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Efficacy of Seed Priming Technique on Seed Germination and Plant Growth Under Salt Stress in *Oryza sativa*

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Abstract

Salinity stress limits agricultural productivity by adversely affecting seed germination and seedling growth. It negatively impacts essential metabolic processes by imposing osmotic and oxidative stress due to ion toxicity. Seed priming is an innovative approach that can ensure seed germination and seedling establishment for ameliorating salinity stress in plants. In this study, response of two popular rice cultivars, PB 1121 and PB 1718 to various priming treatments was evaluated under salt stress. Out of the two, cultivar PB 1718 performed better and showed improved germination, and plant growth when given a priming treatment with Salicylic acid (1mM), KNO3 (1%), Moringa leaf extract (100%) and Neem leaf extract (50%) under salt stress of NaCl (150 mM). In cultivar PB 1121, no significant change was seen in germination indices under salt stress as compared to control. However, Salicylic acid (1mM), KNO_3 (1%), Proline (50mM), Moringa leaf extract (100%), $MgSO_4$ (10mM) and Neem leaf extract (50%) treatments resulted in improved root growth.

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Keywords

Plant Growth;

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Introduction

Plant productivity is known to be severely compromised by soil salinity.[1] Around 190 million hectares of irrigated land have already been lost to salt and are no longer suitable for farming, according to a report by the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services.[2] India faces a similar issue with soil salinity affecting major agricultural states like Gujarat (2.23 M ha), Uttar Pradesh (1.37 M ha), Maharashtra (0.16 M ha), West Bengal (0.44 M ha) and Rajasthan (0.38 M ha). Collectively, these states account for 75% of saline soil of the 6.73 million hectares of affected land in India.[3]

Oryza sativa L. a widely grown tropical cereal is increasingly being threatened by rising levels of salt. Rise in sea levels and frequent flooding has led to salt intrusion into fresh inland water. Additionally, the use of salted water for irrigation in rice adversely affects

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plant growth and subsequent crop yield.[4] Pusa Basmati (PB) an indigenously developed variety of rice through breeding is one of the most preferred rice varieties. It is coveted for its grain attributes and aroma. The PB cultivars 1121 and 1718 have been developed for improved resistance against several bacterial diseases.[5] However, their ability to sustain salinity stress is not known. In rice, seed germination is severely affected by salinity impairing seedling establishment and growth.[6] Breeding of salt tolerant cultivars and the development of technologies that enable efficient seed germination is the key to ensuring high yield.[7,8]

Seed priming technology offers the possibility of enhancing seed germination and crop productivity.[9] It is one of the most promising pre-sowing seed treatments to boost crop output under stressful conditions and is one of the efficient procedures for micronutrient administration during planting in field crops.[10,11] Seed priming facilitates quicker germination in plants under abiotic stress by induction of protective cellular and metabolic events thus facilitating speedy emergence and uniform stand establishment of seedlings which eventually enhances the crop productivity.[12] Various priming treatments like hydropriming, PGR-based (plant growth regulators) hormonal priming, osmopriming, organic priming and chemical priming are used to increase the resilience of crop plants towards various abiotic stresses.[13-16] The effect of seed priming methods on mitigating abiotic stress has been well documented on various plants like *Zea mays*, *Vigna radiata*, *Triticum sp*, and *Oryza sativa*.[17-19] In this study, selected organic, chemical and hormonal primers have been tested for their efficacy in promoting seed germination and growth in *Oryza sativa* Pusa Basmati cultivars PB 1121 and PB 1718 under salt stress.

Materials and Methods

Procurement of Plant Material

Seeds of *Oryza sativa* var. Pusa Basmati (cultivars PB 1121 and PB 1718) were procured from Seed Bank, IARI, New Delhi, India. The seeds were washed with a mild detergent, rinsed thoroughly with water and treated with various priming agents for 24 hours at room temperature. Seed priming treatments were given in a randomized manner with three replicates per treatment. The primers used in the study were.

- Organic Priming- Cow urine (2%), Moringa leaf extract (MLE; 100%), Neem leaf extract (NLE; 50%)
- Chemical Priming- NaCl (100 mM), KNO_3 (1 %), $MgSO_4$ (10 mM)
- Hormones- Salicylic Acid

The concentrations of seed priming agents used in this study were based on the published reports. The seeds were washed thoroughly in water, air dried for 24 hrs and stored till further use. The primed seeds were subjected to salt stress by exposure to 150 mM NaCl for 24 hrs. All the experiments were performed in triplicates.

Estimation of Germination Parameter

The seed germination parameters were assessed using the following formulas

- Germination percentage (GP) $=(N_1/N_2) \times 100$
  N_1 = number of germinated seeds
  N_2 = total number of seeds
- Mean daily germination (MDG) = Final GP/ number of days to reach final GP
- Peak value (PV) = Final GP/ number of days required to reach the peak value of the germination
- Germination value = PV × MDG
- Germination energy percentage = (Number of seeds germinated at 2 days after sowing /
- total number of the seeds tested) × 100

Estimation of Growth Parameter

Using a thread and a scale the shoot length was measured from the tip of the growing shoot to the base of the cotyledons. Similarly, for root length the length from tip of the growing root to the base of cotyledons was measured.

Biochemical Analysis

For biochemical analysis total protein content was assessed spectrophotometrically by using Lowry's method,[20] total phenolics content using Folin-Ciocalteu method[21,22] and antioxidant levels using 2, 2-Diphenyl-1- Picryl Hydrazyl (DPPH) assay.[23]

Statistical Analysis

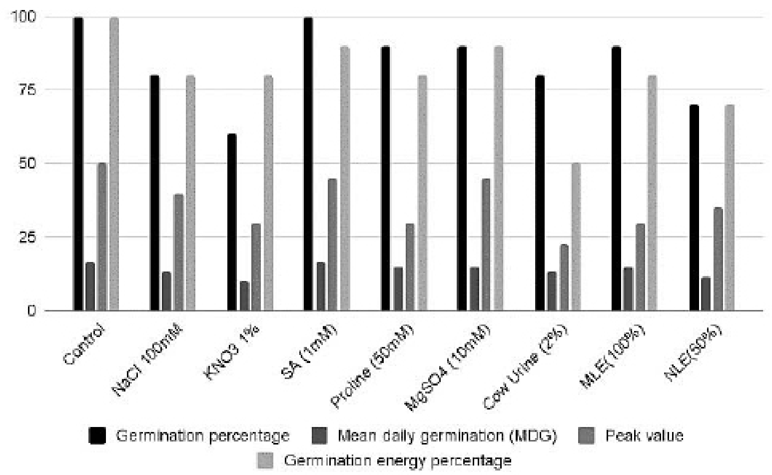
Control and primed samples were analyzed for significant differences between various groups by two tailed t-test and ANOVA using MS Excel software.

Results

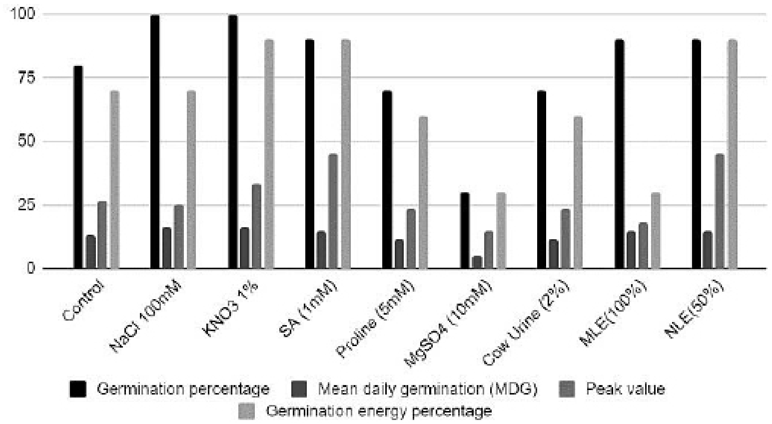
Effect on Seed Germination Indices

The two rice cultivars varied in their responses. In case of cultivar PB 1121, no significant change was seen in any of the germination indices, under salt stress as compared to control (Fig 1 A). The rice cultivar PB 1718 responded positively and an increase was observed in various germination indices (Fig 1 B). An increase in percentage germination and mean daily germination was observed in the seeds primed with NaCl (100mM), KNO3 (1%), salicylic acid (1mM), Moringa leaf extract (100%) and Neem leaf extract (50%) (Fig 1B). Increase in peak value and germination energy percentage was seen in primed seeds as compared to control (Fig 1B). Nearly 20% increase in germination energy percentage was observed in seeds primed with salicylic acid (90%), KNO_3 (90%) and Neem leaf extract (90%) as compared to control (70%) (Fig 1B).

Effect on Growth Parameters



A.



B.

Fig 1: Effect of seed priming on various germination indices in rice cultivars A. PB 1121 B. PB 1718

In comparison to control, no significant increase in shoot length was observed in seeds subjected to various priming treatments in both rice cultivars (Fig 2, 3).

In cultivar PB 1121, an increase in root length was

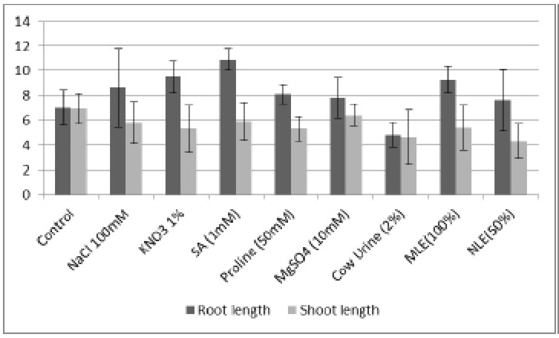
A

B

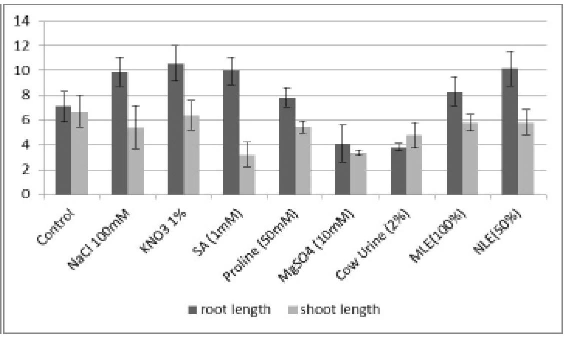
Fig. 2: Effect of different priming agents on *Oryza sativa* (A) PB 1718 (B) PB 1121 seedlings under 150mM NaCl stress. (Red block shows the control)

seen in seedlings treated with Salicylic acid (1mM), KNO_3 (1%), Moringa leaf extract (100%) and Proline (50mM) (Fig. 3A). However, a significant increase in root length was observed in cultivar PB 1718 seedlings treated with NaCl, Salicylic acid (1mM), KNO_3 (1%), Moringa leaf extract (100%) and Neem leaf extract (100%) (Fig. 3B). The increased root growth is important for enhancing water uptake and mineral acquisition.

In cultivar PB 1121, no significant increase was observed in fresh and dry weight in primed seeds(Fig. 4 A). In cultivar PB 1718 a moderate increase in dry weight was observed in seeds primed with MgSO4 (10mM), Cow urine (2%), Neem leaf extract (50%) and Moringa leaf extract (100%) (Fig. 4 B).

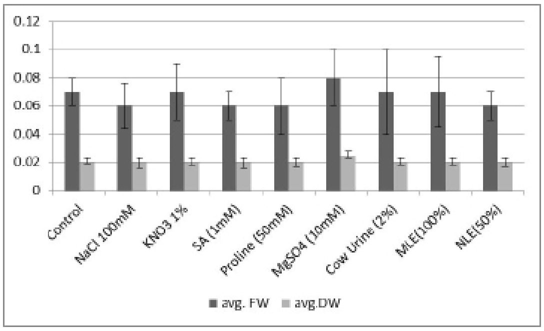


A

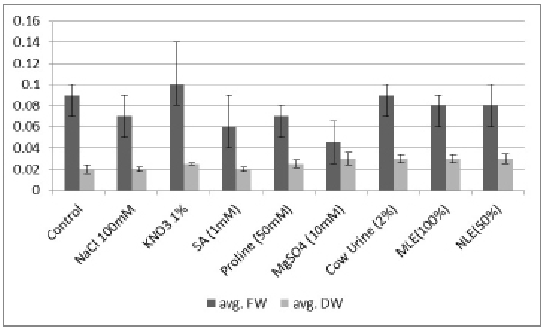


B

Fig. 3: Effect of seed priming on shoot and root length in A. PB 1121, B. PB 1718



A



B

Fig. 4: Effect of priming on fresh and dry weight of seedlings in rice cultivars A. PB 1121 B. PB 1718

Biochemical Analysis

In cultivar PB 1121, an increase in total protein content was observed in seeds primed with Salicylic acid, Moringa leaf extract and Neem leaf extract (40% increase) (Fig. 5B). In cultivar PB 1718 slight increase in total protein was noted in Cow urine (8% increase) and NaCl (2% increase) as compared to control (Fig 5B).

A significant increase of more than 30% was observed in total phenolics content (TPC) in cultivar PB 1121 in NaCl, KNO_3, Salicylic acid, $MgSO_4$ and Neem leaf extract priming treatments as compared to control (Fig 6). In cultivar PB 1718, 40 % increase in TPC was noted in KNO_3 and 19 % in $MgSO_4$ primed seeds as compared to control (Fig 6).

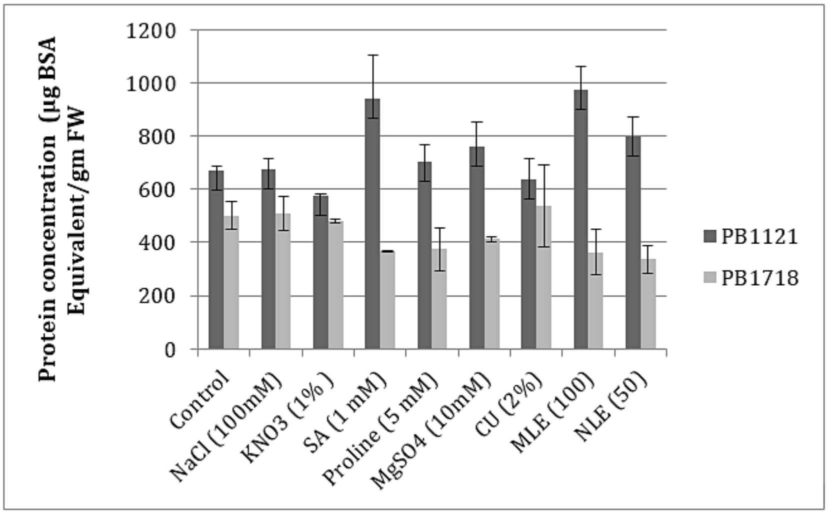


Fig. 5: Effect of priming treatments on total protein content (µg BSA equivalent/gm fresh wt.) in control and primed seeds of rice cultivars PB 1121 and PB 1718

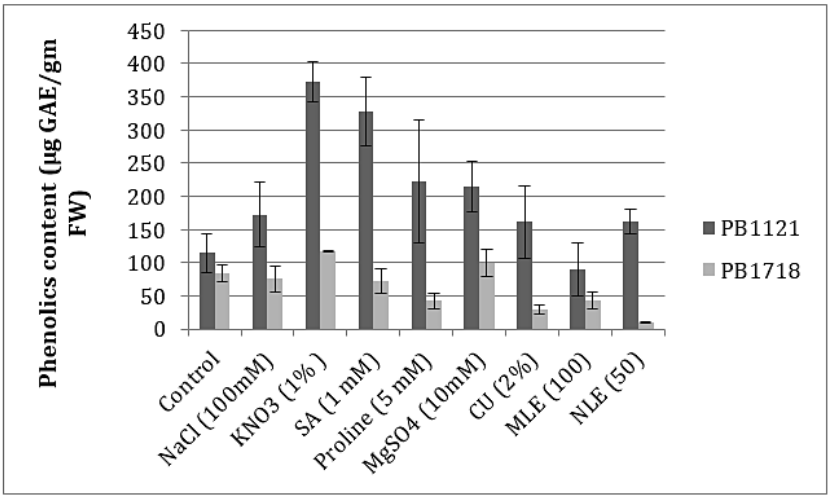


Fig. 6: Effect of seed priming on total phenolics content (µg GAE equiv./gm fresh wt.) in rice cultivars PB 1121 and PB 1718.

Analysis of antioxidant activity was done by DPPH assay. In cultivar PB 1121, three priming treatments were found to have a moderately higher % reduction than control. The highest % reduction was observed in seeds primed with proline [58.11 ± 4.54 %] followed by, salicylic acid [45.27 ± 18.36 %] and KNO_3 [39.88 ± 9.60 %] as compared to control [38.29± 6.93%] (Fig 7). In cultivar PB 1718, all the priming treatments were observed to have significantly higher % reduction than control. The highest % reduction was observed in seeds primed with $MgSO_4$ [77.82 ± 3.91 %], followed by NaCl [77.20 ± 4.59 %], Moringa leaf extract [74.77 ± 3.90 %], Cow urine [74.21 ± 5.37 %], KNO_3 [74.38 ± 5.52 %] as compared to control [22.38 ± 2.51 %] (Fig. 7).

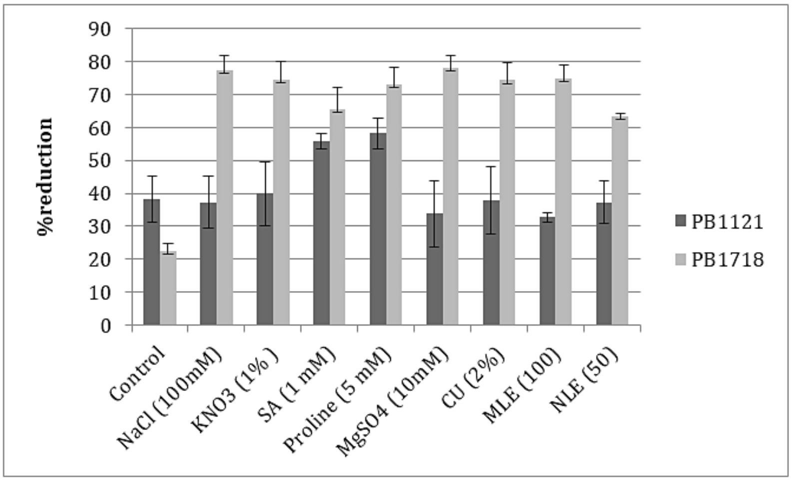


Fig. 7: Effect of priming on DPPH activity (% reduction) in rice cultivars PB 1121 and PB 1718

Discussion

In the present study, efficacy of different seed priming treatments was tested for amelioration of salt stress in two popular varieties of *Oryza sativa*. Out of the two cultivars that were tested, cultivar PB 1718 showed better plant growth and improved germination when subjected to priming treatment with Salicylic acid (1mM), KNO_3 (1%), Moringa leaf extract (100%) and Neem leaf extract (50%) under salt stress of NaCl (150 mM). In cultivar PB 1121, no significant change was seen in germination indices under salt stress as compared to the control. However, this cultivar showed better root growth upon priming treatments with Salicylic acid (1mM), KNO_3 (1%), Proline (50mM), Moringa leaf extract (100%), $MgSO_4$ (10mM) and Neem leaf extract (50%).

KNO_3 and Salicylic acid are reported to have a significant impact on emergence, seedling growth, and biochemical attributes as well as antioxidant properties of rice seedlings. KNO_3 acts by maintaining ion homeostasis which is found to be essential for osmotic adjustment under salt stress. It facilitates membrane stability under stress, prevents chlorophyll degradation and regulates various physiological processes.[24] The application of salicylic acid accelerates the growth of cells by both division and elongation. It also helps in scavenging enzymes such as amylase, dehydrogenase and acid phosphatase in primed seeds by reactive oxygen species (ROS) and thereby stimulating seedling growth.[25] The Salicylic Acid pre-treatment also leads to the accumulation of various osmolytes, antioxidants and antioxidative enzymes to normal levels.[26]

Moringa and Neem leaves have been reported to facilitate cell division due to the presence of zeatin which is a natural cytokinin, in addition to minerals like potassium, calcium, magnesium, vitamin c etc.[27] Similar results involving seed priming with leaf extracts have been found to improve seed quality and overall yield in maize hybrids.[28] Priming treatments with MLE has been reported to improve photosynthetic pigment levels thereby promoting plant growth under stress conditions.

Conclusion

In the present study seed priming treatment of two popular rice, cultivars (PB 1121 and PB 1718) showed promising results for overcoming salt stress with improved germination, seedling establishment and growth. The cultivars assessed showed variable responses towards different priming treatments. Out of the two cultivars assessed, PB 1718 exhibited robust seedling establishment upon priming treatments as compared to PB 1121. In cultivar PB 1718, priming treatments with KNO3 (1%), SA (1 mM), NLE (50%) and MLE (100%) resulted in better germination, growth and biochemical parameters, leading to improved alleviation of salinity stress. In cultivar PB 1121, Salicylic acid (1mM), KNO_3 (1%), MLE (100%), $MgSO_4$ (10mM), Proline (50Mm) and NLE (50%) treatments resulted in improved root growth. Therefore, the present study has the potential for developing cost-effective, easy-to-use, accessible and sustainable priming methods for overcoming salt stress in rice.

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Conflict of Interest

The authors declare no conflict of interest.

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