Seed priming for increased seed germination and enhanced seedling vigor of winter rice

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Abstract. Low seed emergence rate, poor seedling growth and low survival rate of winter rice due to cold spell are very common in Bangladesh. Pre-sowing seed priming could be a viable approach to overcome this problem. A laboratory screening of different priming agents was conducted at the Agro Innovation Laboratory, Department of Agronomy, Bangladesh Agricultural University in November 2018. The experiment comprised two factors. Factor A includes five rice varieties, namely i) BRRI dhan28 ii) BRRI dhan29 iii) BRRI dhan36 iv) BRRI dhan55 and v) Hybrid SL-8H; factor B includes 22 seed priming methods comprised different concentrations of NaCl, KCl, CaCl2, CuSO4, ZnSO4, Na2MoO4, PEG and control (no priming). The experiment was laid out in a completely randomized design (CRD) with four replications. A positive influence of seed priming on seed emergence rate (%), germination time, seedling vigor and seedling growth (length and dry matter) was evident from this study. Among the priming agents, KCl and CaCl2 performed best; while priming with NaCl, Na2MoO4 and PEG showed no advantages over no priming for germination rate and seedling vigor & growth indices. In conclusion, pre-sowing seed priming approach can be explored as a viable tool for increased seed germination and better seedling growth of winter rice under stress condition.

1. Introduction
Rice (Oryza sativa L.), the leading cereal and staple food for the billions of people, is mostly grown in tropical and sub-tropical regions following direct seeding or transplanting method depending on the agro-climatic conditions and management facilities. Apart from low quality seed, chilling and moisture stresses and weed interference in nursery bed and/or main field are amongst the major reasons for low seed germination, poor seedling establishment and reduced plant growth resulting significant yield losses of rice. Winter rice is greatly affected by low temperature stress at different growth stages in sub-tropical condition of Bangladesh. Especially in northern part of the country, seedling mortality often goes up to 90% when temperature goes below 10 °C [1]. Under Bangladesh condition, transplanted winter rice has to face low temperature during seed germination and seedling growth stages in nursery bed (when early planted) or during seedling establishment stage just after transplanting in the main field (when planted late); both resulted in chilling stress with significant growth and yield losses [2]. On the other hand, late planted dry direct seeded (aerobic) winter rice may enjoy moisture stress and huge weed pressure at early growth stage and expose to high temperature during reproductive stage resulting poor growth and lower yield [3,4].
Seed priming is an approach where seeds are allowed to be hydrated partially without radicle emergence following re-drying to original moisture content [5]. Thus, primed seeds imbibe and revive metabolic activities soon after sowing resulting in increased germination rate, higher germination uniformity, faster emergence and vigorous seedlings [6,7]. Moreover, pre-sowing seed priming leads to crops growing faster and yielding higher [8].

Different seed priming techniques include hydropriming, osmopriming, chemical priming, hormonal priming, redox priming and so on. Juraimi et al. [9] confirmed faster and increased germination rate along with vigorous seedling growth in rice as the consequence of seed priming. A positive effect of seed priming on germination and seedling growth of rice under chilling and drought stresses has been revealed by many researchers [10,11]. On the other hand, Anwar et al. [7] reported increased competitiveness of rice plants grown from primed seeds against weeds. Therefore, it was hypothesized that seed priming could help stress management in rice. Keeping those in mind present study was carried out to evaluate the potentiality of different seed priming approaches for increasing seed germination and seedling vigor to combat different biotic and abiotic stresses under field condition.

2. Materials and Methods

2.1. Experimental site and materials

The laboratory experiment was conducted at the Agro Innovation Laboratory, Department of Agronomy, Bangladesh Agricultural University in November 2018. The experimental site was situated at 23°77’ N latitude and 90°33’ E longitude at an altitude of 18.6 meter above sea level under subtropical climate. Seeds of five Boro (winter) rice varieties namely, BRRI dhan28, BRRI dhan29, BRRI dhan36, BRRI dhan55 and Hybrid SL-8H (harvested in the same season) used as plant materials were collected from Bangladesh Rice Research Institute (BRRI) and transferred to the Agro Innovation Laboratory. Seeds were kept in air-tight containers and preserved in a refrigerator at a temperature of 5 ± 1°C till used. Initial moisture content of seeds was between 9-9.5% as determined by oven dry method. Germination test using between papers on 4 replicates of 25 seeds revealed the germination between 90-94%. Seven priming agents used in the experiment were of laboratory grade. Details of the priming agents are presented in Table 1.

| Priming agent      | Chemical formula | Manufacturer    |
|--------------------|------------------|-----------------|
| Sodium chloride    | NaCl             | MERCK, India    |
| Potassium chloride | KCl              | MERCK, India    |
| Calcium chloride   | CaCl₂            | MERCK, India    |
| Copper sulphate    | CuSO₄·5H₂O       | MERCK, India    |
| Zinc sulphate      | ZnSO₄·7H₂O       | MERCK, India    |
| Sodium molybdate   | Na₂MoO₄·2H₂O     | MERCK, India    |
| Polyethylene glycol 4000 | PEG 4000 | LOBAL Chemie, India |

2.2. Experimental treatments and design

The experiment comprised two factors. Factor A includes five rice varieties, namely i) BRRI dhan28 ii) BRRI dhan29 iii) BRRI dhan36 iv) BRRI dhan55 and v) Hybrid SL-8H; factor B includes 22 seed priming methods such as i) 10000 ppm NaCl, ii) 20000 ppm NaCl, iii) 30000 ppm NaCl, iv) 10000 ppm KCl, v) 20000 ppm KCl, vi) 30000 ppm KCl, vii) 10000 ppm CaCl₂, viii) 20000 ppm CaCl₂, ix) 30000 ppm CaCl₂, x) 25 ppm CuSO₄, xi) 50 ppm CuSO₄, xii) 75 ppm CuSO₄, xiii) 5000 ppm ZnSO₄, xiv) 10000 ppm ZnSO₄, xv) 15000 ppm ZnSO₄, xvi) 1 ppm Na₂MoO₄, xvii) 2 ppm Na₂MoO₄, xviii) 3 ppm Na₂MoO₄, xix) 50 ppm PEG, xx) 100 ppm PEG, xxi) 150 ppm PEG and xxi)
Control (no priming). The experiment was laid out in a completely randomized design (CRD) with four replications.

2.3. Seed priming

Seeds of all the rice varieties were separately soaked in different priming agent solutions (previously prepared using distilled water) as per treatments for 24 hours at room temperature (25±2 °C). The ratio of seed weight to solution volume was 1:5 (g m L⁻¹). Then, seeds were removed from the priming agent solution followed by washing several times with distilled water to remove the traces of chemicals. Seeds were then dried back closer to the original moisture content (calculated as 9.5% by oven-dry method) by forced air for 48-60 hrs at 28±2°C. Dried seeds were kept in sealed polythene bags and stored in a refrigerator at 5±1°C for 15 days before placed for germination. While, control treatment received no prior seed priming.

2.4. Preparation of germination media and seed placement

Sterilized sand was used as germination media and plastic pots of 15 cm diameter with 6 cm depth was used as container. The moisture content of the media was maintained at around 80% of the field capacity by watering with distilled water every morning. One hundred seeds were sown in 0.5 cm depth of moist sand in each pot. Pots were put on the desk of the laboratory at room condition (25±2 °C temperature, 70±5% relative humidity and 11/13 h light/dark).

2.5. Observations made

Germination pattern, germination percentage and seedling vigor parameters were observed. Number of germinated seeds were counted every morning. Appearance of plumule over sand layer was considered as germination. Germination percentages (GP), days to 50% germination (T₅₀), mean germination time (MGT) and germination index (GI) were calculated as follows:

\[
GP = \frac{\text{Number of germinated seeds at final count}}{\text{Total number of seeds tested}} \times 100
\]

\[
T_{50} = ti + \frac{2}{nj-ni} (\frac{N}{2} - ni)(nj-ti)
\]

where, N is the final number of germination and nᵢ, nᵣ are cumulative numbers of seeds germinated by adjacent counts at time tᵢ and tᵣ (days), respectively; when nᵢ < N/2 < nᵣ.

\[
\text{MGT} = \sum \frac{Dn}{n}
\]

where, n is the number of seeds germinated on day D and D is the number of day counted from beginning of germination.

\[
\text{GI} = \frac{\text{Number of germinated seeds}}{\text{Day of first count} + \ldots + \text{Day of final count}}
\]

After 14 days of seed placement for germination, 20 seedlings from each replicate were randomly selected. Root and shoot lengths were measured, and then oven dried at 70°C for 72 hrs to record root and shoot dry weight of seedlings. Seedling vigor index (SVI) was calculated as follows:
Seedling Vigor Index (SVI) = \frac{\text{Seedling length (cm)} \times \text{Germination percentage}}{100}

where, Seedling length = Root length + Shoot length

2.6. Statistical analysis
The recorded data were compiled and tabulated for statistical analysis. Analysis of variance was done with the help of computer package MSTAT. The mean differences among the treatments were adjudged by Duncan’s Multiple Range Test at 5% level of significance. Since varietal effect was not significant for any parameter average values of five rice varieties are presented.

3. Results and Discussion
Priming agent exerted significant effect (p<0.01) on seed germination rate, germination pattern and seedling vigor of winter rice (Table 2 and Figure 1). In general, seed priming showed a positive effect on seed germination and seedling vigor of winter rice. Only priming with NaCl at any concentration reduced emergence rate, while CaCl₂ priming enhanced germination rate the most. Compared to control, seed priming resulted in faster germination observed in terms of days to 50% germination and mean germination time. Priming with 50 ppm PEG reduced days to 50% germination the most (> 2 days). On the other hand, priming with 10000 ppm CaCl₂ took the least mean germination time (2.8 days). Priming with CaCl₂, KCl and PEG found the best for germination index, while CaCl₂, KCl and CuSO₄ resulted in the most vigorous seedlings.

Table 2. Effect of seed priming method on seed germination behavior of rice (average of six varieties).

| Seed priming method | Germination % | Days to 50% germination | Mean germination Time (days) |
|---------------------|---------------|-------------------------|-----------------------------|
| Priming agent       | Concentration |                          |                             |
| NaCl                | 10000         | 89.75 de                | 2.80 b-f                    | 3.07 c-g |
|                     | 20000         | 87.75 ef                | 2.85 b-f                    | 3.30 b-g |
|                     | 30000         | 85.00 f                 | 2.92 b-f                    | 3.50 b-d |
| KCl                 | 10000         | 94.50 a-c               | 2.80 b-f                    | 2.95 e-g |
|                     | 20000         | 94.50 a-c               | 3.05 b-f                    | 3.05 c-g |
|                     | 30000         | 92.00 b-e               | 3.25 b                      | 3.15 b-g |
| CaCl₂               | 10000         | 96.25 ab                | 2.55 ef                    | 2.80 g   |
|                     | 20000         | 97.75 a                 | 2.70 c-f                    | 2.97 d-g |
|                     | 30000         | 96.25 ab                | 2.85 b-f                    | 2.95 e-g |
| CuSO₄               | 25            | 95.50 a-c               | 2.62 d-f                    | 3.20 b-g |
|                     | 50            | 96.75 ab                | 2.80 b-f                    | 3.45 b-f |
|                     | 75            | 93.75 a-d               | 2.92 b-f                    | 3.55 bc  |
| ZnSO₄               | 5000          | 95.50 a-c               | 2.85 b-f                    | 3.40 b-f |
|                     | 10000         | 94.75 a-c               | 2.97 b-f                    | 3.55 bc  |
|                     | 15000         | 92.75 b-d               | 3.17 bc                    | 3.47 b-e |
| Na₂MoO₄             | 1             | 92.25 b-d               | 2.97 b-f                    | 3.45 b-f |
|                     | 2             | 93.25 a-d               | 3.10 b-d                    | 3.50 b-d |
|                     | 3             | 90.75 c-e               | 3.07 b-e                    | 3.62 b   |
| PEG                 | 50            | 94.00 a-d               | 2.52 f                      | 2.92 fg  |
|                     | 100           | 95.25 a-c               | 2.57 d-f                    | 2.85 g   |
|                     | 150           | 93.00 b-d               | 2.80 b-f                    | 3.20 b-g |
| No priming (control)|               | 92.75 b-d               | 4.60 a                      | 5.17 a   |
| Sx                  |               | 1.39                    | 0.159                       | 0.159    |
| Level of significance| **           | **                      | **                         |
| CV (%)              | 2.98          | 10.81                   | 9.57                        | ** = Significant at 1% level of probability
Table 3. Effect of seed priming method on seedling growth of rice (average of six varieties).

| Seed priming method | Priming agent | Concentration (ppm) | Root length (mm) | Shoot length (mm) | Seedling length (mm) | Root dry matter (mg) | Shoot dry matter (mg) | Seedling dry matter (mg) |
|---------------------|---------------|---------------------|------------------|-------------------|----------------------|---------------------|----------------------|------------------------|
|                     | NaCl          | 10000               | 44.72 j          | 97.32 e-g         | 142.1 hi             | 8.40 c-e            | 13.27 ef             | 21.67 de               |
|                     |               | 20000               | 45.17 ij         | 95.78 g           | 140.9 hi             | 8.20 c-e            | 13.45 ef             | 21.65 de               |
|                     |               | 30000               | 47.05 g-j        | 96.50 f-g         | 143.6 g-i            | 8.32 c-e            | 13.50 d-f            | 21.83 de               |
| KCl                 | 10000         | 55.95 a-c           | 113.9 ab         | 169.8 ab          |                      | 10.18 a-c           | 16.52 a-e            | 26.70 a-c              |
|                     | 20000         | 57.75 ab            | 115.8 a          | 173.5 a           |                      | 10.52 ab            | 16.92 a-d            | 27.45 ab               |
|                     | 30000         | 59.67 a             | 117.2 a          | 176.8 a           |                      | 10.75 a             | 17.23 a-c            | 27.98 a                |
| CaCl₂               | 10000         | 52.80 b-g           | 109.0 a-c        | 161.8 b-d        |                      | 8.92 a-e            | 17.75 ab             | 26.67 a-c              |
|                     | 20000         | 55.75 a-d           | 113.4 a-b        | 169.2 a-c        |                      | 9.27 a-e            | 18.50 a              | 27.77 a                |
|                     | 30000         | 54.67 a-e           | 113.8 a-b        | 168.5 a-e        |                      | 9.40 a-e            | 18.27 a              | 27.67 a                |
| CuSO₄               | 25            | 51.65 c-h           | 107.4 b-d        | 159.1 c-e        |                      | 10.25 a-c           | 17.25 a-c            | 27.50 ab               |
|                     | 50            | 53.13 b-f           | 109.8 a-c        | 162.9 b-d        |                      | 10.23 a-c           | 17.20 a-c            | 27.42 ab               |
|                     | 75            | 53.95 b-f           | 109.2 a-c        | 163.1 b-d        |                      | 10.60 ab            | 17.45 a-c            | 28.05 a                |
| ZnSO₄               | 5000          | 50.88 c-i           | 100.1 d-g        | 151.0 e-h        |                      | 9.10 a-e            | 16.45 a-e            | 25.55 a-d              |
|                     | 10000         | 51.63 c-h           | 101.7 c-g        | 153.3 d-g        |                      | 9.47 a-d            | 16.67 a-e            | 26.15 a-c              |
|                     | 15000         | 53.55 b-f           | 101.5 c-g        | 155.1 d-f        |                      | 9.52 a-d            | 17.08 a-c            | 26.60 a-c              |
| Na₂MoO₄             | 1             | 48.15 f-j           | 105.5 b-e        | 153.7 d-g        |                      | 8.27 c-e            | 14.03 c-f            | 22.30 c-e              |
|                     | 2             | 48.30 f-j           | 106.8 b-d        | 155.1 d-f        |                      | 8.80 a-e            | 14.25 b-f            | 23.05 b-e              |
|                     | 3             | 48.38 f-j           | 107.0 b-d        | 155.9 d-f        |                      | 8.57 b-e            | 14.23 b-f            | 22.80 c-e              |
| PEG                 | 50            | 49.20 c-e           | 97.95 e-g        | 147.1 f-i        |                      | 7.30 e              | 12.30 f              | 19.60 e                |
|                     | 100           | 49.80 c-e           | 101.3 c-g        | 151.1 e-h        |                      | 7.70 de             | 12.88 f              | 20.58 e                |
|                     | 150           | 50.15 d-j           | 104.8 e-f        | 154.9 d-f        |                      | 8.15 c-e            | 12.98 f              | 21.13 de               |
|                     | No priming (control) | 46.83 b-j         | 93.60 g          | 140.4 i          |                      | 8.15 c-e            | 12.35 f              | 20.50 e                |

S² = Significant at 1% level of probability, NS = Not significant
A significant effect (p<0.01) of seed priming on seedling growth of winter rice was evident (Table 3). With a very few exceptions all the priming agents positively influenced seedling growth of winter rice. Priming with CaCl₂ and KCl performed the best in terms of root length, shoot length and seedling length of rice, while NaCl priming performed the worst. It is interesting to note that NaCl priming resulted in shorter root length compared to control. For enhancing root, shoot and dry matter of winter rice, seed priming with KCl, CaCl₂, CuSO₄ and ZnSO₄ were found better than priming agents like NaCl, Na₂MoO₄ and PEG.

Seed germination rate, germination pattern, seedling growth and vigor are very crucial for plant growth and productivity under both normal and adverse conditions. As evident from different reports [13, 14], seed priming provides plants with greater tolerance when exposed to stress. It was, therefore, hypothesized that pre-sowing seed treatment could enhance germination, increase germination rate and improve seedling vigor and growth that could be helpful for winter rice seedlings exposed to different abiotic (moisture, temperature) and biotic (weed) stresses. In general, a positive impact of seed priming (except NaCl priming) on seed germination percentage of winter rice is evident from this study; KCl or CaCl₂ priming performed the best. These findings are in conformity with those of many others who confirmed increased and faster germination along with synchronized emergence of primed seeds in rice and other crop species [9, 12].

Arif et al. [15] opined that genetic and structural repair along with rapid development of immature embryo resulted from seed priming are plausible justifications for increased germination rate. Still and Bradford [16], on the other hand, claimed that seed priming reduced physiological non-uniformity in the seed bulk that might contribute to higher seed germination rate and more synchronized germination. Although NaCl priming failed to increase seed germination rate in this study, conflicting findings has also been reported by [12]) in rice, where osmo-hardening with NaCl enhanced germination rate. This might be due to the variation in crop variety and seed quality used and NaCl concentration tested in different studies.

Seedling growth parameters like root and shoot length and dry weight were enhanced due to seed priming. All the priming agents registered an enhanced seedling growth, but in some cases performances of NaCl or PEG were not satisfactory, may be due to their phytotoxic effects on rice seedlings. Several studies confirmed the positive influence of pre-sowing seed priming on seedling growth [2, 11]. Rapid imbibition during priming might disrupt cell membrane, causes localized cells in cotyledons resulting in more vigorous seedlings [17]. Basra et al. [6], on the other hand, opined that seed priming mediated seedling growth might be the consequence of rapid emergence as the consequence of earlier production of emergence metabolites. As stated by [18], starch metabolism, total soluble sugar contents and α-amylase activities are strongly linked to the rice seedling growth.

4. Conclusion

Present study confirms the potentiality of seed priming as a tool for increasing rice seed germination rate, and enhancing seedling growth. Seed priming with KCl or CaCl₂ was found the best priming agents. Conclusively, these findings will provide new avenues for advancing seed priming for higher germination and increased seedling vigor of rice under stress.

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