Original Research Article

Effects of 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 (CS).
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: The outcomes shown that proline (Pro) and glycine betaine (GB) priming had a favorable effect on germination and survival rate, shoot and root length, shoot and root weight, and photosynthetic pigments of leaves 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 a concentration of 10 mM demonstrated insignificant performance over control but considerable over stress in the majority of the parameters. The findings showed that seed priming generally has a favorable effect on germination and survival, shoot and root length and weight, and leaf chlorophyll content.
Conclusion: Proline, betaine, or warm water priming (at 45 0C for 5 min.) can be thought of as a lasting priming agent. This means that priming may be an efficient strategy to boost seed germination and better seedling growth of rice under chilling stress.

Keywords: Germination, survival, chlorophyll content and cold stress

1. INTRODUCTION

Crop productivity is hindered by abiotic factors such as salinity, drought, and extremely high or low temperatures [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. More than 2 million hectares of rice crops in Bangladesh's northern and north-eastern regions were also shown to be partially to completely destroyed by the cold season. Chilling stress affects rice plants especially at the seedling and reproductive stages. Improper seed germination, 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].

The rate, percentage, and uniformity of seed germination and seedling emergence under stress conditions like salt, temperature, and drought stress have all been demonstrated to be improved by seed priming [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. Seed priming enhances germination and early seedling growth, especially in favorable conditions, according to numerous research on vegetables, floriculture, and some field crops [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 conducted at Bangladesh Agricultural University's Department of Seed Science and Technology in Mymensingh. In this experiment, the plant components were seeds of the well-known cultivated rice variety BRRI Dhan 29. The surface of the seeds was cleaned three times with sterile distilled water to reduce contamination during priming.

2.2 Seed priming and experiment conduction

In this investigation, seed priming techniques such as hydro-priming (using warm and regular tap water) and chemical priming (using proline and glycine betaine) were investigated in order to fulfill the role of seed priming in reducing the negative impacts of chilling stress. For continuation of the study seeds were primed with 20 mM solution of proline and glycine betaine. When hydro-priming, warm water heated to about 45°C and tap water to about 25°C were used to prime or soak the seeds. With the exception of warm water, seeds were primed for 30 minutes in all treating agents. It took just five minutes to prime with warm water. Following the completion of the soaking, the prepped seeds were surface-dried with blotting paper after being cleaned with distilled water for 2 minutes. After washing and drying, the seeds (20 seeds per dish) were placed on Petri dishes (150 x 20 cm^2) with three layers of Whatman filter papers, covered with a lid, and kept in a standard laboratory environment (room temperature: 25±1°C, relative humidity: 95%), while the primed seeds dishes were exposed to cold stress (below 5°C) in the refrigerator. To prevent desiccation, distilled water was sprayed on the seeds. The stressed seeds were placed in the growth chamber for 23 days after being planted after 7 days, when germination had just begun. While the temperature for the control treatment was maintained at 25°C in a separate growth chamber, the chilling stress was imposed in the growth chamber by maintaining the day and night temperatures at 15°C. 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 gathered on the percentage of seeds that germinated, the survival rate, the seed vigor index (SVI), the length and weight of the shoots and roots (both fresh and dried), and the amount of chlorophyll. At 10 days following sowing, the ultimate germination percentage was determined (DAS). At 20 DAS, sample seedlings (10 per replicate) were randomly chosen to obtain information on the percentage of survival, the length of the shoots and roots, and the fresh weight of the shoots and roots. The number of seedlings that survived (at 20 DAS) divided by the number of seedlings that emerged (at 10 DAS) is known as the survival percentage, and it is given as a percentage. The length of the roots, indicated in centimeters, was calculated from the plant's base to the tip of the longest root. These were placed in an oven set at 60 °C for 72 hours after being freshly weighed. Both the fresh and dried weights of the shoot and root were given in mg. The total of the root and shoot dry weights, reported in mg, was used to determine the seedling's dry weight. The following formula was used to compute seed vigor index, survival percentage, and germination:

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

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

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

Following Lichtenthaler's approach, the amounts of the photosynthetic leaf pigments chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll were measured spectro-photometrically [11]. Fresh leaves weighing 0.5g were put into a tiny vial with 10mL of acetone at an acetone concentration of 80%. For the purpose of extracting the pigments, the containers were sealed with aluminum foil and kept in the dark for 7 days. A spectrophotometer was used to measure the absorbance from leaf extraction at 663 and 645 nm wave lengths for Chl a, Chl b, and total chlorophyll concentrations (Shimadzu UV-2550, Kyoto, Japan). The following equations were used to determine the amounts of Chl a, Chl b, and total chlorophyll:

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

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

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

2.4 Statistical Analysis

When comparing statistical differences between the mean values of various treatments and cold stress at the 5% significance level, the collected data were subjected to a one-way analysis of variance (ANOVA) using Minitab 17.0 statistical software (Minitab Inc., State College, PA, USA).

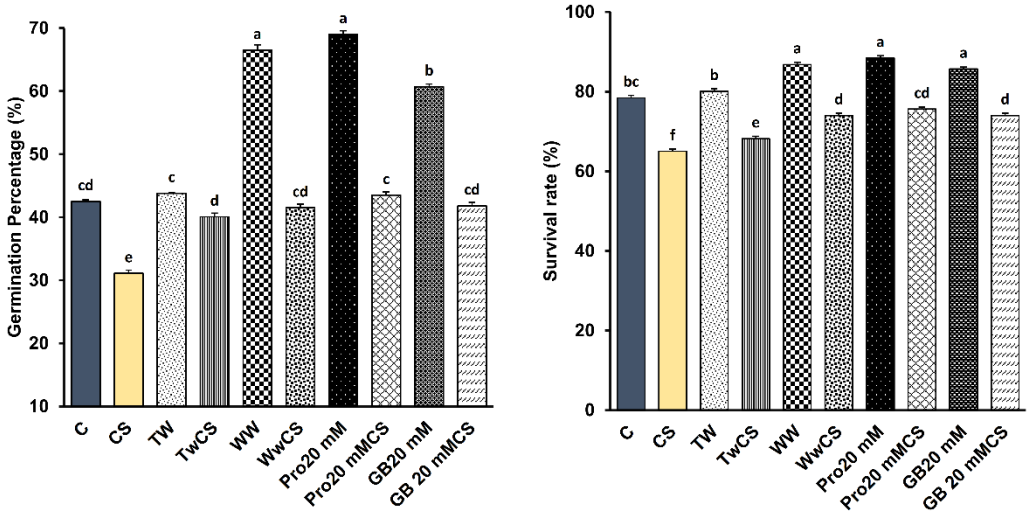
3. RESULTS

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).

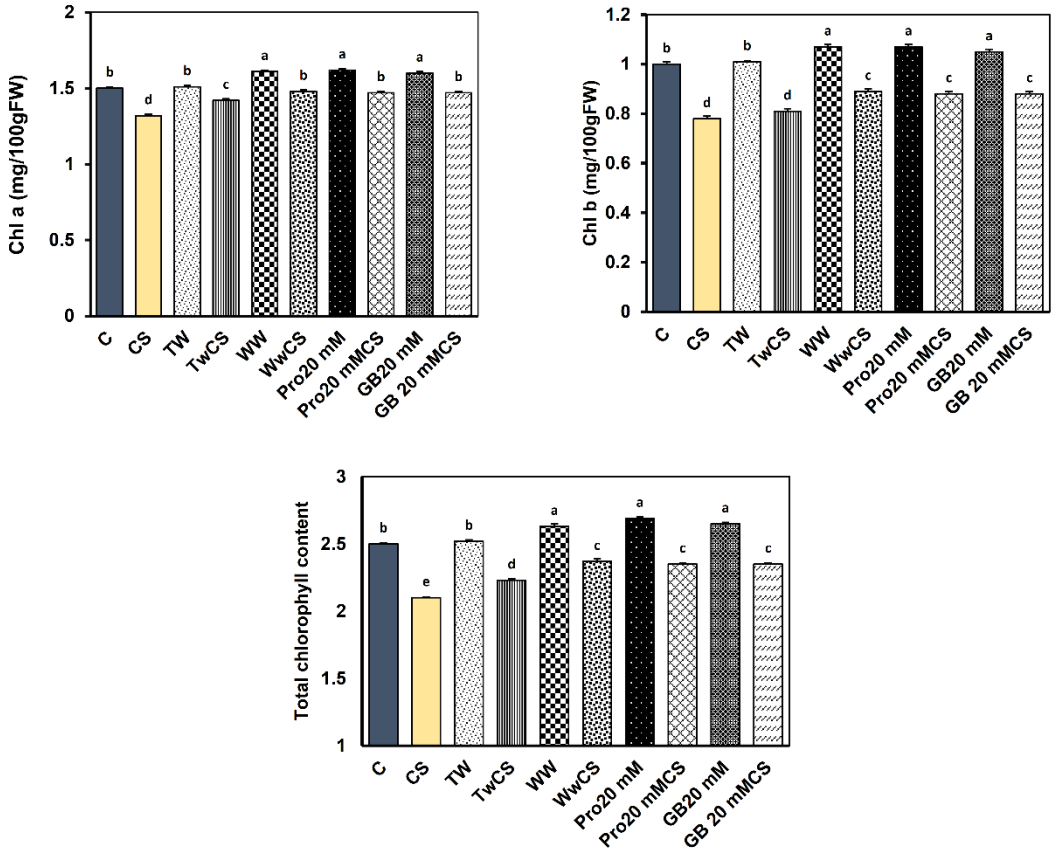


Figure 2: Effects of different priming agents on photosynthetic pigments of rice under chilling stress

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. Plants are known to be more tolerant when seeds are primed before being exposed to any stress [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 results are consistent with studies that found that pre-sowing seed priming in a variety of crop species, including rice, boosted germination rates and accelerated and synchronized emergence [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]. According to numerous earlier research, the reduced water intake, cell membrane damage, decreased cellular respiration, and raised reactive oxygen species (ROS) levels were the main causes of the delayed and uneven germination of rice under cold stress [21]. Li et al. [22] and Du et al. [23] observed that low temperatures hampered water intake and root growth, which in turn reduced seed germination.

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]. Respiration is one of the most significant metabolic processes during seed germination, providing the energy for coleoptile elongation, according to Hussain et al. [30]. The rate of respiration during seed germination is highly connected to temperature. Additionally, Cheng et al. [31] came to the conclusion that seeds' respiration is reduced by low temperatures, and that the reduction in respiration rate increases with the duration of low temperatures. In the current study, primed seed treatments led to higher seedling growth under cold stress, which may have been caused by quicker emergence from early production of emergence metabolites. According to Khan et al. [32], fast imbibition during priming disrupts the cell membrane, causes localized cells in the cotyledons, and results in seedlings that are more vigorous.

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.

Ultimately, careful analysis from this study suggested that each priming agent had a beneficial effect on seed germination, seedling emergence, seedling growth, and leaf chlorophyll content under cold stress, with the same results from whereas, seed priming with 20 mM proline or betaine, or warm water priming performed better.

4. CONCLUSION

Rice seedlings exposed to chilling stress had significantly lower rates of germination, survival, chlorophyll content, and other growth metrics. Seed priming with water, proline and

betaine improves morpho-physiological attributes of rice seedlings along with reducing the harmful effects of cold stress. When it came to reducing the negative impacts of Cold stress, proline performed better than hydro-priming and glycine betaine in some of the measures. Larger field experiments are required, nevertheless, to get a deeper understanding of priming-induced mechanisms and to validate these findings.

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