Original Research Article

Seed priming with proline and glycine betaine on germination, seedling growth, and photosynthetic pigments of rice (*Oryza sativa*) under chilling stress

.

ABSTRACT

Aims: Rice grown in late Rabi season frequently results poor germination and establishment because of exposure to extreme low temperature or chilling stress. Seed priming is an effective seed treating technique for uniform and good germination in various crops under different abiotic stresses. The present study was undertaken to assess the role of different seed priming agents in seed germination and seedling growth of rice (BRRI Dhan 29) under chilling stress.
Place and Duration of Study: a pot experiment was carried out at the Department of Seed Science and Technology, Bangladesh Agricultural University during Late November – Mid December 2021.
Methodology: The rice seeds (BRRI Dhan 29) were soaked in different priming agents (hydro-priming, 20 mM proline and glycine betaine) for 30 min. The primed seeds were exposed to cold stress (under 50C) in the refrigerator. The untreated seeds were used as control.
Results: In this single factor experiment, the treatments of Pro and GB priming has a positive impact on germination and survival rate, shoot and root length, shoot and root weight and photosynthetic pigments of leaf under CS. Among the treatments, Pro and GB at 20 mM and warm water performed better; while priming with tap water and Pro and GB at 10 mM showed insignificant performance over control but significant over stress in most of the parameters. Results indicated that (in most of the cases) seed priming has a positive impact on germination and survival, shoot and root length, shoot and root weight and chlorophyll content of leaf.
Conclusion: Thus, priming may be an effective tool to increase seed germination, better seedling growth of rice under cold stress, and proline or betaine or warm water priming (at 45 0C for 5 min) can be considered as an enduring priming agent.

Keywords: Germination, survival, chlorophyll content and cold stress

1. INTRODUCTION

Abiotic stresses like salinity, drought, extreme temperature (heat or cold) is the barrier for crop production [1]. Rice is one of the major field crops grown in the tropical and sub-tropical region of the world which has a great influence of temperature on its vegetative and reproductive growth. In Bangladesh, it is frequently endangered by low temperature stress and this stress during crop establishment and reproductive phases is a major problem for winter rice in Bangladesh's subtropical climate. A study conducted by Rashid et al. [2] found that seedling mortality in nursery beds and major fields, especially in the northern portion of the country, can reach 90% due to excessive cold temperatures in December and

January. They also showed about more than 2 million hectares of rice crop in Bangladesh's northern and north-eastern regions are being affected by cold period causing the partial to total crop destruction. Chilling stress affects rice plants especially at the seedling and reproductive stages. Diminished germination, poor seedling establishment, stunted seedlings, yellowing or whitening, and reduced tillering can all occur from low temperatures [3]. This stress stops a rice variety's full genetic expression by inhibiting metabolic reactions directly and indirectly through cold-induced osmotic, oxidative, and other stress [4]. Low temperatures hinder photosynthesis, limiting growth and yield since there is less glucose available for seed formation [5].

Seed priming has been shown to be an efficient seed invigoration strategy, enhancing the rate, percentage, and uniformity of seed germination and seedling emergence under stress conditions such as salinity, temperature, and drought stress [6-7]. Priming is commonly practiced to shorten the time between seed sowing and seedling emergence, as well as to coordinate emergence [8-9]. In this method seeds are soaked in a variety of solutions with a high osmotic potential to prevent them from collecting enough moisture to form radicles, thereby suspending them in the lag phase along with metabolic activation which is required for germination. Several studies on vegetables, floriculture, and some field crops have shown that seed priming improves germination and early seedling growth, particularly in plenty of conditions [10]. On this aspect, to mitigate this cold stress problem in rice yields in a sustainable way significant research is needed. The objectives of this paper were to see the effectiveness of different seed priming agents in improving seed emergence, seedling growth, vigour, and survival capacity of winter rice under cold stress, as well as to find out the responses of primed and un primed seeds when exposed to cold stress.

2. MATERIAL AND METHODS

2.1 Plant materials

The experiment was carried out at the Department of Seed Science and Technology of Bangladesh Agricultural University, Mymensingh. Seeds of well-known cultivated rice variety released by BRRI named BRRI Dhan 29 were used as plant materials in this experiment. To reduce contamination during priming, seeds surface was washed with sterile distilled water in three times.

2.2 Seed priming and experiment conduction

To accomplish the role of seed priming in alleviating the unwanted effects of chilling stress, seed priming approaches like, hydro-priming (normal tap water and warm water), and chemical priming (proline and glycine betaine) were examined in this experiment. For continuation of the study seeds were primed with 20 mM solution of proline and glycine betaine. In case of hydro-priming tap water around 25^0C and warm water about 45^0C was used for priming or soaking of the seeds. In all treating agent's seeds were primed for 30 minutes except warm water. Priming with warm water time duration was only 5 minutes. After the completion of soaking, the primed seeds were washed with distilled water for 2 min, surface-dried using blotting paper. Subsequently washing and drying, the seeds (twenty seeds per dish) were positioned on Petri dishes (150×20 cm^2 diameter) having three layers of Whatman filter papers, covered with lid and kept in normal laboratory (the room temperature was 25±1ºC and relative humidity was 95%) conditions while, the primed seeds dishes were exposed to cold stress (under 5^0C) in the refrigerator. Distilled water was sprayed to seeds to avoid desiccation. After 7 days of seed placement, when the germination just started the stressed seeds were placed in the growth chamber for 23 days to record the data. The chilling stress was imposed in growth chamber by maintaining the day and night temperatures at 15 ^{0}C, while temperature for control treatment was set at 25 ^{0}C in a separate growth chamber. With three times replication in a completely randomized design (CRD) this study was completed with the following treatments: Control, C; Chilling stress, CS; Hydro-priming with warm water, WW; Hydro-priming with warm water under chilling stress, WwCS; Hydro-priming with room temperature tap water, TW; Hydro-priming with room temperature water under chilling stress, TwCS; Priming with 20 mM Proline, $Pro_{20\ mM}$; Priming with 20 mM Proline under chilling stress, $Pro_{20mM}CS$; Priming with 20 mM Glycine betaine, $GB_{20\ mM}$; and Priming with 20 mM Glycine betaine under chilling stress, $GB_{20mM}CS$.

2.3 Data collection

Data were collected on germination (%), survival rate (%), seed vigour index (SVI), shoot and root length, shoot and root weight (both fresh and dry) and chlorophyll content. The final germination percentage was calculated at 10 DAS. At 20 DAS representative seedlings (10) from each replicate were selected randomly selected to get the data of percent survival, shoot and root length and the fresh weight of shoot and root. The survival percentage is the ratio of the number of survived seedlings (at 20 DAS) to the number of seedlings that emerged (at 10 DAS) and expressed as a percent. Root length was measured from the base of the plant up to the end of the longest root and expressed in cm. Shoot length was measured from the base of the plant up to the tip of the longest leaf and expressed in cm. After taking the fresh weight, those were kept in an oven at 60°C for 72 hrs. Both the weight (fresh and dry) of shoot and root was expressed in mg. The dry weight of the seedling was calculated as the sum of root and shoot dry weight and expressed in mg. Germination, survival percentage and seed vigour index were calculated by the following formula:

$$\text{Germination percentage (GP \%)} = \frac{Number\ of\ seeds\ germinated\ at\ 10\ DAS}{Number\ of\ seeds\ placed\ in\ the\ Petridish} \times 100$$

$$\text{Survival rate (\%)} = \frac{Number\ of\ survived\ seedlings}{Number\ of\ emerged\ seedlings} \times 100$$

$$\text{Seed vigour index (SVI)} = \frac{Germination\ percentage \times Seedling\ length (cm)}{100}$$

The contents of photosynthetic leaf pigments chlorophyll a (*Chl a*), chlorophyll b (*Chl b*) and total chlorophyll were determined spectro-photometrically based on the method described by Lichtenthaler [11]. 0.5g fresh leaves were collected into a small vial containing 10mL of 80% acetone. The containers were covered by an aluminium foil and preserved in the dark for 7 days for extraction of pigments. The absorbance was measured from leaf extraction at 663 and 645 nm wave length for *Chl a*, *Chl b*, and total chlorophyll contents by using a spectrophotometer (Shimadzu UV-2550, Kyoto, Japan). The *Chl a*, *Chl b* and total chlorophyll were calculated using the following equations:

$$\text{Chlorophyll a} = 0.999 \times A663 - 0.0989 \times A645$$

$$\text{Chlorophyll b} = -0.328 \times A663 + 1.77 \times A645$$

$$\text{Total chlorophyll} = \text{chlorophyll a} + \text{chlorophyll b}$$

2.4 Statistical Analysis

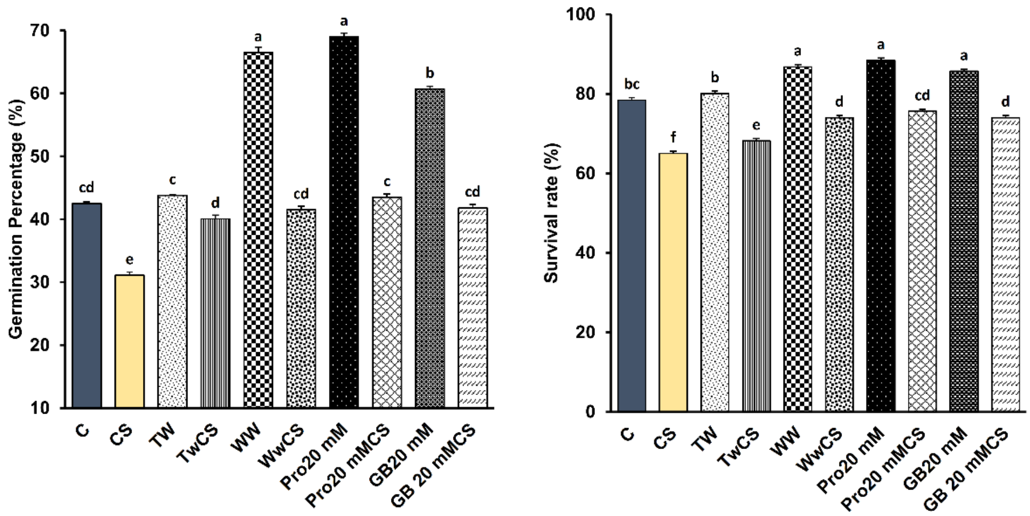
Collected data were subjected to a one-way analysis of variance (ANOVA) using Minitab 17.0 statistical software (Minitab Inc., State College, PA, USA) and Tukey's pair wise comparisons were used to compare statistical differences among the mean values of different treatments and cold stress at 5% significance level.

3. RESULTS

3.1 Germination and Survival

Findings from this experiment revealed that germination percentage of rice seeds significantly increased in response to various priming treatments. Highest GP was recorded for 20 mM proline primed seeds and it was 69.0% (Figure 1). The second highest GP was found for warm water primed seeds. Basically, there was no significant difference between the treatments of proline priming and warm water priming. Among the treatments, seeds grown under chilling stress gave lowest GP and the recorded value was 31.13%. GP obtained from hydro-primed (normal temperature water) and un-primed control seeds did not show any difference significantly. Along with GP, seeds under cold stress significantly decreased the survival percentage (65.03%) of the rice seedlings too. The best performance in survivalist (88.4%) of the seedlings was observed in proline primed seeds. No significant variation was found among the findings from proline, betaine and warm water priming. Hydro-priming with tap water also increased survival percentage (80.06%) in comparison to un-primed (78.43%) and stressed seeds (65.03%) (Figure 1).

Figure 1: Effects of different priming agents on germination percentage and survival rate of rice under chilling stress



3.2 Growth and Vigor

Growth of the seedlings actually determined from the length of shoot and root of the rice seedlings. Results showed that the length of both shoot and root increased because of different priming treatments and simultaneously unprimed cold stressed seeds produced smaller size shoot and root at the same DAS. Maximum shoot and root length were recorded as 19.1 cm and 11.4 cm for warm water primed seedlings (Table 1). Whereas, the lowest length of shoot and root was obtained for cold stressed unprimed seedlings and the values were 12.43 cm and 6.57 cm respectively. Moreover, seed vigor index (SVI) also calculated from percent germination and total length of the seedlings. Proline treatment increased SVI about 71.52% in comparison to stressed condition (Table 1). Priming with betaine and water (both in room temperature and warm) also increased SVI in compared to unprimed stressed seedlings.

Table 1: Effects of different priming agents on seedling attributes of rice under chilling stress. Values are means of 3 replicates and means with different letters in a column indicating significant differences ($p \leq 0.05$).

Treatments	Shoot length cm)	Root length (cm)	Seedling height (cm)	SVI
C	15.57 bc	9.43 abcd	25 bcd	10.63 c

CS	12.4 d	6.57 d	19 e	5.92 d
TW	17 ab	9.8 abc	26.8 abcd	11.74 c
TwCS	14.1 cd	8.17 bcd	22.27 de	8.94 c
WW	19.1 a	11.4 a	30.5 a	20.26 ab
WwCS	15.2 bcd	7.2 abcd	22.4 de	9.28 c
Pro_{20mM}	18.9 a	11.2 ab	30.1 ab	20.76 a
$Pro_{20mM}CS$	15.5 bc	8.7 abcd	24.2 cde	10.54 c
$GB_{20\ mM}$	18.4 a	10.67 ab	29.1 abc	17.67 b
$GB_{20mM}CS$	15.4 bc	8.5 abcd	23.93 cde	10.02 c
SE	0.68	0.51	1.18	1.61

3.3 Shoot and Root weight

Weight of shoot and root parts of the seedlings were taken in both fresh and oven dried condition. Samples collected from the priming treatments showed more weight (both in fresh and dry) in compared to unprimed stressed treatment. The maximum weight of shoot in fresh and dry condition was found for warm water primed seedlings and the values were 2.2 gm and 1.2 gm respectively (Table 2). The next higher weight of fresh and dry shoot was recorded for warm water primed and betaine primed seedlings but statistically among these treatments there was no variation. The minimum fresh (1.2 gm) and dry (0.6 gm) weight of shoot was recorded for unprimed stressed seedlings. Similarly, unprimed stressed seeds produced the seedlings with minimum weight of root in fresh (0.48 gm) and dry (0.03 gm) condition. On the contrary, maximum weight of root in fresh (0.66 gm) and dry (0.10 gm) condition were recorded from the proline primed seedlings (Table 2). All the treatments except CS, increased root weight than control treatment.

Table 2: Effects of different priming agents on seedling attributes of rice under chilling stress. Values are means of 3 replicates and means with different letters in a column indicating significant differences ($p \leq 0.05$).

Treatments	SFW (gm)	SDW (gm)	RFW (gm)	RDW (gm)
C	1.78 abc	0.78 bc	0.53 c	0.07 bcd
CS	1.2 d	0.65 cd	0.48 d	0.03 e
TW	1.6 bcd	0.6 d	0.54 c	0.07 bcd
TwCS	1.5 cd	0.66 cd	0.5 cd	0.04 de
WW	2.2 a	1.2 a	0.64 ab	0.09 abc
WwCS	1.4 cd	0.75 bcd	0.53 c	0.07 bcd
Pro_{20mM}	2.1 a	0.9 b	0.66 a	0.105 a
$Pro_{20mM}CS$	1.4 cd	0.73 cd	0.52 cd	0.06 cde
$GB_{20\ mM}$	2 ab	1.1 a	0.61 b	0.095 ab
$GB_{20mM}CS$	1.38 cd	0.72 cd	0.52 cd	0.06 cde
SE	0.11	0.06	0.01	0.007

3.4 Chlorophyll content

A significant variation in leaf chlorophyll content was observed due to priming treatments and stressed condition. Among the treatments, leaves from proline primed gave highest content of Chl a (1.62 mg/100gFW) (Figure 2) and warm water primed gave highest content of Chl b (1.07 mg/100gFW) (Figure 2), but the total Chl content was found maximum for proline treatment. Betaine and tap water priming also increased Chl content in compared to control and CS treatment, though no significant difference among proline, betaine and water priming was found. As, the treatment CS produced less vigorous seedlings and smaller size leaves so, minimum content of Chl a and b also recorded under this stressed treatment. However, the obtained Chl a and Chl b content in response to CS condition were 1.32 and 0.78 mg/100gFW respectively (Figure 2).

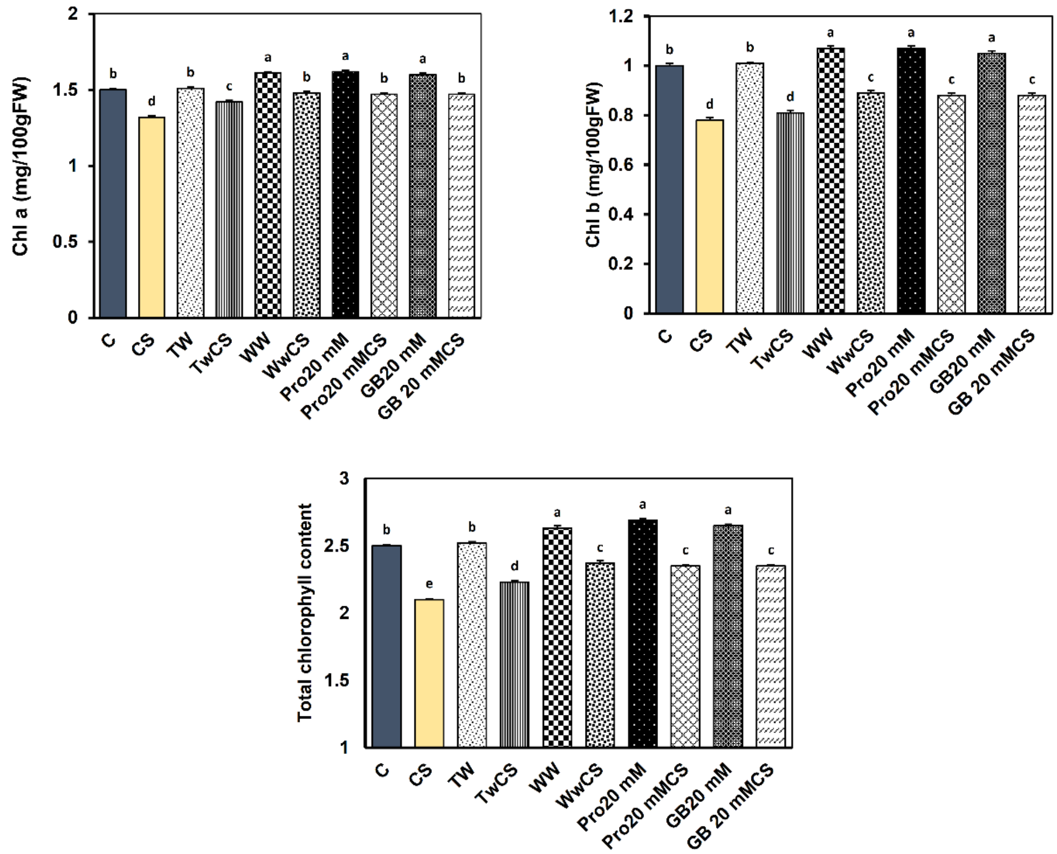


Figure 2: Effects of different priming agents on photosynthetic pigments of rice under chilling stress

4. DISCUSSION

Abiotic stresses such as salinity, drought, and cold are detrimental proper seed germination and crop production [12]. Seed germination and seedling establishment is very crucial for plant growth and productivity which often interrupted by some adverse conditions called stress like moisture, salinity, temperature, pathogen, weed etc. Seed priming a prior encounter to any stress, is known to bestow plants with greater tolerance [13-14]. In this study, it was investigated that seed priming could be helpful for faster and higher seed emergence, better survival and growth of rice seedlings when exposed to cold stress. From our experiment, we found that seed priming enhanced seed germination and seedling performance compared with the non-primed seed which actually shows the potentiality of seed priming in cold stress mitigation during early stage of rice. Every priming agent had a significant impact on seed germination, seedling emergence and survival percentage, and seedling growth both in cold stress and in combine condition of priming and stress. These findings are in line with those who also confirmed increased germination rate along with faster and synchronized emergence as a consequence of pre-sowing seed priming in different crop species including rice [15-16]. The development of the immature embryo along with genetic repairment during seed priming are plausible justifications for increased germination rate [17-18].

In this study, priming (with proline) effect presented in Figure 1 was observed as significant for germination and survival at early stage of rice seedlings (20 DAS). Glycine betaine treatment of seeds also helped in improvement of seed germination along with survival of rice seedling through cold stress mitigation. Although seed priming with tap water did not show significant germination percentage and survival with other priming but significantly over the only stressed condition, where warm water primed seeds showed significant results of percent germination. In case of germination percentage, proline performed better than the other priming agents like betaine and water. This was might be due to the variation in crop species and the nature of the priming agents. Similar findings were also reported by Ambreen et al. [19], and Tania et al. [20]. It was reported from the many previous studies that the delayed and non-uniform germination of rice under cold stress was mainly due to reducing water uptake, cell membrane injury, less cellular respiration, and elevated reactive oxygen species (ROS) levels [21]. In low temperatures, hindered the water uptake and root growth, resulting in a decreased seed germination was reported by Li et al. [22], and Du et al. [23].

Seedling growth parameters like shoot and root length and seed vigour index (SVI) were significantly influenced by priming agent and cold stress. It was found proline performed better in terms of seedling growth and vigour than betaine both in stress and un stressed condition. On the other hand, warm water priming was found in better shoot and root length along with higher vigor index of rice seedling in comparative to tap water priming under cold stress. These results showed the similarities with the findings of Tania et al. [24], Cao et al. [25], Dikilitas et al. [26], El Mokhtari et al. [27], and Sadeghipour [28]. A report on cold stress in producing metabolic imbalance in plant tissues and reducing the growth of the seedlings by suppressing cell elongation and division was given by Oliver et al. [29]. In 2016, Hussain et al. [30] reported respiration as one of the most important metabolic events during seed germination which provides the energy for coleoptile elongation, and the respiration rate during seed germination is closely related to temperature. Moreover, Cheng et al. [31] also concluded that, low temperature reduces the respiration of seeds, and the longer the duration of low temperature, the greater the reduction of respiration rate. Higher seedling growth under cold stress in the current study from the primed seed treatments was might be the consequence of rapid emergence due to earlier production of emergence metabolites. Khan et al. [32] opined that rapid imbibition during priming disrupt the cell membrane, cause localized cells in cotyledons resulting in more vigorous seedlings.

Dry matter accumulation in seedling shoots and root observed from the study revealed that seed priming with warm water, pro or GB performed similarly and there is no significant variation among the priming treatments of warm water, proline and betaine which have the similarities with the results of Tania et al. [33], Karalija et al. [34], and Anwar et al. [35]. Though, seedlings grown from proline primed seeds gave the highest value of fresh and dry weight of shoot and root in compared to other treatments and treatment combinations.

Leaf pigment is one of the most important photosynthetic components in producing food. Chlorophyll a and chlorophyll b are major part of leaf pigment which also varied due to different priming treatments along with proline and betaine in higher concentration having the similarities with the findings of Kahloui et al. [36]. A significant increase of Chl a, b and total Chl was found for the treatment of proline, betaine and warm water. There was actually no significant difference among them in case of leaf pigment. But all the priming treatments increase the content of leaf pigment over the stress. As, the leaf size and growth improved due to priming so the more accumulation of pigments might be occurred in the leaf from primed seeds.

After all, thorough observation from this study suggested that, each priming agents had a positive impact on seed germination, seedling emergence, seedling growth and leaf chlorophyll content under cold stress with the similar findings from whereas, seed priming with 20 mM proline or betaine, or warm water priming performed better.

4. CONCLUSION

Chilling stress led to a significant decrease in germination, survival, chlorophyll content and other growth parameters of rice seedlings. Seed priming with water, proline and betaine improves morpho-physiological attributes of rice seedlings along with reducing the harmful effects of cold stress. Proline was more effective in some of the parameters than hydro-priming and Glycine betaine in mitigating the detrimental effects of Cold stress. However, to obtain a better understanding of priming-induced mechanisms and for validation of this results, larger field trials are needed.

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