimered seeds (Fig. 4a). In general, haloprimering increased the PS II activity of all three varieties of *V. radiata* seedlings raised under non-stressed and stressed (NaCl and PEG) conditions as compared to their non primed controls. The seedlings raised from NaCl primed seeds of Pusa Vishal showed maximum increase in PS II activity, it was 67 % (0 stress), 25 % (NaCl stress) and 60 % (PEG stress) respectively.

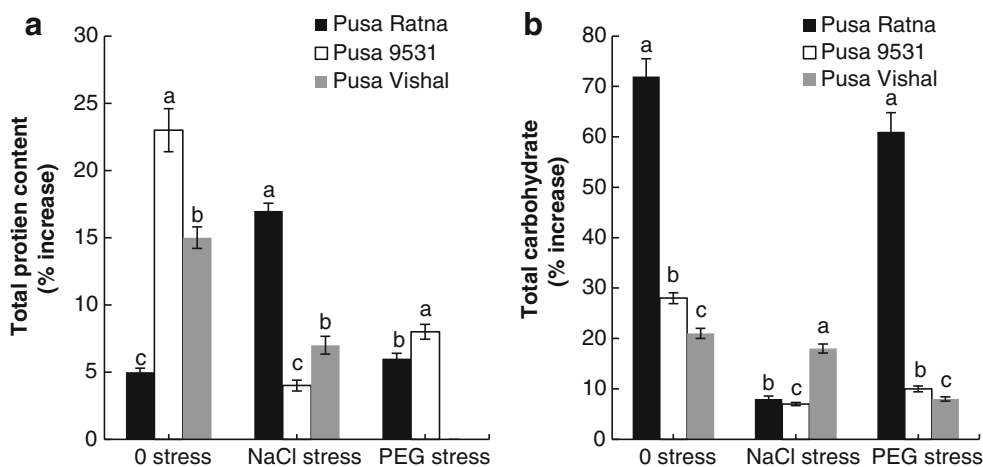


Fig. 1 Percentage of increase in protein (a) and total carbohydrates (b) of seedlings raised from haloprimered seeds of three different varieties of *V. radiata* as compared to that of seedlings raised from non primed seeds. Total protein content in seedlings raised from non primed seeds of Pusa Ratna in 0, NaCl and PEG stress is 105, 231 and 186 mg/g dw, respectively; for Pusa 9531 in 0, NaCl, PEG stress is 239, 241, 227 mg/g dw, respectively and for Pusa Vishal in 0, NaCl, PEG stress is 258, 146 and 149 mg/g dw, respectively. Total carbohydrate content in seedlings raised

from non primed seeds of Pusa Ratna in 0, NaCl and PEG stress is 145, 173, 187 mg/g dw, respectively; for Pusa 9531 in 0, NaCl, PEG stress is 126, 124, 103 mg/g dw, respectively and for Pusa Vishal in 0, NaCl, PEG stress is 161, 202, 204 mg/g dw, respectively. The vertical bars represent SE of the mean value of recordings from three independent experiments each with a minimum of three replicates. Values super scribed with same alphabet is homogenous for each stress at 1 % level significance ($p < 0.01$; ANOVA)

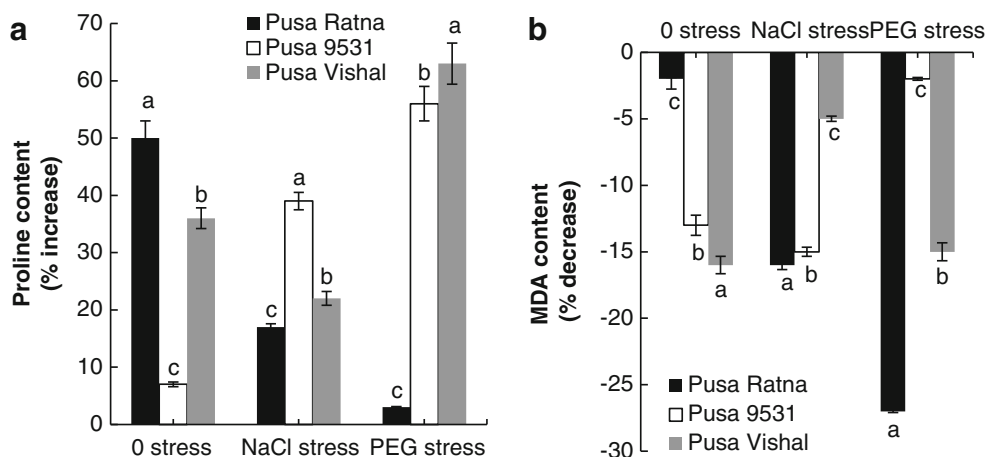


Fig. 2 Percentage of change in proline (**a**) and MDA (**b**) of seedlings raised from haloprimered seeds of three different varieties of *V. radiata* as compared to that of seedlings raised from non primed seeds. Proline content in seedlings raised from non primed seeds of Pusa Ratna in 0, NaCl, PEG stress is 634, 641, 778 $\mu\text{g/g dw}$, respectively; for Pusa 9531 in 0, NaCl, PEG stress is 793, 1,384, 3,149 $\mu\text{g/g dw}$, respectively and for Pusa Vishal in 0, NaCl, PEG stress is 1,237, 1,912, 1,896 $\mu\text{g/g dw}$, respectively. MDA content in seedlings raised from non primed seeds

of Pusa Ratna in 0, NaCl and PEG stress is 117, 127, 133 $\mu\text{mol/g dw}$, respectively; for Pusa 9531 in 0, NaCl, PEG stress is 61, 67, 66 $\mu\text{mol/g dw}$, respectively and for Pusa Vishal in 0, NaCl, PEG stress is 92, 104, 103 $\mu\text{mol/g dw}$, respectively. The vertical bars represent SE of the mean value of recordings from three independent experiments each with a minimum of three replicates. Values super scribed with same alphabet is homogenous for each stress at 1 % level significance ($p < 0.01$; ANOVA)

Whereas, in Pusa Ratna, the increase in PS II activity on haloprimering was significant only under NaCl stress (73 %). Haloprimering of Pusa 9531 brought about increase in PS II activity, the increase was 34 % (NaCl stress) and 76 % (PEG stress) (Fig. 4b).

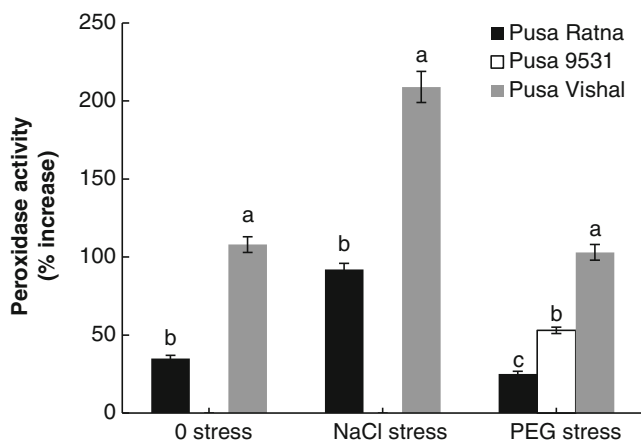


Fig. 3 Percentage of increase in peroxidase activity of seedlings raised from haloprimered seeds of three different varieties of *V. radiata* as compared to that of seedlings raised from non primed seeds. Peroxidase activity in seedlings raised from non primed seeds of Pusa Ratna in 0, NaCl, PEG stress is 178, 433, 331 $\mu\text{mol guaiacol oxidized/min/g dw}$, respectively; for Pusa 9531 in 0, NaCl, PEG stress is 473, 536, 963 $\mu\text{mol guaiacol oxidized/min/g dw}$, respectively and for Pusa Vishal in 0, NaCl, PEG stress is 314, 353, 980 $\mu\text{mol guaiacol oxidized/min/g dw}$, respectively. The vertical bars represent SE of the mean value of recordings from three independent experiments each with a minimum of three replicates. Values super scribed with same alphabet is homogenous for each stress at 1 % level significance ($p < 0.01$; ANOVA)

Mitochondrial activity

Pusa Ratna on haloprimering resulted in 15 % (0 stress), 136 % (NaCl stress) and 35 % (PEG stress) increase in mitochondrial activity when compared to respective non primed controls. In Pusa 9531, haloprimering resulted in 20 % (0 stress), 8 % (NaCl stress) and 36 % (PEG stress) increase in mitochondrial activity. In Pusa Vishal haloprimering resulted in 14 % (0 stress), 69 % (NaCl stress) and 12 % (PEG stress) increase in mitochondrial activity (Fig. 5).

Discussion

As the three varieties of *V. radiata* are known to exhibit different levels of tolerance towards NaCl, it became essential to check the optimal concentration of NaCl for haloprimering and it was found that Pusa Ratna, a known abiotic stress sensitive variety was effectively haloprimered (in terms of better shoot length, fresh weight and dry weight) at a lower NaCl concentration (35 mM), whereas Pusa 9531 (a known drought tolerant) and Pusa Vishal (a known NaCl tolerant) was effectively haloprimered at 50 mM concentration of NaCl.

As the three varieties selected had varied level of NaCl and PEG tolerance potential, the NaCl and PEG stress tolerance potential of the three *V. radiata* varieties was tested against a concentration which imparted 40–50 % growth retardation and it varied for each variety (75 mM NaCl and 15 % PEG for Pusa Ratna; 75 mM NaCl and 20 % PEG for Pusa 9531; and 100 mM NaCl and 15 % PEG for Pusa Vishal).

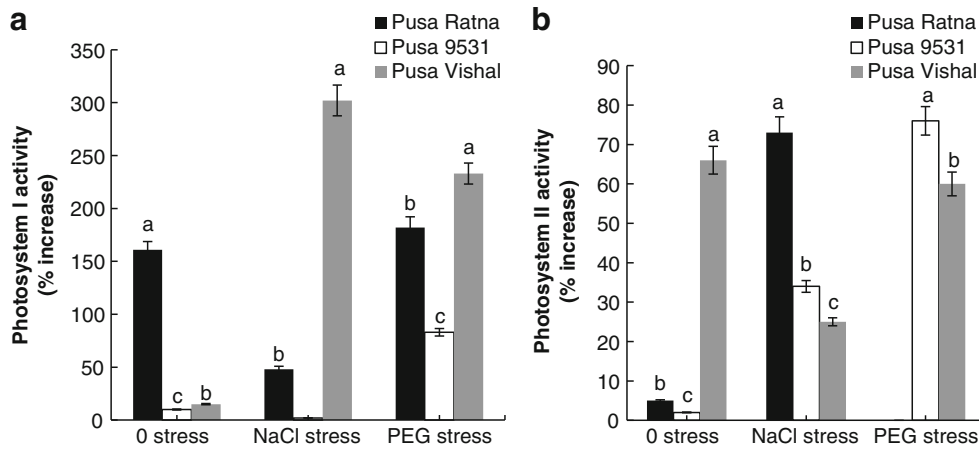


Fig. 4 Percentage of increase in PS I (a) and PS II (b) activity of seedlings raised from haloprimered seeds of three different varieties of *V. radiata* as compared to that of seedlings raised from non primed seeds. PS I activity in seedlings raised from non primed seeds of Pusa Ratna in 0, NaCl, PEG stress is 116, 155, 101 μmol of O_2 consumed/mg chl/min, respectively; for Pusa 9531 in 0, NaCl, PEG stress is 168, 165, 113 μmol of O_2 consumed/mg chl/min, respectively and for Pusa Vishal in 0, NaCl, PEG stress is 127, 103, 105 μmol of O_2 consumed/mg chl/min, respectively. PS II activity in seedlings raised from non primed seeds of Pusa

Ratna in 0, NaCl, PEG stress is 41, 14, 24 μmol of O_2 evolved/mg chl/min, respectively; for Pusa 9531 in 0, NaCl, PEG stress is 30, 28, 24 μmol of O_2 evolved/mg chl/min, respectively and for Pusa Vishal in 0, NaCl, PEG stress is 38, 26, 22 μmol of O_2 evolved/mg chl/min, respectively. The vertical bars represent SE of the mean value of recordings from three independent experiments each with a minimum of three replicates. Values super scribed with same alphabet is homogenous for each stress at 1 % level significance ($p < 0.01$; ANOVA)

The beneficial effects of halopriming with NaCl were reported in many crops like sugar beet (Bourgne et al. 2000), melon (Sivritepe et al. 2003, 2005), wheat (Afzal et al. 2005), sugarcane (Patade et al. 2009), sunflower (Bajehbaj 2010), milk thistle (Sedghi et al. 2010) etc. The

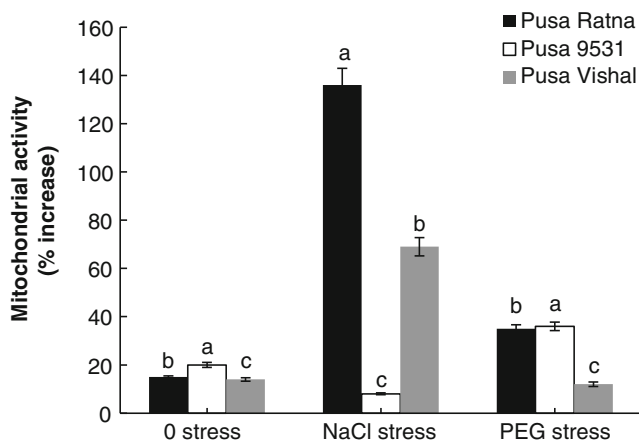


Fig. 5 Percentage of increase in mitochondrial activity of seedlings raised from NaCl primed seeds of three different varieties of *V. radiata* as compared to that of seedlings raised from non primed seeds. Mitochondrial activity in seedlings raised from non primed seeds of Pusa Ratna in 0, NaCl, PEG stress is 44, 11, 27 μmol of O_2 consumed/mg protein/min, respectively; for Pusa 9531 in 0, NaCl, PEG stress is 40, 37, 22 μmol of O_2 consumed/mg protein/min, respectively and for Pusa Vishal in 0, NaCl, PEG stress is 40, 17, 30 μmol of O_2 evolved/mg protein/min, respectively. The vertical bars represent SE of the mean value of recordings from three independent experiments each with a minimum of three replicates. Values super scribed with same alphabet is homogenous for each stress at 1 % level significance ($p < 0.01$; ANOVA)

enhancement in the growth attributes like shoot length, fresh weight and dry weight after halopriming as compared to unprimed controls is the direct evidence of increased seedling vigour due to halopriming. Among the three varieties studied, Pusa Ratna showed maximum increase in shoot length and fresh weight under priming whereas the increase in seedling dry weight was highest in Pusa Vishal. This increase may be due to the metabolic changes associated with halopriming which resulted in better germination and better seedling establishment. Moreover, the halopriming enables the seedlings to cope with the stress imposed by NaCl or PEG. There are many reports that halopriming reduces the inhibitory effects of salinity on seed germination and seedling establishment. Halopriming significantly reduced the inhibitory effects of osmotic stress in melon (Sivritepe et al. 2003), canola (Farhoudi and Sharifzadeh 2006), chickpea (Sarwar et al. 2006) and sugar cane (Patade et al. 2009), which further resulted in better seedling establishment. In green gram the reduction in seedling growth under NaCl stress was ameliorated significantly by halopriming (Saha et al. 2010). The enhanced growth of seedlings indicates the stimulative role of priming solution (NaCl) in acclimation process of the seedlings as reported in soyabean (Umezawa et al. 2000) and rice (Djanaguiraman et al. 2006). Khan et al. (2009) reported that, halopriming was much efficient in improving germination and seedling growth of pepper seeds and our results also confirmed it. In earlier studies, it was observed that seedlings from NaCl primed canola seeds maintained greater mean seedling dry weights and cell membrane stability than untreated seedlings (Farhoudi et al. 2007). Halopriming

has shown improved germination and growth of many crops under stressed conditions (Cayuela et al. 1996; Sivritepe et al. 2003; Farhoudi et al. 2007; Shahi et al. 2009).

There are reports for the accumulation of stress proteins and transcription factors under priming (Conrath et al. 2006). But in the present study there was no significant increase in total protein content on halopriming in all three varieties of *V. radiata* studied. It gives an indication that the major representative proteins (with storage and structural function) in the seedlings are not getting enhanced as a result of NaCl priming. At the same time, it would be premature to cite any specific role for proteins in combating stress (NaCl/PEG) in these varieties, based on the insignificant enhancement of proteins in NaCl primed plants. Pusa Ratna showed highest increase in total carbohydrate under halopriming. The significant enhancement in the carbohydrates in NaCl primed plants on exposure to stress could be a probable mechanism of countering the stress by increasing the osmoticum. There are earlier reports that accumulations of soluble carbohydrates increase the tolerance to drought in plants (Kameli and Losel 1993). Increase in carbohydrate concentration under halopriming was also reported in muskmelon plants (Farhoudi et al. 2011). According to Cayuela et al. (1996), the increased salt tolerance of NaCl-primed seedlings seems to be the result of higher capacity for osmotic adjustment since plants from primed seeds have more Na^+ and Cl^- in roots and more sugars and organic acids in leaves than plants from non-primed seeds. The increased accumulation of osmolytes in the seedlings raised from primed seeds could facilitate the increased uptake of water resulting in a turgor which ultimately promotes the expansion of cells. It was earlier reported that improved seed performance under halopriming could be attributed partially to osmotic adjustment, metabolic repair processes or due to the buildup of metabolites necessary for germination (Haghpanah et al. 2009).

The elevated level of proline content observed in seedlings raised from seeds subjected to halopriming may be due to its prominent role in osmotic stress tolerance. Increase in the synthesis of proline is known as a common metabolic reaction of plants under stress (Behairy et al. 2012). Proline, in addition to its major role as compatible solute, provides carbon and nitrogen source for post stress recovery growth, stabilizes membranes and protein machinery, scavenges free radicals etc. From the difference in proline accumulation between the three varieties of *V. radiata*, it could be inferred that Pusa Vishal showed a higher dependence on proline accumulation for stress tolerance (both in NaCl and PEG) than Pusa Ratna and Pusa 9531. MDA content increased in seedlings raised from both NaCl primed and non-primed seeds under NaCl and PEG stress which indicates that peroxidation of membrane lipids occurred under these stresses. However, halopriming reduced the level of MDA in all the three varieties of *V. radiata* studied. The reduction in MDA content upon halopriming was

more or less same in all the three varieties. The reduction in MDA content under halopriming was already reported in maize (Randhir and Shetty 2005), bitter melon (Yeh et al. 2005) and mung bean (Saha et al. 2010).

A correlation between the antioxidant enzyme activities and osmotic stress tolerance was reported in many plants (Munns 2002; Ashraf and Ali 2008). From the present result it was clear that halopriming caused an increase in PER in seedlings of all the three varieties of *V. radiata* indicating that priming altered the metabolic pathway for the increased production of guaiacol peroxidase. Ashraf and Ali (2008) reported that enhanced antioxidant enzyme activities of catalase and peroxidase improved salt tolerance of canola seedlings.

The observed reduction in chlorophyll content under NaCl and PEG stress may be due to the degradation of chlorophyll pigments or interference in the synthesis of chlorophyll. Ashraf and Rasul (1988) reported that the reduction in chlorophyll content under osmotic stress is due to the suppression of enzymes required for chlorophyll synthesis. Moreover, salinity may cause destruction of chloroplast and instability of pigment protein complex (El-Samad et al. 2011). From the results it was clear that halopriming improved the photosynthetic efficiency in all the three varieties but it was significant in Pusa Vishal which was evident from the high chlorophyll content, high PS I and PS II activities in the primed seedlings. On halopriming, the rate of chlorophyll degradation was not to the extent as that observed in the case of non-primed seedlings. Therefore, the energy harvested by the chlorophyll would have effectively channelized into the photosystems, resulting in an enhanced PS I and PS II activities. The better photosynthetic capacity of Pusa Vishal is also reflected in the dry matter accumulated.

Under NaCl and PEG stress, the mitochondrial activity got reduced in all the varieties studied. Whereas the extent of reduction was lesser in seedlings emerged from seeds subjected to halopriming and it was lowest in Pusa Ratna followed by Pusa Vishal and then Pusa 9531. The reduction in mitochondrial activity under both NaCl and PEG stress could be due to the closure of stomata and the limited entry of CO_2 , which in turn leads to reduction in synthesis of photosynthates. The less availability of photosynthates reflects in the rate of mitochondrial activity by reduced respiration. The probable improvement in the mitochondrial intactness by maintaining the membrane integrity and concomitant increase in the number of mitochondria achieved through halopriming of the seed would have sufficed the increased energy demand of the establishing seedlings. The better growth parameters of the seedlings which have emerged from the haloprimed seeds are confirmation to our view. Seedlings of Pusa Ratna raised from haloprimed seeds showed enhanced growth vigour in terms of shoot length and fresh weight and coinciding with this result the mitochondrial activity in the same variety was least

affected. Earlier studies suggest that salt stress negatively impacts mitochondria causing impaired electron transport activities and resulting release of reactive oxygen species in the mitochondria which ultimately causes lipid peroxidation (Mittova et al. 2003; Chen et al. 2009). Priming is known to improve the integrity of the outer membrane of mitochondria and even increases the number of mitochondria (Ashraf and Bray 1993; Benamar et al. 2003; Varier et al. 2010).

Conclusion

From the present investigation it can be concluded that among the green gram varieties studied, halopriming improved the PEG and NaCl stress tolerance potential of seedlings and it was significantly evident in the NaCl tolerant variety Pusa Vishal as compared to Pusa 9531 (drought tolerant) and Pusa Ratna (abiotic stress sensitive). Therefore, by halopriming the seeds we can evidently make the NaCl tolerant variety more tolerant and the drought tolerant variety and sensitive variety better tolerant towards NaCl and PEG stress. Moreover, halopriming was found to be simple and cheap, and therefore could be recommended to the farmers, so they can get better synchrony of seedling emergence and better crop stand in *V. radiata*.

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