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Seed Priming Improves Germination and Early Seedling Growth in Wheat Under Control and Drought Conditions

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Abstract

Drought is a serious threat of wheat seed germination, growth and productivity in Bangladesh. A germination test was set to examine the influence of seed priming with various agents on seed germination and early seedling growth in two wheat cultivars (BARI Gom 28 and BARI Gom 29) under control and drought conditions. The experiment was conducted following completely randomized design with three replications. Four priming treatments were used such as i) hydropriming (distilled water, control), ii) 10% Polyethylene Glycol (PEG-6000), iii) 100 mM $CaCl_2$ and iv) 1 mM ascorbic acid (AA). The primed seeds of two wheat cultivars were set in petri dishes for germination under two growth conditions *viz.* i) control and ii) drought (10% PEG-6000). In both cultivars and growth conditions, all priming agents increased final germination percentage (FGP), root and shoot length and root and shoot biomass in comparison to control. All these parameters were significantly affected by drought stress in both wheat cultivars. The highest FGP was observed in AA and $CaCl_2$ priming (73%) in both cultivars under drought stress compared to the others. The root length and root dry weight were also higher in AA and $CaCl_2$ priming in both cultivars and growth conditions but the variations between AA and $CaCl_2$ were statistically similar. All seed priming techniques increased the stress tolerance indices and root-shoot ratio in both wheat cultivars showing an increased tolerance to drought. The higher values of stress tolerance indices in BARI Gom 28 compared to BARI Gom 29 indicated that the cultivar BARI Gom 28 performed better in response to drought and priming agents. In conclusion, the priming agents AA and/or $CaCl_2$ (depending on availability and cost effectiveness) could be used for improving seed germination and early seedling growth both under control and drought stress conditions.

Introduction

Wheat (*Triticum aestivum* L.) is the second most important cereal crop after rice in Bangladesh. The crop is cultivated all over Bangladesh during Rabi season with or without irrigation. During the year 2018–2019, a total of 10, 17,000 m tons of wheat were produced from 3, 30,348 ha of land with an average grain yield of 3.014 m tons ha^{-1} in Bangladesh (BBS, 2019). The area of wheat cultivation and production in Bangladesh are decreasing (5.94% less than the year 2017-2018). This could be because of climate change consequences and lack of improved production technology as well as lack of tolerant and high yielding varieties. Drought is one of the most important environmental factors limiting plant growth and productivity all over the world (Araus et al., 2002). Major wheat growing areas of the world are faced water deficit that adversely affect grain yield (Semenov and Shewry, 2011).

The northern-western part of Bangladesh is severely affected by drought stress especially during the later part of the Rabi season. Drought stress initially affects germination and impaired seedling establishment (Kaya et al., 2006; Farooq et al., 2012). Various studies have reported the reduction in germination potential, early seedling growth, root and shoot dry weight, hypocotyl length and vegetative growth in plants under drought (Jisha et al., 2013; Marthandan et al., 2020).

Seed germination and early seedling growth in many crops are the most sensitive stages to drought stress and water deficit may delay the onset and reduce the rate of uniformity of germination, leading to poor crop growth and yield (Demir et al., 2006). Seed priming is a pre-sowing strategy for influencing seedling development by modulating pre-germination metabolic activity prior to

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emergence of the radicle and generally enhances germination rate and plant performance (Taylor and Harman, 1990). It improves seed performance by rapid and uniform germination, normal and vigorous seedlings, which resulted in faster and better germination and emergence of different crops in normal and stress conditions (Powell et al., 2000; Cantliffe, 2003; Ashraf and Foolad, 2005; Carbineau and Come, 2006; Jisha et al., 2013). Seed priming has been proved to be an effective method in imparting stress tolerance to plants (Van Hulten et al., 2006).

Primed seeds usually exhibit an increased germination rate, uniform germination with lower time and greater germination percentage and these attributes have practical agronomic implications especially under adverse growth conditions (Barsa et al., 2005). Therefore, seed industries need to find suitable priming agent(s) with strong emphasis for increasing the tolerance of plants under abiotic stress conditions. Various priming approaches have been employed including hydropriming, osmopriming (soaking seeds in osmotic solutions), halopriming (soaking seeds in salt solution), hormonal priming (soaking seeds in plant growth regulator solutions) etc. to increase the speed and synchrony of seed germination (Jisha et al., 2013). In wheat, among the different seed-priming agents like salicylic acid, ascorbic acid, kinetin, GA3 and ascorbic acid showed better results (Khan et al. 2011). Seed priming is a rapid and cost-effective technology which can be adopted by farmers without any complication to enhance the germination and seedling growth of wheat under drought condition. The objectives of this study were i) to investigate the influence of different priming agents on seed germination and seedling growth in two wheat cultivars under drought stress and ii) to select suitable priming agent(s) for the enhancement of seed germination in both control and drought conditions.

Materials and Methods

The experiment was conducted in the Plant Physiology Laboratory, Department of Crop Botany, Bangladesh Agricultural University in 2019. The seeds of two wheat cultivars viz. BARI Gom 28 and BARI Gom 29 were collected from Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. A two factorial experiment was set following completely randomized design (CRD) with three replications. The experimental factors were a) Priming agents - 4 and b) Stress - 2 (control and drought). Four priming techniques were implied such as i) hydropriming with distilled water (control), ii) osmopriming with 10% Polyethylene Glycol (PEG-6000) (LR grade, Merck, Mumbai, India), iii) osmopriming with 100 mM $CaCl_2$ (LR grade, Research-lab Fine Chem Industries, Mumbai, India) and iv) hormonal priming with 1 mM ascorbic acid (AA) (LR grade, Research-lab Fine Chem Industries, Mumbai, India). The priming was done by immersing 75 seeds of each cultivar under 20 mL of respected priming solutions in 50 mL glass beakers and kept for 24 h at 25°C in dark conditions. The ratio of seed weight to solution volume was maintained as 1:6 (g mL^{-1}). After 24 h, primed seeds were rinsed with distilled water for two min and then dried to the original moisture level and left overnight at room temperature to their original dry weight. The primed seeds of two wheat cultivars immediately getting their original dry weight were placed in the petri dish (9 cm diameter) as per treatment specifications. Germination test for primed seeds was performed for 10 days in petri dishes on a layer of filter paper (Whatman # 41) under two growth conditions i) Control (0% PEG-6000, 0 MPa osmotic potentials) and ii) Drought (10% PEG-6000, -0.30 MPa). For control, 24 petri dishes (2 cultivars x 4 priming agents x 3 replications) containing 10 seeds/dish were applied 5 ml of distilled water daily to keep it moist. For drought condition, another set of 24 petri dishes containing 10 seeds/petri dish were applied 5 ml of distilled water for first two days and then 5 mL of 10% PEG-6000 solution was applied for each petri dish for drought imposition.

The number of sprouted seeds (2 mm radicle length) was counted every day. The final count was done on day eight and final germination percentage (FGP) was calculated using the following formulae.

$$\text{Final Germination Percentage (FGP)} = \frac{\text{Total no. of seeds germinated at day 8}}{\text{Total no. of seeds set for germination}} \times 100$$

Shoot Length (SL) was measured from shoot base to the tip of the longest leaf and Root Length (RL) was measured from root base to the root tip. The dry weight of root and shoot (g) were determined by drying the sample in an oven at 80±2^0 C for three days. Germination Stress Tolerance Index (GSTI), Root Length Stress Tolerance Index (RLSI), Shoot Length Stress Tolerance Index (SLSI) and Root Dry Weight Stress Tolerance Index (RDSI) were estimated using the following formula (Ashraf et al., 2008):

GSTI = (FGP of stressed seeds / FGP of control seeds) X 100

where, RLSI = (Radicle length of stressed plants / radicle length of control plants) X 100; SLSI = (Shoot length of stressed plants / shoot length of control plants) X 100; and RDSI = (Root dry weight of stressed plants / root dry weight of control plants) X 100.

The Root Shoot Ratio (RSR) was calculated by root dry weight over shoot dry weight.

The statistical software 'R' was used for two-way ANOVA considering the factors priming and stress for each cultivar. The multiple comparison of means was done by Tukey test of R program.

Results

Final Germination percentage (FGP) was significantly declined in drought condition compared to control in both wheat cultivars (Fig. 1). The FGP also varied in priming agents, but the variations were insignificant in each growth condition in both cultivars. The highest FGP was observed in ascorbate and $CaCl_2$ priming (73%) in both cultivars while the lowest FGP was found in hydropriming (67% in BARI Gom 28 and 63% in BARI Gom 29) under drought stress (Fig. 1).

Root length (RL) and root dry weight (RDW) were significantly differed among the priming agents in both growth conditions in both cultivars except the control condition in BARI Gom 28 (Fig. 2). In BARI Gom 28, the maximum RL and RDW were found in ascorbate priming followed by $CaCl_2$ whereas the minimum RL and RDW were observed in hydropriming in both control and stress conditions (Fig. 2A, C). In BARI Gom 29, the RL was higher in $CaCl_2$ and lower in PEG priming but the RDW was higher in ascorbate and lower in PEG priming in both control and stress condition in comparison to other priming agents (Fig. 2B, D). The RL and RDW in both cultivars were significantly affected by drought stress in comparison to control (Fig. 2).

Priming agents had significant effect on shoot length (SL) and shoot dry weight (SDW) in both the cultivars (Fig. 3). A significant decrease in SL and SDW was also observed in drought compared to control in both cultivars. The SL was higher in ascorbate followed by $CaCl_2$, PEG and hydropriming in both cultivars under control condition (Fig. 3A, B). In drought condition, ascorbate priming showed higher SL while lower SL was recorded in hydropriming in both cultivars. The ascorbate, $CaCl_2$ and PEG priming in both cultivars resulted in significantly higher SDW compared to hydropriming but the variations in SDW among the three priming agents were statistically similar in both control and drought conditions (Fig. 3C, D).

A significant variation in root shoot ratio (RSR) was observed among the priming agents and between the growth conditions in both cultivars (Fig. 4). In BARI Gom 28, the highest RSR was observed in hydropriming followed by $CaCl_2$, ascorbate and PEG in control whereas the maximum RSR was recorded in ascorbate followed by hydro, $CaCl_2$ and PEG under drought condition (Fig. 4A). In BARI Gom 29, the RSR was greater in ascorbate and hydropriming in control and drought conditions, respectively (Fig. 4B). The PEG priming showed significantly lower RSR in both growth conditions in BARI Gom 29. A significant increase in RSR considering each priming agent was found in drought condition compared to control (Fig. 4).

The stress tolerance indices were significantly varied by the application of priming agents in two wheat cultivars (Fig. 5). The germination stress tolerance index (GSTI) was maximum in $CaCl_2$ and ascorbate (76%) followed by PEG (72%) and hydropriming (69%) but the variations were not significant in BARI Gom 28. In contrast, GSTI was significantly lower in hydropriming (66%) in comparison ascorbate (76%), $CaCl_2$ (76%) and PEG (72%) in BARI Gom 29 (Fig. 5A). The root length stress index (RLSI) was maximum in ascorbate in both cultivars (74% in BARI Gom 28 and 71% in BARI Gom 29) whereas the lowest RLSI was observed in hydropriming (63% in BARI Gom 28 and 64% in BARI Gom 29) (Fig. 5B). The ascorbate priming showed significantly higher shoot length stress index (SLSI) in comparison to other priming agents (71% in BARI Gom 28 and 68% in BARI Gom 29) (Fig. 5C). In BARI Gom 28, ascorbate (79%) and $CaCl_2$ (78%) priming showed greater root dry weight stress index (RDSI) followed by PEG (69%) and hydropriming (62%) (Fig. 5D). The RDSI values were 74%, 73%, 66% and 63% in ascorbate, $CaCl_2$, hydro and PEG priming, respectively in BARI Gom 29 (Fig. 5D).

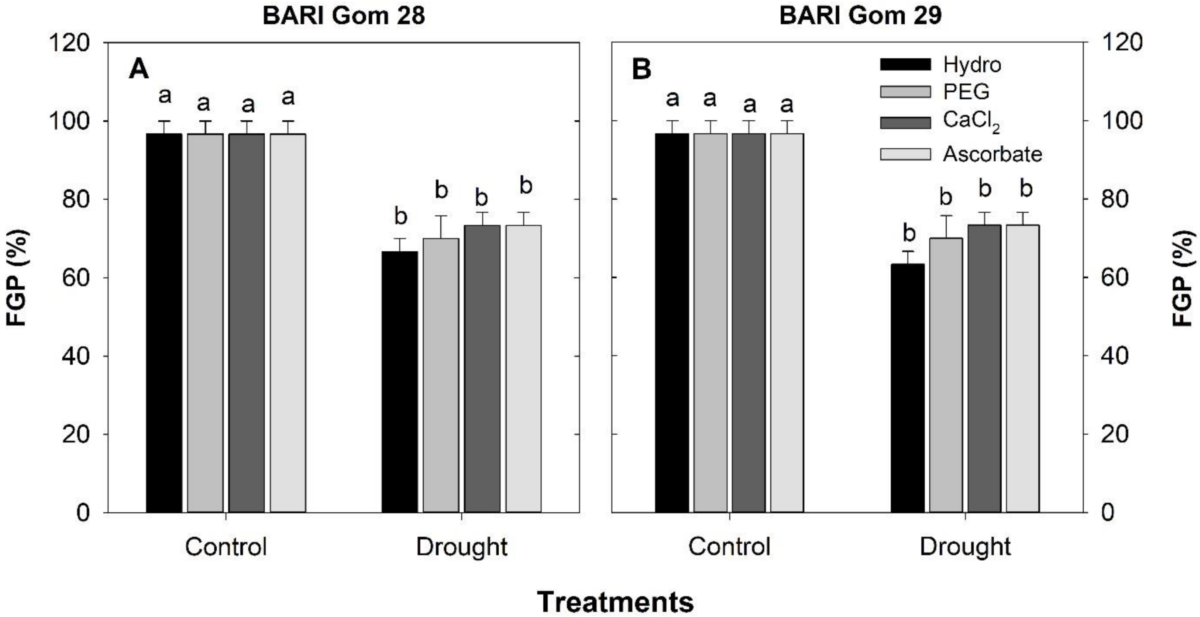

Figure 1. Final germination percentage (FGP, %) of two wheat cultivars grown under control and drought conditions using four priming agents (hydro, PEG, $CaCl_2$ and ascorbate). Treatment means with different letters within the growth conditions are significantly different at 5% level of probability. Vertical bars are SEM (n = 3).

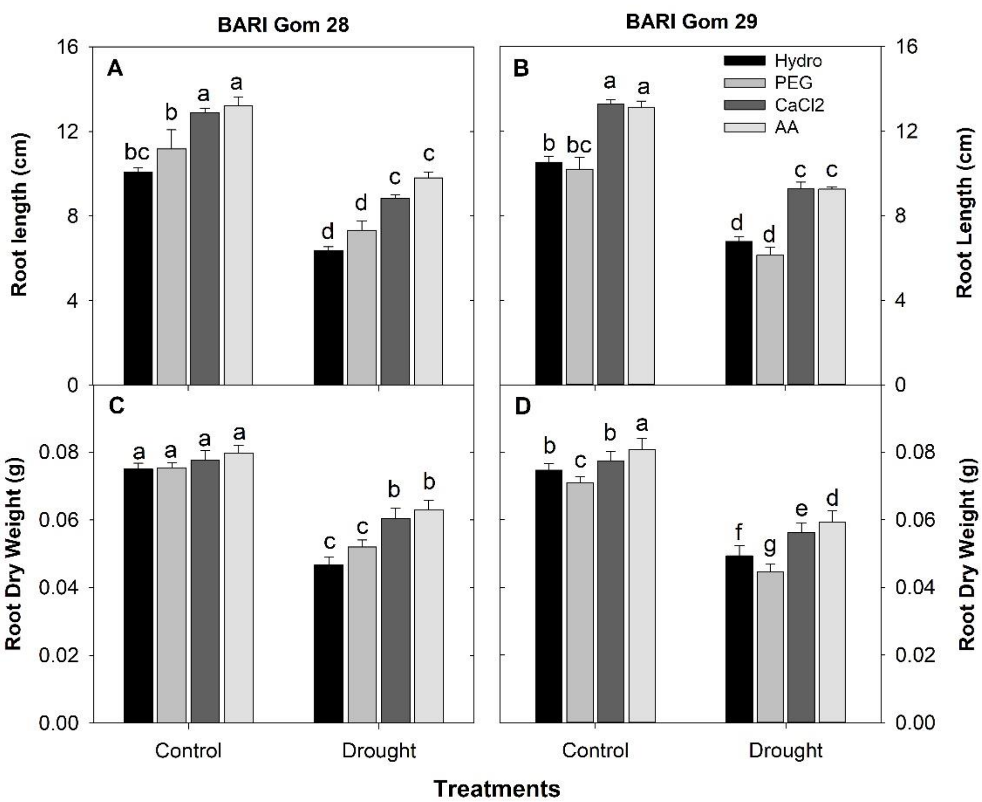

Figure 2. Root length (cm) and root dry weight (g) of two wheat cultivars grown under control and drought conditions using four priming agents (hydro, PEG, $CaCl_2$ and ascorbate). Treatment means with different letters within the growth conditions are significantly different at 5% level of probability. Vertical bars are SEM (n = 3).

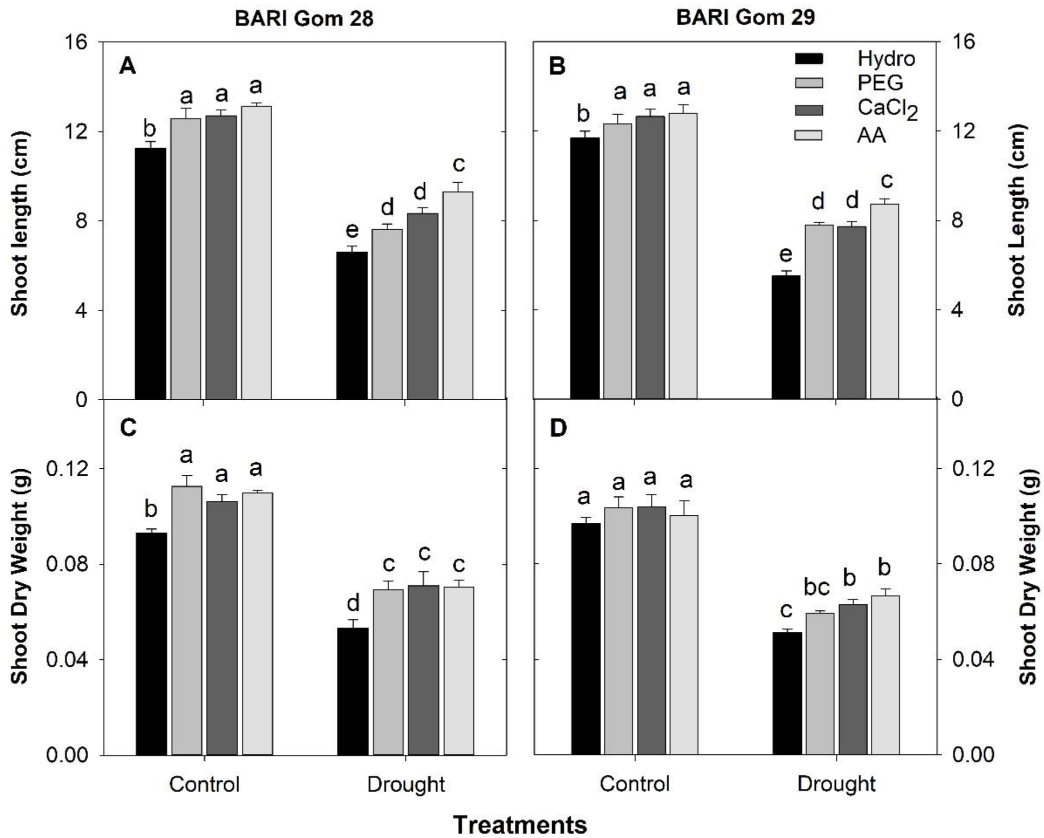

Figure 3. Shoot length (cm) and shoot dry weight (g) of two wheat cultivars grown under control and drought conditions using four priming agents (hydro, PEG, $CaCl_2$ and ascorbate). Treatment means with different letters within the growth conditions are significantly different at 5% level of probability. Vertical bars are SEM (n = 3).

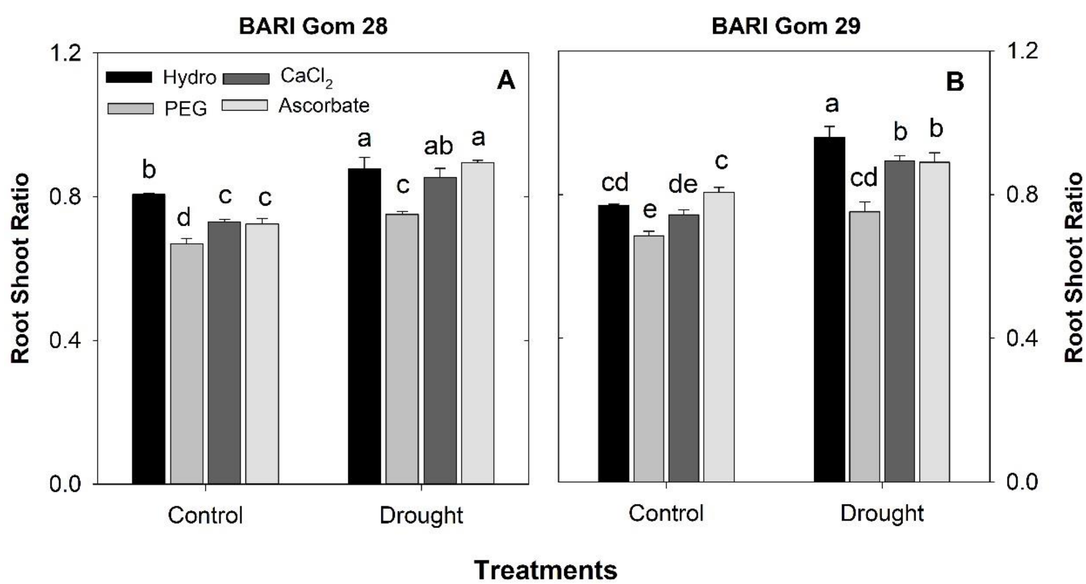

Figure 4. Root Shoot Ratio (RSR) of two wheat cultivars grown under control and drought conditions using four priming agents (hydro, PEG, $CaCl_2$ and ascorbate). Treatment means with different letters within the growth conditions are significantly different at 5% level of probability. Vertical bars are SEM (n = 3).

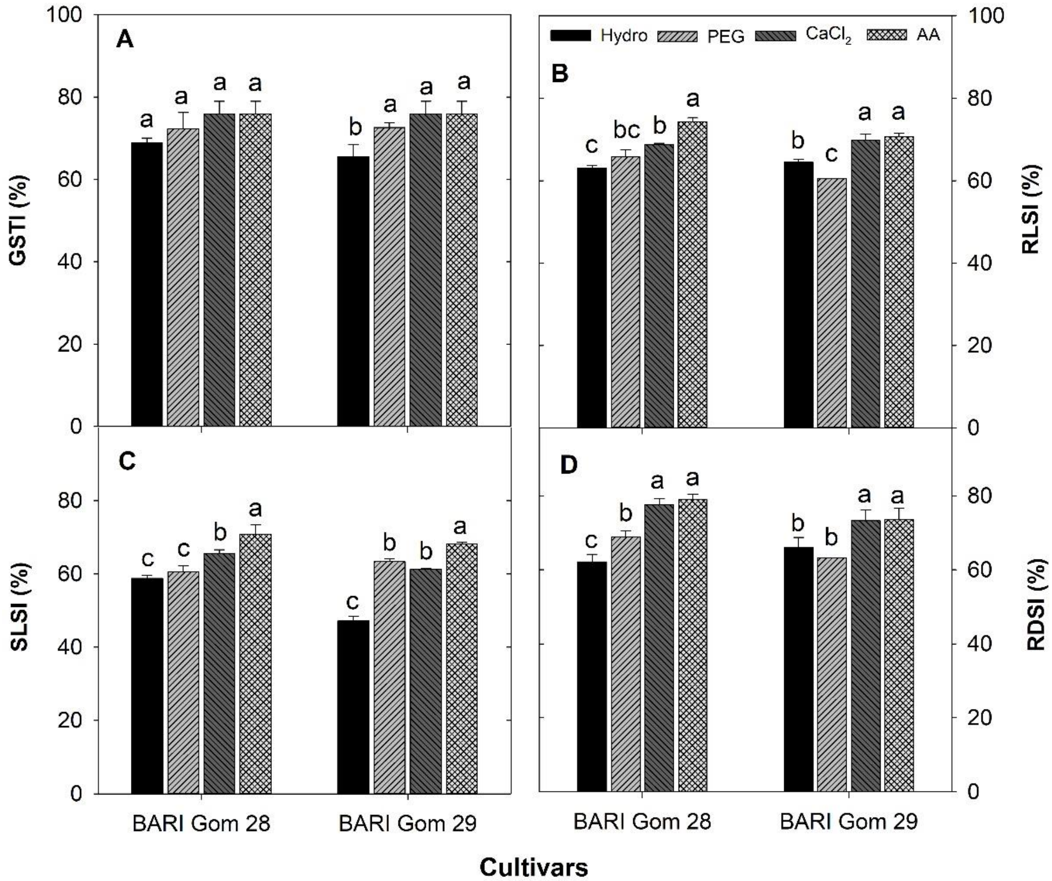

Figure 5. Germination stress tolerance index (GSTI), root length stress index (RLSI), shoot length stress index (SLSI) and root dry weight stress index (RDSI) of two wheat cultivars using four priming agents (hydro, PEG, $CaCl_2$ and ascorbate). Treatment means with different letters within each cultivar are significantly different at 5% level of probability. Vertical bars are SEM (n = 3).

Discussion

The frequency and severity of drought stress are expecting to increase in the world including Bangladesh due to global climate change (Trenberth et al., 2014). The first and foremost effect of water deficit is on seed germination or seedling establishment in crops due to the decrease in entrance of water into the seeds (Kaya et al., 2006). Seed priming is an easy and cost-effective and widely used technique to enhance drought tolerance in many crops including wheat (Jisha et al., 2013; Tabassum et al., 2018; Marthandan et al., 2020). A germination test was conducted to evaluate the performance of seed germination and seedling growth of two wheat cultivars under drought condition using different seed priming agents.

The results of this study revealed that all priming agents increased the seed germination and early seedling growth as well as increased the drought tolerance in both wheat cultivars. Many studies have reported the negative impacts of drought stress on germination potential, early seedling growth, root and shoot biomass and hypocotyl length in several crops (Kaya et al., 2006; Farooq et al., 2009). Among the priming agents implied in this study, ascorbate and $CaCl_2$ priming had given better results in seed germination, root and shoot length and biomass in both wheat cultivars in control and drought conditions. It is well documented that the priming of seeds with different priming agents increased the rate of germination and seedling growth by reducing imbibition time, increasing pre-germinative enzyme activation and metabolite production and regulating osmosis (Jisha et al., 2013; Hossain et al., 2016; Marthandan et al., 2020).

Osmopriming with different agents such as KNO_3, KCl, K_3PO_4, KH_2PO4, $MgSO_4$, $CaCl_2$, NaCl, Mannitol, polyethylene glycol (PEG), etc. essentially exposes seeds to a low external water potential to restrict the rate and extent of imbibition and activate pre-germinative metabolic processes without radicle protrusion (Farooq et al., 2005; Lutts et al., 2016). The PEG is widely and commonly used as lowering water potential of a solution because it is nontoxic in nature with large molecular in size which restricts its diffusion into the seeds to avoid cytotoxic effects (Thomas et al., 2000; Singh et al., 2014). Compared to hydropriming, PEG priming was found to have a better effect on seed germination and seedling growth under drought (Yuan-Yuan et al., 2010) and our study found the similar observation.

In present study, seed priming with $CaCl_2$ performed better in comparison to PEG and hydropriming in both control and drought condition. Seed priming with calcium salts was more effective and economical in improving stress tolerance in plants (Jafar et al., 2012; Tabassum et al., 2018) because calcium increases cell wall integrity and acts as secondary messenger in signaling pathways (Xu et al., 2013). $CaCl_2$ also enhances osmolytes accumulation and antioxidant activity under stressed conditions (Farooq et al., 2017). An increase in external Ca^{2+} could help to maintain an optimum K^+/Na^+ ratio in the cytosol by regulating K^+ transport across the plasma membrane (Laohavisit et al., 2013). Wheat seeds primed with $CaCl_2$ completed germination earlier and had more total sugars and non-reducing sugars than non-primed seeds (Jafar et al., 2012). Ascorbic acid used as a hormonal priming agent has been found to be effective in many studies (Khan et al., 2011; Jisha et al., 2013; Marthandan et al., 2020). Among the priming agents applied in this study, the greater germination and seedling growth in two wheat cultivars were observed in ascorbate priming. Seed priming with ascorbic acid resulted in maximum final germination and emergence percentage (FGP and FEP), radicle and plumule length, root and shoot length, number of secondary roots, root shoot ratio, root dry weight, shoot dry weight and seedling dry weight compared to control in wheat (Khan et al., 2011).

All priming agents used in this study exhibited greater drought tolerance compared to hydropriming showing higher stress tolerance indices (GSTI, SLSI, RLSI, SDSI) and greater root shoot ratio. The cultivar BARI Gom 28 performed slightly better than BARI Gom 29 due to the influence of different priming agents in both control and drought growth conditions. Finally, the results of this study suggested that the osmo-priming of wheat seeds with $CaCl_2$ would be more exacting than other priming agents because it would produce quicker and easier results with less expenses and could offer farmers a highly attractive approach for improving seed germination and seedling growth as well as better yield in drought condition.

Conclusion

Seed priming with ascorbate, $CaCl_2$ and PEG increased seed germination and early seedling growth in two wheat cultivars in both control and drought conditions. All these agents enhanced drought tolerance in both wheat cultivars while BARI Gom 28 performed better in relation to drought tolerance in comparison to BARI Gom 29. The study recommends farmers to use $CaCl_2$ priming for its easy availability and low cost compared to other priming agents. Further field trial is required to investigate the influence of seed priming using the recommended agent and dose of this study on growth, physiology and yield in wheat cultivars.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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