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COLD SURVIVAL POTENTIALITY OF SELECTED RICE CULTIVARS AT SEEDLING STAGE IN NORTHWEST BANGLADESH

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ABSTRACT

Cultivation of cold tolerant rice genotypes is the best way to ascertain maximum possible yield in sudden low temperature stress prone areas. In this study the morpoho-physiological traits of rice seedlings like proline content, plant pigments (chlorophyll-a, chlorophyll-b, total chlorophyll and total carotenoid), relative water content, shoot and root length, shoot and root dry weight, protein content, starch content and seed germination percentage of selected seven rice cultivars were investigated to screen out rice cultivar having comparatively higher surviving potentiality during low temperature environment. The tested rice cultivars were Poshusail, Jirasail, BRRI dhan28, NERICA-4, GSR-IRRI-1, Purple rice and BRRI dhan50 (Banglamoti), cultivated in Northwest Bangladesh where chilling cold is common in winter. A significant variation was found among the studied rice cultivars regarding the investigated parameters. This study resulted that the NERICA-4 and Jirasail (a local variety) showed comparatively better potentiality to survive at low temperature (below 15ºC) in the Northwest Bangladesh. Rice cultivars contained increased amount of proline, chlorophyll, protein, relative water content, starch and dry weight percentage having the highest survival potentiality in low temperature stress condition. Though the cultivars studied expressed morpho-physiological responses differently to low temperature environment, the increased leaf proline content might be a reasonable indicator for responses to sudden low temperature stress in all cultivars.

Key words: Chlorophyll, low temperature stress, physiology, proline, rice seedling

INTRODUCTION

Low temperature in Northwest Bangladesh occasionally retard the seedling growth resulted in late planting for boro rice cultivation (Sarker*et al*. 2015). Low temperature stress is crucial one among the major plant abiotic stress factors that limits the agricultural crop productivity in a drastic rate especially in the tropical and subtropical region where low temperature is appeared as a sudden limiting environmental factor. Crops induct various changes in its body which may be at molecular, cellular as well as at physiological level to survive during this sudden low temperature stress period (Xin and Browse 2000). Rice (*Oryza sativa* L.), one of the most three important cereals, is consumed by more than 3 billion people worldwide and its annual production is more than 600 million tons which must be more than 800 million tons as early as 2025 to meet the world rice demand (Green *et al.* 2005). But the sudden low temperature stress inhibits seedling establishment effecting early growth stages of rice and resulting in poor crop maturation and consequently lowers the total crop productivity. Various phenotypic symptoms in response to sudden low temperature stress may be appeared which include reduced leaf expansion, wilting, chlorosis (yellowing of leaves) and may lead to necrosis (death of tissue). Graham and Patterson (1982) observed that many plants of tropical origin cannot tolerate sudden low temperature and total crop might be damaged even at moderate temperatures that are below 20ºC. This exposure of rice seedling to sudden low temperature stress condition usually induct a variety of biochemical, physiological and enzymatic changes in its body as an acclimation response which might be characterized by a greater ability to resist injury or survive during further lethal low temperature stress (Howard and Ougham 1993, Hughes *et al.* 1996).

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Similar to other cultivated crops, rice prefers a relative variable temperature during its growing period and any deviation from this optimum temperature range at any growth stage will alter various biochemical and physiological processes. Rice plant may produce some special metabolites in an elevated amount to cope with the sudden temperature changes as well (Sarker*et al.* 2013). At temperatures below 15-20ºC, rice plants exhibit a wide range of phenotypic and physiological responses depending on the length of exposure and the developmental stage. Seed germination, seedling and flowering stage is most critical period regarding low temperature stress which directly affect the crop yield. The imbibition phase of germination is considered to be the most sensitive, which leads to the escape of solutes from the seeds (Khan and Ahmed 2009). The occurrence of low temperature stress during the early growth stages of rice inhibits seedling establishment, eventually leads to non-uniform crop maturation and dramatically reduce its production (Aghaee*et al.* 2011).

The Northwest Bangladesh is experiencing the effect of comparatively short duration low temperature on the crop production especially during the seedling-raising period for rice crops similar to other parts of the world. In order to gain stable rice production, low temperature tolerance at seedling stage is an important character. If the seedling damage due to low temperature stress could be avoided by screening out the cold tolerate rice cultivar at seedling stage, achieving the target rice production for future food security would be possible and the efficiency of using farm resources could be improved by saving 10-20% seeds. Therefore, the present study was conducted to screen out the relatively low temperature stress tolerant rice cultivar(s) based on some morpho-physiological parameters.

MATERIALS AND METHODS

Planting materials and experimental design: The study was conducted at the research field of the Department of Biochemistry and Molecular Biology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh located in 25.13ºN latitude and 88.23ºE longitude and at an elevation of 34.5 m above the mean sea level. The experimental field soil was the Old Himalayan Piedmont Plain soil belonging to Agro Ecological Zone 1 (AEZ-1). The selected local varieties were Poshusail, Jirasail and HYV were BRRI dhan28, NERICA-4, GSR-IRRI-1, Purple rice and BRRI dhan50 (Banglamoti). The field experiment was designed following Randomized Complete Block Design (RCBD) with three replications. The prevailing air temperature was recorded accordingly over the field experiment period at six hours interval, which is shown in Figure 1,and the field experiment was lasted for 36 days during December 2011 to February 2012.

Physiological parameters: Samples for analyzing plant pigments and proline were collected from the experimental plot at four days interval and the determination of plant protein and starch content was done at 36 days after sowing. Determination of plant pigments (chlorophyll-a, chlorophyll-b and total carotenoid) content was performed by following Arnon (1949) and total chlorophyll was estimated by following Porra (2002). For proline content, about 50 mg fresh leaf samples from rice seedlings at 16, 20, 24, 28, 32 and 36 days after sowing was collected in a 2-mL eppendorf tube. Free plant proline content was estimated by following acid ninhydrin method described by Bates *et al.* (1973). For protein and starch determination, the aerial parts of rice seedlings were collected from each plot and oven dried at 60ºC for 72 hours. The estimation of protein content from rice seedlings was done by Kjeldahl method. The starch content of rice seedling was determined by Fehling's solution method. The starch of sample plant parts was first simplified to glucose and total glucose content was measured. The total glucose content was then used to calculate the amount of starch present accordingly.

The estimation of relative water content (RWC) was done in last three sampling periods. For RWC, the fresh weight of leaves was taken immediately after collecting sample from the experimental field. Then the leaves were dipped in distilled water for about 6 hours to ensure full turgid or saturation condition. The leaves were blotted quickly and then the weight of the fully saturated leaves was taken. Then the leaf samples were dried in oven for 72 hours maintaining the temperature at 60ºC. After that the dry weight (fully dehydrated) of the leaves were taken. Dried leaves were weighed using digital balance (accuracy 0.0001g). The RWC measurement was carried out by following formula (Barr *et al.* 1962).

The germination percentage was determined at field level at the initial stage of the experiment. This was done by counting the number of germinated seed and total number of seed within a marked area. This counting was done after 10 days of first germination (DAG). The percent germination was first calculated using following formula.

$$\%\ \text{Germination} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

Statistical analysis

The collected data were analyzed statistically for the analysis of variance (ANOVA) and means were compared by Duncan's Multiple Range Test (DMRT) as described by Gomez and Gomez (1984) using the statistical computer package program MSTAT C (Russell 1986).

RESULTS AND DISCUSSION

The screening of comparatively more cold tolerant rice cultivar was made based on some physiological parameters studied among selected seven rice cultivars grown in same field condition. Though the amount of chlorophyll-a content (Table 1) of rice seedlings was varied with the change of temperature, the NERICA-4 rice cultivars was found as a statistically superior cultivar in relation to the amount of chlorophyll-a synthesized over the experimental period. Similar trend of NERICA-4 rice cultivar was also found in case of chlorophyll-b (Table 2) and total chlorophyll content (Table 3). In case of total carotenoid content (Table 4), local rice cultivar Jirasail was found to synthesize in elevated amount but not statistically significant. The V7 (BRRI dhan50) rice cultivar was more vulnerable to sudden low temperature in producing plant pigments throughout the sampling periods.

The chlorophyll-a, cholorophyll-b, total chlorophyll and total carotenoid were noticed being negatively affected by low temperature. Decrease in environmental temperature resulted in decreased plant photosynthetic pigments content and vice-versa. But in the same environmental condition, rice cultivar having relatively high plant pigment content is definitely more potential to survive in low temperature stress condition. NERICA-4 rice cultivar showed the better performance among the cultivars studied. Among the local cultivars, Jirasail rice was noticed to perform relatively better. The photosynthesis, a vital physiological process of plants, activity of plant is primarily dependent on the presence of plant pigments and its intensity which also limits the final crop productivity (Sarker*et al.* 2013). As sudden low temperature limits the synthesis of chlorophyll and other plant pigment, the decrease of photosynthetic activity might be a common physiological response of low temperature stress sensitive plant (Yadegari*et al.* 2007). Chlorophyll-a and chlorophyll-b are most important plant pigment whose synthesis gets affected after the plant being subjected to sudden low temperature stress. Both high temperature and low temperature other than optimum one is a major limited factor of plant photosynthetic pigments synthesis (Harding 1974). Carotenoid, another light-harvesting plant pigment associated with photosynthetic process, is also affected and resulted in a decreased amount by low temperature stress condition (Haghjou*et al.* 2007).

Rice cultivars produced increased amount of proline with the decrease of temperature and vice-versa but this correlation is not statistically significant ($p<0.05$) (Table 5). At 36 days after sowing, the NERICA-4 rice cultivar synthesized the highest proline but the other tested varieties decreased markedly. The cultivar especially NERICA-4 rice cultivar produced higher amount of proline in comparison to other tested cultivars. The synthesis of proline is highly dependent on its genetical make up. The rice cultivar having high proline content had genetical potentiality to survive under low temperature stress. Proline, a non-essential amino acid, is considered as an effective metabolite which is generally produced by plants to cope with sudden abiotic stress factor (Sarker*et al.* 2005). It is, however, regarded as a primary defensive agent of plants against sudden unfavourable environmental condition. Gilmour *et al.* (2000) reported that plants produce proline in response to cold stress but its amount may vary species to species as well as cultivar to cultivar. In this present study, the proline content of rice cultivars varied much depending upon the environmental temperature.

Figure 2 shows that the protein content of studied rice varieties were statistically similar but the mean value of protein content of NERICA-4 rice cultivar is greater than that of others. In case of starch content (Figure 3), the local cultivar Jirasail synthesized the lowest amount while the highest content was in NERICA-4 rice varieties. Proteins are compounds of fundamental importance for all functions in plant cell. It has also been reported that plant produces some specific enzymes or proteins to overcome suddenly imposed stress condition (Hieng*et al.* 2004). Nagy *et al.* (1972) revealed that some thermogenic plants of temperate region produces therapeutic stress enzyme named alternative oxidase (AOX) to be produce heat during low temperature stress. In this study, the protein content of rice cultivar at 36 days after sowing was statistically similar but NERICA-4 rice cultivar contained the highest protein compounds. Again, carbohydrate metabolism of plant is generally affected by low temperature stress and are subjected to produce some soluble sugars which are generally thought to act as protective compatible solutes (Korn et al. 2008) and are important mean of freezing tolerance (Sarker*et al.* 2013). In case of present study, the NERICA-4 rice cultivar possessed highest mean starch content providing a strong evidence of enriched genetical structure allowing the seedling to fight enough against low temperature stress. Sarker*et al.* (2015) revealed that the rice seedling synthesized more proline during cold environment which is in well agreement with the present study.

The RWC of rice leaves was statistically similar during sampling periods but at the 36 days after sowing (Table 6). In this study, NERICA-4 rice cultivar had highest mean RWC at final sampling period which ensures the less membrane injury and quick recovery of stress condition. At the same time, the BRRI dhan50 had statistically lower relative water content indicating highly low temperature prone cultivar. Leaf water status of plant is directly related to several physiological variables including photosynthesis (Kramer and Boyer 1995) and measuring of relative water content (maximum water holding capacity of leaf tissues) is an important parameter for studying stress tolerance of a variety.

Table 1. Chlorophyll-a content of different rice cultivars at different days after sowing

Variety	Chlorophyll-a (mg g^{-1})					
	16 DAS	20 DAS	24 DAS	28DAS	32 DAS	36 DAS
Poshushail	6.29 ab ± 0.42	5.09ab ± 1.79	5.12 abc ± 1.02	4.18ab ± 0.86	4.15 ab ± 0.60	4.33ab ± 1.52
Jirasail Local	5.59 bc ± 0.07	4.32 bc ± 1.30	6.29 ab ± 2.91	2.89bc ± 0.35	3.95ab ± 0.17	3.50 ab ± 1.40
BRRI dhan28	4.63cd ± 1.10	3.23cd ± 0.80	4.26abcd ± 0.12	3.01bc ± 0.33	3.24 bc ± 0.68	4.20 ab ± 0.58
NERICA- 4	7.07 a ± 0.58	5.79 a ± 1.11	6.67 a ± 0.89	5.06 a ± 1.74	5.15 a ± 0.88	5.70 a ± 1.35
GSR- IRRI-1	4.42cd ± 0.57	3.07 cd ± 1.12	4.09bcd ± 0.65	3.44bc ± 0.25	2.86bc ± 1.11	3.63ab ± 0.83
Purple rice	5.61bc ± 0.75	3.64 cd ± 1.38	3.79 cd ± 1.12	2.27 c ± 1.10	2.65 c ± 0.71	3.08 b ± 0.99
BRRI dhan50	3.41d ± 0.61	2.81 d ± 0.71	2.40 d ± 0.42	1.89 c ± 0.49	2.22 c ± 0.75	2.22 b ± 0.62
LSD (5%)	1.17	1.16	2.25	1.54	1.19	2.02

± indicates standard deviation and different letters in a column differ significantly at 5% level of significance (as per DMRT)

Table 2. Chlorophyll-b content of different rice cultivars at different DAS

Variety	Chlorophyll-b (mg g^{-1}) synthesized by rice cultivars					
	16 DAS	20DAS	24 DAS	28 DAS	32 DAS	36 DAS
Poshushail	2.74 ab ± 0.29	2.32ab ± 0.54	2.68 ab ± 0.69	1.88 ab ± 0.13	1.61ab ± 0.27	1.89 abc ± 0.43
Jirasail Local	2.37 bc ± 0.08	1.99 bcd ± 0.47	2.28 bc ± 0.32	1.32 bcd ± 0.16	1.63ab ± 0.14	1.71bc ± 0.49
BRRI dhan28	2.09 cd ± 0.23	1.71cd ± 0.32	2.39 abc ± 0.42	1.56 bc ± 0.14	1.50 b ± 0.37	2.17 ab ± 0.33
NERICA- 4	3.20 a ± 0.28	2.81a ± 0.42	3.34 a ± 0.27	2.27a ± 0.56	2.04 a ± 0.26	2.58 a ± 0.57
GSR- IRRI-1	2.04 cd ± 0.31	1.52 d ± 0.36	2.68 ab ± 0.83	1.62 bc ± 0.11	1.31 b ± 0.42	1.58 bc ± 0.31
Purple rice	2.68 ab ± 0.36	2.10 bc ± 0.89	2.00 bc ± 0.65	1.27cd ± 0.48	1.31 b ± 0.22	1.61bc ± 0.39
BRRI dhan50	1.73 d ± 0.35	1.57 cd ± 0.31	1.44 c ± 0.11	0.98 d ± 0.16	1.16 b ± 0.39	1.16 c ± 0.23
LSD (5%)	0.53	0.52	0.91	0.53	0.43	0.77

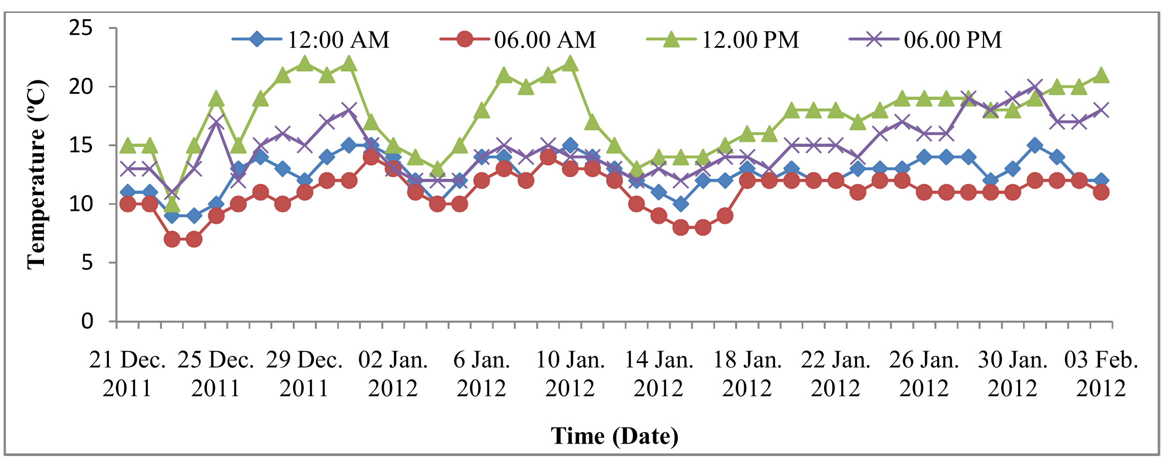

Figure 1. Air temperature trend during the experimental period

Table 3. Total chlorophyll content of different rice cultivars at different DAS

Variety	Total chlorophyll (mg g^{-1}) synthesized in different rice cultivars					
	16 DAS	20 DAS	24 DAS	28 DAS	32 DAS	36 DAS
Poshushail	9.25ab ± 0.70	7.59ab ± 2.38	7.99abc ± 1.73	6.20ab ± 1.01	5.89 ab ± 0.84	6.36ab ± 1.99
Jirasail Local	8.14bcd ± 0.03	6.46bc ± 1.82	8.77 ab ± 2.76	4.31bc ± 0.52	5.71 abc ± 0.32	5.34b ± 1.91
BRRI dhan28	6.88cde ± 1.36	5.05cd ± 1.10	6.81 bc ± 0.32	4.69 bc ± 0.44	4.85bcd ± 1.08	6.53ab ± 0.90
NERICA- 4	10.52 a ± 0.83	8.82 a ± 1.57	10.25 a ± 1.16	7.50 a ± 2.33	7.36a ± 1.17	8.48 a ± 1.96
GSR- IRRI-1	6.61de ± 0.90	4.71cd ± 1.52	6.93bc ± 1.37	5.19 bc ± 0.27	4.28bcd ± 1.56	5.34b ± 1.17
Purple rice	8.49 bc ± 1.14	5.88bcd ± 1.33	5.93cd ± 1.80	3.63c ± 1.59	4.05cd ± 0.95	4.81b ± 1.38
BRRI dhan50	5.27e ± 0.97	4.49d ± 1.04	3.93 d ± 0.54	2.93c ± 0.66	3.46 d ± 1.08	3.46 b ± 0.87
LSD (5%)	1.68	1.65	2.59	2.07	1.62	2.82

Table 4. Total carotenoid content of different rice cultivars at different DAS

Variety	Total carotenoid content (mg g^{-1}) in different rice cultivars					
	16 DAS	20 DAS	24 DAS	28 DAS	32 DAS	36 DAS
Poshushail	1.12 ab ± 0.05	0.83ab ± 0.34	0.68b ± 0.13	0.78ab ± 0.26	0.99a ± 0.10	0.87 a ± 0.21
Jirasail Local	1.23 a ± 0.20	0.95a ± 0.25	1.25 a ± 0.40	0.73ab ± 0.08	1.01a ± 0.03	0.80 ab ± 0.26
BRRI dhan28	0.97 ab ± 0.30	0.71abc ± 0.12	0.80 b ± 0.09	0.57ab ± 0.11	0.84 ab ± 0.13	0.79 ab ± 0.11
NERICA- 4	1.06 ab ± 0.15	0.80abc ± 0.29	0.80 b ± 0.12	0.73ab ± 0.29	0.80ab ± 0.50	0.94 a ± 0.06
GSR- IRRI-1	0.97 ab ± 0.13	0.63bc ± 0.26	0.50 b ± 0.06	0.91a ± 0.23	0.69ab ± 0.32	0.79 ab ± 0.22
Purple rice	0.85 bc ± 0.10	0.53 c ± 0.18	0.58 b ± 0.21	0.42b ± 0.13	0.68 ab ± 0.14	0.66ab ± 0.13
BRRI dhan50	0.64 c ± 0.12	0.62 bc ± 0.20	0.48 b ± 0.19	0.43b ± 0.15	0.45b ± 0.30	0.45b ± 0.12
LSD (5%)	0.28	0.26	0.35	0.33	0.43	0.33

Table 5.Proline content of different rice cultivars at different DAS

Variety	Total proline content (mg g^{-1}) in different rice cultivars					
	16 DAS	20 DAS	24 AS	28 DAS	32 DAS	36 DAS
Poshushail	1.34 a ± 0.37	0.72 a ± 0.74	0.60 c ± 0.18	1.09 ab ± 0.24	1.08 ab ± 0.24	0.35 b ± 0.03
Jirasail Local	1.02 a ± 0.55	0.79 a ± 0.54	0.71 bc ± 0.01	0.78 c ± 0.08	1.03ab ± 0.14	0.37 b ± 0.05
BRRI dhan28	1.09 a ± 0.16	0.44 a ± 0.21	0.72bc ± 0.15	0.86 bc ± 0.13	1.03ab ± 0.12	0.36 b ± 0.07
NERICA- 4	0.69 a ± 0.31	0.51 a ± 0.24	0.97 b ± 0.06	0.99 abc ± 0.06	1.12 a ± 0.18	1.26 a ± 1.13
GSR- IRRI-1	0.92 a ± 0.32	0.46 a ± 0.09	0.47 c ± 0.14	0.87 bc ± 0.09	1.09 a ± 0.20	0.79 ab ± 0.42
Purple rice	1.22 a ± 0.15	0.95 a ± 0.57	1.35a ± 0.27	1.22 a ± 0.16	1.11 a ± 0.08	0.53 ab ± 0.05
BRRI dhan50	0.93 a ± 0.50	0.40 a ± 0.14	0.71bc ± 0.11	0.89 bc ± 0.06	0.57 c ± 0.14	0.41 ab ± 0.22
LSD (5%)	0.63	0.66	0.27	0.23	0.27	0.81

Table 6. Relative water content (RWC) of different rice cultivars at different DAS

Variety	Relative water content (RWC) (%)		
	28 DAS	32 DAS	36 DAS
Poshushail	90.12 a ± 4.82	90.32 a ± 1.73	90.09 a ± 4.50
Jirasail Local	87.04 a ± 2.68	87.92 a ± 7.93	85.06 ab ± 1.90
BRRI dhan28	91.87 a ± 4.21	88.42 a ± 4.03	85.31 ab ± 4.75
NERICA- 4	89.82 a ± 6.79	91.22 a ± 4.81	90.14 a ± 1.59
GSR- IRRI-1	87.95 a ± 2.53	86.93 a ± 6.45	87.23 a ± 4.88
Purple rice	86.08 a ± 7.29	85.32 a ± 9.56	84.39 ab ± 5.09
BRRI dhan50	90.90 a ± 4.52	89.97 a ± 3.75	78.05 b ± 2.51
LSD (5%)	8.233	10.70	7.044

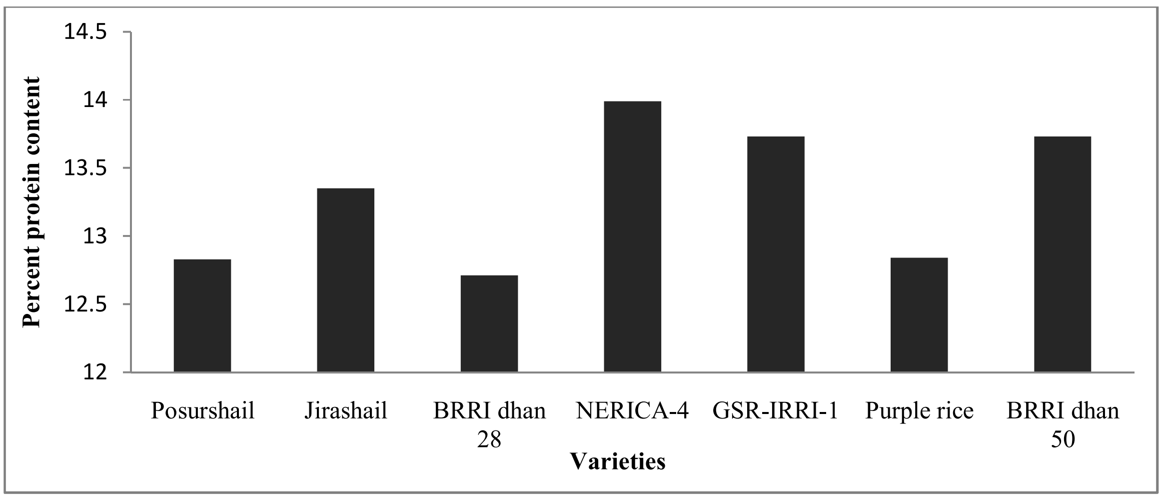

Figure 2. Percent protein content of rice seedlings at 36 DAS

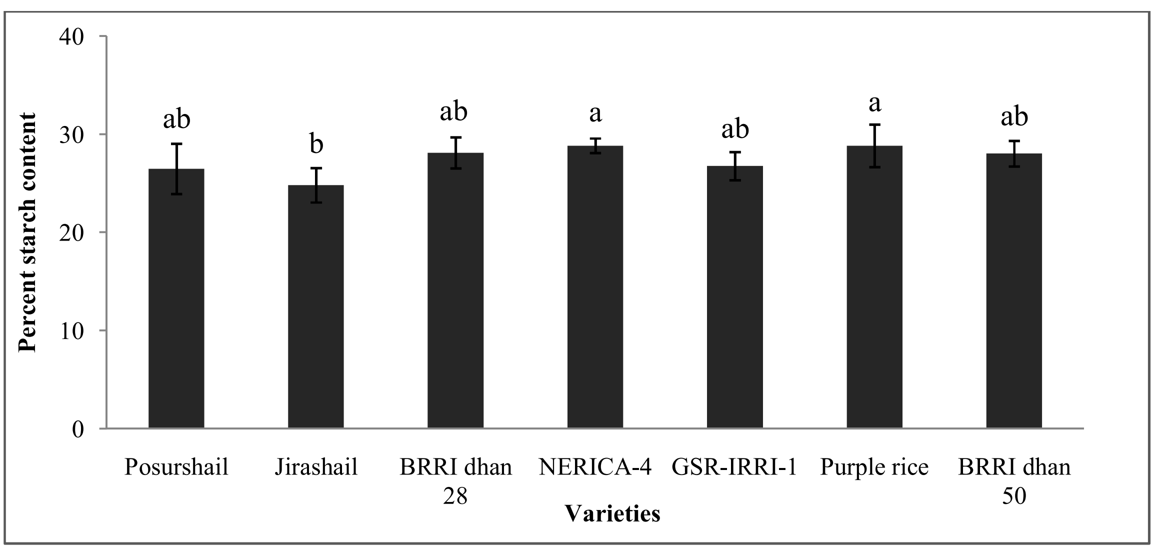

Figure 3. Percent starch content of rice seedlings at 36 DAS

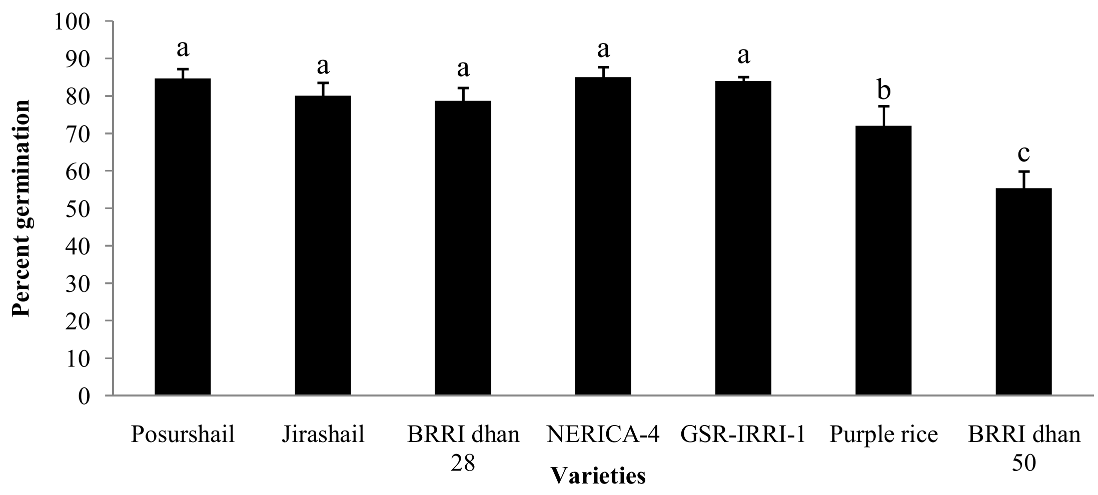

Figure 4. Percent seed germination of rice cultivars at 10 DAG (Days after first germination)

Plants that are highly susceptible to stress get deformed leaf tissues quickly just after subjected to stress environment and leaf water status is decreased. Jamil*et al.* (2012) revealed that the cellular membrane injury increases with the increase of abiotic stress resulting low leaf relative water content of plant.

The seedling emergence of rice cultivars was the first critical period due to prevailing low temperature. The statistically lowest percent germination was recorded in BRRI dhan50 rice cultivar (Figure 4). Though the value obtained regarding seed germination percentage of Poshusail, Jirasail, BRRI dhan28, NERICA-4 and GSR-IRRI-1 rice cultivars are statistically similar, the highest mean seed germination percentage was recorded in NERICA-4 rice cultivar. For germination of every seed, there is a range of optimum temperature and any deviation in this range resulting in poor germination as well as germination failure (Yari*et al.* 2012). This study period was characterized by the air temperature below 15ºC experienced low temperature stress condition. NERICA-4 rice cultivar showed the highest germination percentage while the statistically lowest seed germination was obtained in BRRI dhan50 cultivar. Thus, the germplasm of NERICA-4 rice cultivar is might have more low temperature tolerant potentiality.

CONCLUSION

Though the cultivars studied expressed morpho-physiological responses differently to low temperature environment, the increased leaf proline content might be a reasonable indicator for responses to sudden low temperature stress in all cultivars. In this study, NERICA-4 rice cultivar was found enriched genetical potentiality to survive in low temperature stress period in compare to other six rice cultivars studied. Thus, the NERICA-4 rice cultivar might solve temporarily for facing the upcoming low temperature stress in the Northwest Bangladesh. It is inferred that the genetical make up of this rice cultivar might be incorporated to a rice cultivar using modern biotechnological tools to develop low temperature tolerant rice cultivar with high yielding potentiality as well.

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