

Published with Open Access at **Journal BiNET**

Vol. 12, Issue 01: 976-985

Journal of Bioscience and Agriculture ResearchJournal Home: www.journalbinet.com/jbar-journal.html

Biochemical investigation and seedling mortality rate of some selected boro rice cultivars at low temperature stress

Basunia S. C.^a, Sarker, B. C.^a, Md. Omar Kayess^b, Md. Imanur Rahman^b, Md. Kajal^c and Md. Rakibul Alam^b

^aDept. of Agricultural Chemistry, Hajee Mohammad Danesh Science & Technology University, Dinajpur

^bDept. of Genetics and Plant Breeding, Hajee Mohammad Danesh Science & Technology University, Dinajpur

^cDept. of Agroforestry and Environment, Hajee Mohammad Danesh Science & Technology University, Dinajpur, Bangladesh

✉ For any information: ask.author@journalbinet.com, Available online: 25 January 2017.

ABSTRACT

A field study was conducted for biochemical investigation and seedling mortality rate determination of some selected Boro rice seedlings during the period of December, 2014 to January, 2015. Experiment time was characterized by prevailing low environmental temperature of below 15°C. Eight rice cultivars (V_1 : Pariza; V_2 : BRRI dhan29; V_3 : Shaita Boro; V_4 : BRRI dhan 28; V_5 : Jotapari; V_6 : BRRI dhan 16; V_7 : Pashushail; V_8 : Hybrid SL-8H) of different genetic constituents were evaluated in randomized complete block design with four replications. Two temperature regime was applied for seedling culture. Ambient temperature (T_0) for field investigation and 4°C temperature (T_1) environment was maintained only for physiological parameters. Changes of leaf Proline content, chlorophyll content (a and b), total carotenoid content and mortality rate were investigated. Result showed that V_8 (Hybrid SL-8H) and V_2 (BRRI dhan 29) seedling synthesized the maximum leaf Proline at low temperature than other cultivars. Highest amount of chlorophyll-a, was in V_8T_0 seedlings (10.03 mg g⁻¹), while chlorophyll-b (2.063 mg g⁻¹) and total carotenoid (4.0470 mg g⁻¹) were highest in V_3T_0 . Maximum chlorophyll-a/b ratio (6.729 mg) was recorded in V_7T_0 . Mortality rate was lowest in Hybrid SL-8H. Results of the study revealed that Hybrid SL-8H cultivars showed comparatively better potentiality to survive at low temperature that might be selected for cultivation and popularization in the low temperature region of Bangladesh.

Key Words: Boro rice, Low temperature, Bio chemicals, Mortality and Selection

Cite Article: Basunia, S. C., Sarker, B. C., Kayess, M. O., Rahman, M. I., Kajal, M. & Alam, M. R. (2017). Biochemical investigation and seedling mortality rate determination of some selected boro rice cultivars at low temperature stress. *Journal of Bioscience and Agriculture Research*, 12(01), 976-985.



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I. Introduction

Being the important food item of the world, rice (*Oryza sativa* L.) is the source of 76% of the total caloric demand of the south East Asia (Ma et al., 2007; Melissa et al., 2009). Rice stands next to wheat in the global food grain production (Roy et al., 2015). In the national economy of Bangladesh, rice

plays an important role likewise it is a staple food of many developing countries of the world (Faruq et al., 2010; Trans, 2001). In low laying areas, the type of rice cultivated on stored or residue after harvesting of Kharif is known as Boro (Singh, 2002). The Third Assessment Report of IPCC showed that climate change impacts create most vulnerability in south Asia (McCarthy et al., 2007). Among the most vulnerable countries of the world Bangladesh ranks high due to climate change (Climate Change Cell, 2008). Agriculture of Bangladesh is already under pressure due to the degradation of agricultural land and water endowments (Ahmed et al., 2000). For crop yield low temperature is the main limiting factor (Lee, 2001). Due to low temperature at high latitude and altitude areas yield loss were well documented in Northeast and southern China, Bangladesh, India, Nepal and other countries (Sanghera et al., 2011; Lee, 2001; Kanada, 1974). Below 15°C rice plant is susceptible (Warth and Ougham, 1993; Fujino et al., 2004). Physiology of crop changes due to exposure to low temperature crop (De Los Reyes et al., 2015) like reduction in total chlorophyll content (Ghaee et al., 2011), limitation of photosynthetic activity (Allen and Ort, 2001; Díaz et al., 2006) and oxidative stress. Non-uniform plant height is the result of low temperature effect at seedling stage (Da Cruz et al., 2006). During seedling stage low temperature results poor germination, stunted seedling growth and seedling mortality (Pathak et al., 2003). Low temperature causes irreversible injury in leaves, such as necrosis (Suzuki et al., 2008; Ye et al., 2009) and chlorosis (Andaya and Mackill, 2003). From October to early March the usuallu low temperature prevails and or expected in Bangladesh. At this time the Boro rice which is commonly known as winter rice suffer from cold injury in different growth stages due to low temperature. It has been noted that some of the local rice cultivars arc very popular due to their wider environmental adaptability. For breeding the rice plant has available genotypes for cold and cold-tolerant ecotypes. Keeping the above facts in mind the present research was carried out to screen suitable cultivars that can be utilized in future breeding programs for the development of cold tolerant Boro rice (Winter rice) varieties.

II. Materials and Methods

Plant material: Eight hybrid as we as local rice cultivars were used in this investigation. Seeds of these selected rice cultivars were collected from Bangladesh Agricultural Development Cooperation (BADC) (Table 01).

Table 01. Name of the selected rice cultivars

Designation	Name of rice cultivars	Nature of rice cultivars
V ₁	Pariza	Local
V ₂	BRRRI dhan 29	HYV
V ₃	Saita boro	Local
V ₄	BRRRI dhan 28	HYV
V ₅	Jotapari	Local
V ₆	BRRRI dhan16	HYV
V ₇	Pashushail	Local
V ₈	Hybrid dhan SL-8H	Hybrid

Experimental site, design and seedling: The research work was conducted at the research field of the department of Agricultural Chemistry, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh. The site is located in 25.13°N latitude and 88.23°E longitude and at an elevation of 34.5 m above the mean sea level. This site belongs to AEZ-1 (Agro-ecological Zone-1) (UNDP and FAO, 1988). All of eight cultivars are raised in small plots laid out on randomized complete block design with four replications. The whole area was divided into three blocks and each block was divided into five units maintaining plot dimension to 2×2 m². Plot to plot distance was 30 cm. Recommended rice production practice of Bangladesh Agricultural Research Council (BARC) was followed (BARC, 2005). Experimental site is situated in the tropical climatic zone, characterized by heavy rainfall during the months from May to September and minimum rainfall during winter season. Soil of the experimental field was sandy loam in texture with medium high land having soil with soil pH 6.0. The seeds of selected eight rice cultivars were sown in previously prepared seedbed. The sprouted seeds were placed in individual plots without any biasness at the rate of 330 g per plot. Proper irrigation was made as and when needed and weeding was done at 20 and 27 days after sowing (DAS).

Proline content of rice seedling: Fresh leaf samples from rice seedlings at different times were used for the determination of Proline. Free Proline content was estimated using the acid Ninhydrin method (Bates, 1973). About 40 to 50 mg of fresh leaf sample by weight was collected in an eppendorf tube containing 0.5 ml 3% sulfosalicylic acid and homogenized well using eppendorfpastle. It was then placed on a vortex mixer for about 10 minutes. Then 0.5 mL of 3% sulfosalicylic acid was added in it again. The eppendorf tube was then centrifuged for 20 minutes at 25°C temperature with 15000 rpm. Then the supernatant was collected in a test tube carefully with the help of micropipette. Then 1.0 mL of 3% sulfosalicylic acid was added again to the eppendorf tube and centrifuged for 20 minutes at 25°C temperature with 15000 rpm followed by mixing well using vortex mixer for 10 minutes. The supernatant was collected and added to the previously collected supernatant. Acid ninhydrin solution was made by adding 1.25 g ninhydrin with 30 mL glacial acetic acid and 20 mL of 6 M phosphoric acid and warmed it until it dissolved. Standard proline solution was also prepared by adding 0, 1, 5, 20, 50, 100, 150, 200 and 300 µg per 2 mL of 3% sulfosalicylic acid in test tubes for preparing standard curve. Then 2 mL each of glacial acetic acid and acid ninhydrin solution were added to the test tubes containing sample and standard Proline solution. Test tubes were then heated for 15 minutes in dry block heater maintaining 96-100°C temperature and the reaction was terminated in an ice bath. Optical densities of the solutions (sample and standard solution) were measured at 520 nm wave length using UV-visible spectrophotometer. Amount of Proline was determined from a standard curve.

Chlorophyll content of rice seedling: Chlorophyll content of rice seedling was determined by the method described by (Arnon, 1949). On the other hand, total chlorophyll was determined using the formulae given by (Porra, 2002). Concentration of chlorophyll-a (Chl-a), chlorophyll-b (Chl-b), total chlorophyll and total carotenoid was measured by using following formula.

$$\text{Chl-a} = 12.21 A_{663} - 2.81 A_{646} \quad (\mu\text{g ml}^{-1} \text{ of plant extract or } \text{mgg}^{-1} \text{ fresh weight})$$

$$\text{Chl-b} = 20.13 A_{646} - 5.03 A_{663}$$

$$\text{Total chlorophyll} = 17.76 (A_{646}) + 7.34 (A_{663})$$

$$\text{Total carotenoid} = \frac{(1000A_{470} - 2.05 \text{ Chl-a} - 114.8 \text{ Chl-b})}{245}$$

Mortality rate: 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.

$$\text{Mortality rate} = 100 - \left(\frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100 \right)$$

However, data were collected on various morphological and cold stress related biochemical characters namely Proline content, chlorophyll content (chlorophyll a and b), total carotenoid content and seedling mortality rate. 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).

III. Results and Discussion

Proline, a non-essential amino acid have multiple roles in stress tolerance in plants and also considered as key factor in metabolism and development of higher plants (Hare et al., 1998; Nanjo et al., 1992). Changes of Proline synthesis by selected cultivars at different experimental periods are shown in Table 02. Amount of Proline synthesis of different rice cultivars showed greatly variable at different times of the experiment. Proline content increases with the rising of stress period. Result showed that the rice seedlings tended to accumulate the higher Proline up to (1.717 mg g⁻¹) in V₂T₁ rice cultivar at 30 DAS and the lowest Proline (0.7867 mg g⁻¹) in V₁T₀ rice cultivar at 10 DAS while the temperature was below 13°C. Among the cultivars, the V₈T₁ cultivar showed the more potential to low temperature by over producing Proline. Similar result was revealed by (Sarker et al., 2013; Kamata and Uemura, 2004) as they concluded that prolonged low temperature for 14 to 21 days generally increased the Proline and total amino acids content.

Chlorophyll

One of the major component of photosynthesis is Chlorophyll content and also an important physiological trait closely related to photosynthetic ability of rice.

Chlorophyll-a content

Chlorophyll-a plays a vital role in the survivability of plants as well as the yield of crops. So the higher chlorophyll-a content in leaf indicated that the cultivar may perform better as their physiological growth and may give higher yield. V_8T_0 seedlings synthesized highest amount of Proline in all the different study period (7.613 mg g⁻¹ at 10 DAS, 8.937 mg g⁻¹ at 20 DAS and 10.03 mg g⁻¹ at 30 DAS) (Table 03). It also revealed that with the increment of cold stress period the Priline biosynthesis was also increased accordingly. The biosynthesis of chlorophyll-a, in V_8T_0 considered the superior among the cultivars studied during the experimental period. Araus et al. (1997) and Thomas et al. (2005) showed positive correlation between chlorophyll content and photosynthetic rate.

Chlorophyll-b content

Considering chlorophyll-b synthesis there were significant variation among the cultivars. V_8T_0 cultivar was found to have more chlorophyll-b content during the experiment period. At 10 DAS, the highest chlorophyll-b content was found in V_8T_0 (1.663 mg g⁻¹) while the lowest chlorophyll-b content was observed in V_7T_0 (0.930 mg g⁻¹). V_2T_0 cultivar showed more superiority to synthesize chlorophyll-b at 20 DAS (1.807 mg g⁻¹) and at 30 DAS, V_8T_0 showed more superiority to synthesize chlorophyll-b (2.063 mg g⁻¹), respectively, when the temperature was below 13°C (Table 03). Likewise, chlorophyll-a, the V_8T_0 rice seedlings showed the more superiority to other tested cultivars. Tested rice cultivars responded differently to same prevailing low air temperature due might be to their genetic variation and their difference in defense mechanism. To same prevailing low air temperature the tested rice cultivars responded differently due might be to their genetic variation and their difference in defense mechanism. Erge et al. (2008) revealed strong relationship between temperature and plant chlorophyll synthesis and stated that chlorophyllase occur due to any deviation from the optimum temperature condition.

Table 02. Mean Proline (mg g⁻¹ FW) synthesized by rice seedlings at different DAS

Varieties	Proline (mg g ⁻¹) synthesized by rice seedlings		
	10 DAS	20 DAS	30 DAS
V_1T_0	0.786f	1.050g	1.203c
V_1T_1	0.856ef	1.320c-f	1.267bc
V_2T_0	1.103a-c	1.580ab	1.610a
V_2T_1	1.007a-e	1.410a-d	1.717a
V_3T_0	0.890d-f	1.083fg	1.080cd
V_3T_1	0.936c-f	1.147e-g	1.140c
V_4T_0	0.993b-e	1.130e-g	1.247bc
V_4T_1	1.123a-c	1.250d-g	1.260bc
V_5T_0	1.020a-e	1.227d-g	0.870d
V_5T_1	1.157ab	1.353b-e	1.237bc
V_6T_0	1.160ab	1.143e-g	1.293bc
V_6T_1	1.077a-d	1.453a-d	1.517ab
V_7T_0	0.863ef	1.037g	1.153c
V_7T_1	0.943c-f	1.033g	1.183c
V_8T_0	1.097a-c	1.547a-c	1.567a
V_8T_1	1.200a	1.653a	1.683a
CV (%)	9.78	10.42	11.44
SE	0.057	0.077	0.087

Mean followed by the same letter(s) did not differ significantly at 5% level

Table 03. Leaf chlorophyll-a and chlorophyll-b content rice cultivars during experiment time

Varieties	Chlorophyll-a (mg g ⁻¹)			Chlorophyll-b (mg g ⁻¹)		
	10 DAS	20 DAS	30 DAS	10 DAS	20 DAS	30 DAS
V ₁ T ₀	6.917c	7.873b	8.370bc	1.223cd	1.437bc	1.950ab
V ₁ T ₁	6.947bc	7.237c	8.090cd	1.090d-f	1.267b-f	1.483c-e
V ₂ T ₀	7.287ab	8.333ab	9.890a	1.497ab	1.807a	1.957ab
V ₂ T ₁	7.270ab	7.933b	8.723b	1.147de	1.550ab	1.507c-e
V ₃ T ₀	6.553d	7.073cd	8.003cd	1.030d-f	1.280b-f	1.477c-e
V ₃ T ₁	6.063ef	6.423d-f	7.827cd	0.953ef	1.100e-g	1.180ef
V ₄ T ₀	6.117ef	6.587c-f	7.217ef	0.940f	1.417b-d	1.640b-d
V ₄ T ₁	5.920e-g	6.187ef	7.040ef	0.953ef	1.167c-g	1.330d-f
V ₅ T ₀	5.697g	6.120f	7.060ef	1.143de	1.533ab	1.427c-f
V ₅ T ₁	5.787fg	6.033f	6.907f	0.950ef	1.207c-g	1.210ef
V ₆ T ₀	6.113ef	6.777c-e	7.560de	1.023ef	1.237c-f	1.500c-e
V ₆ T ₁	6.153ef	6.810c-e	7.187ef	0.936f	1.137d-g	1.123f
V ₇ T ₀	6.183e	6.843c-e	7.160ef	0.930f	1.017fg	1.310d-f
V ₇ T ₁	5.663g	6.110f	7.017ef	0.966ef	0.9400g	1.267ef
V ₈ T ₀	7.613a	8.937a	10.03a	1.663a	1.793a	2.063a
V ₈ T ₁	7.413a	8.773a	9.563a	1.353bc	1.337b-e	1.737bc
CV (%)	3.04	4.95	3.81	9.55	11.76	11.62
SE	0.114	0.203	0.175	0.060	0.089	0.101

Mean followed by the same letter(s) did not differ significantly at 5 % level

Table 04. Variation in chlorophyll-a/b ratio in different rice seedlings at different DAS

Varieties	chlorophyll-a/b ratio(mg g ⁻¹) synthesized by rice seedlings		
	10 DAS	20 DAS	30 DAS
V ₁ T ₀	5.656	5.479	4.292
V ₁ T ₁	6.373	5.712	5.455
V ₂ T ₀	4.868	4.612	5.054
V ₂ T ₁	6.338	5.118	5.788
V ₃ T ₀	6.362	5.526	5.418
V ₃ T ₁	6.360	5.839	6.633
V ₄ T ₀	6.507	4.649	4.401
V ₄ T ₁	6.210	5.302	5.293
V ₅ T ₀	4.984	3.992	4.947
V ₅ T ₁	6.092	4.998	5.708
V ₆ T ₀	5.976	5.479	5.040
V ₆ T ₁	6.569	5.989	6.400
V ₇ T ₀	6.648	6.729	5.466
V ₇ T ₁	5.858	6.500	5.538
V ₈ T ₀	4.578	4.984	4.862
V ₈ T ₁	5.479	6.562	5.505

Total chlorophyll content

Chlorophyll-a/b is directly related to temperature. Table 04 showed that At 10 DAS when the day lowest temperature was 13°C, the highest chlorophyll-a/b ratio was found in V₇T₀ (6.648) while the lowest value was observed in V₈T₀ (4.578). Chlorophyll-a/b ratio of all cultivars at 20 DAS are statistically similar but V₇T₀ (6.729) and V₇T₁ (6.50) had the highest value among them, respectively. Lowest chlorophyll-a/b ratio at 20 DAS were in V₅T₀ (3.992). Performance of V₃T₁ was found superior at 30 DAS while the lowest chlorophyll-a/b was found in V₁T₀ (4.292) rice seedlings. However, during this study period, the highest chlorophyll-a/b content was found in V₇T₀ (6.729) but the lowest value was observed in V₅T₀ (3.992) at 20 DAS. Performance of V₇T₀ followed by V₃T₁ was found as superior throughout the sampling period. Sarker et al., 2013 also stated that some tolerant rice cultivar produced more chlorophyll during low temperature period. Farooq et al. (2009) stated that both the chlorophyll a and b are prone to soil dehydration.

Total carotenoid content

Highest carotenoid content was found in V₁T₀ (2.720 mg g⁻¹) while the lowest carotenoid synthesis was observed in V₇T₁ (1.567 mg g⁻¹) at 10 DAS. V₃T₀ rice seedlings also contained the highest (4.070

mg g⁻¹) carotenoid content at 20 DAS while the environmental temperature was below 12°C. The lowest total carotenoid content was found at 20 DAS (2.133 mg g⁻¹) and 30 DAS (2.473 mg g⁻¹) in V₂T₁ and V₄T₁ rice seedling. Highest carotenoid content was happening in V₃T₀ (4.070 mg g⁻¹) (Table 05). Performance of V₃T₀ was found as superior throughout the experimental period. In different varieties of rice the variability of protein contents up to 15% have been mentioned by many researcher (Sotelo et al., 1990; Lam-Sanchez et al., 1993; Kennedy and Burlingame, 2003) which verify the precision of the study that showed that protein content of tested rice varieties ranged from 5.98 to 7.75%, respectively.

Table 05. Amount of total carotenoid content (mg g⁻¹) at different DAS

Varieties	Carotenoid content (mg g ⁻¹) synthesized by rice seedlings		
	10 DAS	20 DAS	30 DAS
V ₁ T ₀	2.720a	3.447b-d	3.583a
V ₁ T ₁	1.943de	2.923e-g	2.967a-d
V ₂ T ₀	2.477a-c	2.950e-g	3.303a-c
V ₂ T ₁	1.837de	2.133i	2.727b-d
V ₃ T ₀	2.700a	4.070a	3.250a-d
V ₃ T ₁	2.163b-d	3.273c-e	2.560cd
V ₄ T ₀	2.577ab	3.607bc	3.357abc
V ₄ T ₁	2.210b-d	3.043d-f	2.473d
V ₅ T ₀	2.713a	3.697ab	3.477ab
V ₅ T ₁	2.210b-d	3.277c-e	2.840a-d
V ₆ T ₀	2.443a-c	3.593bc	3.453ab
V ₆ T ₁	1.673e	3.137d-f	2.723b-d
V ₇ T ₀	2.023c-e	2.553gh	2.950a-d
V ₇ T ₁	1.567e	2.043i	2.727b-d
V ₈ T ₀	2.563ab	2.733fg	3.410ab
V ₈ T ₁	2.040c-e	2.303hi	3.157a-d
CV (%)	11.63	7.43	13.61
SE	0.150	0.130	0.241

Mean followed by the same letter(s) did not differ significantly at 5 % level

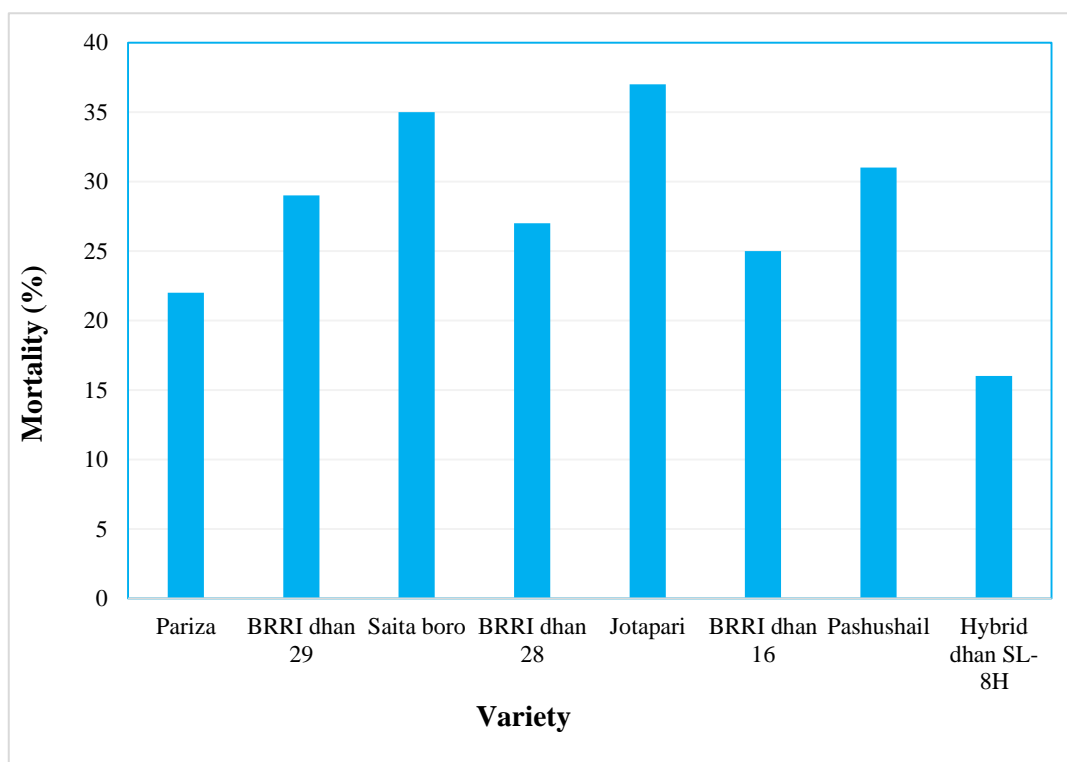


Figure 01. Mortality rate of eight rice cultivars at seedling stage.

Mortality rate

Seeds require a specific certain optimum temperature in order to germinate. Around 25°C the seeds of maximum rice cultivars germinate well (Fujino et al., 2008). Any deviation from this optimum

temperature (low and high temperature) decreases the germination rate. This study period was characterized by the air temperature below 15°C. A significant variation was observed in seed germination percentage of selected rice cultivars (Figure 01). The highest mortality percentage was recorded in Jotapari and Saita boro (37 and 35%) and the lowest was observed in Hybrid dhan SL-8H (16.0%). Cultivar having lower mortality percentage in lower temperature stress is tolerate than other cultivars. Low temperature causes much mortality of seedling were reported by Guan et al. (2009) and Schonbeck and Egley (1980).

IV. Conclusion

Hybrid varieties are more tolerant to low temperature stress as they synthesized more biochemical related to stress and also may be an important gene donors for breeding and genetic studies. Therefore, considering the above facts the Hybrid dhan SLY-8H is suitable for cultivation in Boro (winter) season. Further studies are suggested in different locations and agro-climate.

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<https://doi.org/10.1071/CP09006>

HOW TO CITE THIS ARTICLE?**APA (American Psychological Association)**

Basunia, S. C., Sarker, B. C., Kayess, M. O., Rahman, M. I., Kajal, M. & Alam, M. R. (2017). Biochemical investigation and seedling mortality rate determination of some selected boro rice cultivarss at low temperature stress. *Journal of Bioscience and Agriculture Research*, 12(01), 976-985.

MLA (Modern Language Association)

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