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Effect of Low Temperature on Chlorophyll and Carotenoid Content on the Seedlings of Some Selected Boro Rice Varieties

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Abstract

A study was conducted to screen out the low-temperature tolerant Boro rice seedlings from November 2012 to January 2013 for facing the upcoming rice production challenge in Northwest Bangladesh. The experimental time was characterized by a prevailing low environmental temperature of below 15˚C. Five rice cultivars (V1: BR-2; V2: BR-16; V3: Pariza; V4: Minicate; V5: BRRI dhan 50) were selected for the study. The leaf proline, chlorophyll content and total carotenoid content were investigated. The V2 (BR-16) seedling synthesized the higher leaf proline (1.228 $mg \cdot g^{-1}$) at a low temperature than those of other tested cultivars. Again, the highest amount of chlorophyll-a (3.957 $mg \cdot g^{-1}$), chlorophyll-b (2.118 $mg \cdot g^{-1}$), chlorophyll-a/b ratio (3.6754 $mg \cdot g^{-1}$) and total chlorophyll (5.051 $mg \cdot g^{-1}$) was measured in V2 (BR-16). The maximum total carotenoid (1.213 $mg \cdot g^{-1}$) was also observed in V2. In this experiment, the V2 (BR-16) showed comparatively better potentiality to survive at low temperatures (below 15˚C) than other varieties.

Keywords

Low Temperature, BORO Rice Seedling, Proline, Chlorophyll, Carotenoid Content

1. Introduction

Rice is the most important cereal crops, being a staple food for half of the world population by providing 76% of the total caloric demand of South East Asia [1] [2]. In global food grain production, it stands next to wheat [3]. Not alike the other major cereals, more than 90% of rice are consumed by humans [4]. Rice is the staple food for the people and in the national economy plays a vital role in Bangladesh [5] [6]. In the world, Bangladesh is the fourth largest rice producer and consumer country and provides 75% of the calories and 55% of the protein in the average daily diet [7]. The rice sector contributed one half of the agricultural GDP and one-sixth of the national income in Bangladesh [8]. Rice can be grown in different environments depending upon water availability and temperature conditions. For rice cultivation, the optimum temperature is between 25˚C and 35˚C, but it may change with genotypes, duration of the critical temperature, diurnal changes and physiological status of the plant [9]. Cold stress is a common problem that affects global production as a crucial factor in rice cultivation. Serious yield and yield component losses occur when low temperature prevails during the reproductive stages of rice. At high latitude and altitude area yield loss was well documented in the Northeast and Southern China, Bangladesh, India, Nepal, and other countries due to low temperature [10] [11]. As rice is originated from tropical or subtropical zones, so it's a cold-sensitive plant resulting in serious yield and yield component losses if low-temperature stress prevails during the reproductive stages [12].

Rice plants become susceptible below 15˚C [13] [14]. Due to exposure to low temperature, the physiology of crop changes [15] like total chlorophyll content reduction [16], limitation of photosynthetic activity [17] [18] and oxidative stress. Low temperature causes irreversible injury in leaves, such as necrosis [19] [20] and chlorosis [21]. In general, cold temperatures can reduce the crop survival rate, inhibit photosynthesis, retard growth, and block the synthesis of proteins, lipids, and carbohydrates [22] [23] [24].

In Bangladesh, from October to early March, the usually low temperature prevails and or expected. At this time, the Boro rice, which is commonly known as winter rice suffers from a cold injury in different growth stages due to low temperature [9]. As the cold environment has numerous adverse effects on rice production, we should screen out cold stress-tolerant rice variety or varieties suitable for the Boro season cultivation prevailing the temperature 15˚C in Bangladesh. Keeping the above facts in mind the present research was carried out to know the effect of low temperature on chlorophyll and carotenoid content on some selected Boro rice varieties.

2. Materials and Methods

2.1. Plant Material

Five hybrids as we as local rice cultivars were utilized in this study. Seeds of these chosen rice cultivars were collected from Bangladesh Agricultural Development Cooperation (BADC) (**Table 1**).

2.2. Experimental Site, Design and Seedling Rising

The experiment was conducted at the research field of the Department of

Table 1. Variety number, name and nature of selected rice cultivars.

Variety number	Name of rice varieties	Nature of rice varieties
V1	BR-2	HYV
V2	BR-16	HYV
V3	Pariza	HYV
V4	Minicate	Local
V5	BRRI dhan50	HYV

Agricultural Chemistry, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh. The study site belongs to AEZ-1 (Agro-ecological Zone-1) and is located in 25.13˚N latitude and 88.23˚E longitude with an elevation of 34.5 m above the mean sea level [25]. The selected rice cultivars are grown in small plots laid out in a randomized complete block design with three replications. The whole study area was divided into three blocks and each block was divided into five units maintaining the plot dimension to $2 \times 2\ m^2$. The plot to plot distance was 30 cm. Bangladesh Agricultural Research Council (BARC) [26] recommended rice production practice was followed in this experiment. The experimental site is characterized by heavy rainfall during the months from May to September and minimum rainfall during the winter season as it is situated in the tropical climatic zone. The experimental field soil was sandy loam in texture with medium high land having soil pH 6.0. The seeds of selected five rice cultivars were sown in a previously prepared seedbed. At the rate of 330 g per plot, the sprouted seeds were placed in individual plots without any biases. Proper irrigation was made as and when needed and different intercultural practices were done at 20 and 27 days after sowing (DAS).

2.3. Proline Content Determination of Rice Seedling

For the determination of Proline Fresh leaf samples from rice seedlings at different times were used. By using the acid Ninhydrin method [27] free Proline content was estimated. The fresh leaf sample (about 45 mg) 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 blender for approximately 10 minutes. After that 0.5 ml of 3%, sulfosalicylic acid was added in it again. The eppendorf tube was at that point centrifuged for 20 minutes at 25˚C temperature with 15,000 rpm. With the help of a micropipette then the supernatant was collected in a test tube carefully. Then, in the eppendorf tube, 1.0 mL of 3% sulfosalicylic acid was added again and centrifuged for 20 minutes at 25˚C temperature with 15,000 rpm followed by mixing well using vortex mixer for 10 minutes. The supernatant was collected and included in the already collected supernatant. Acid ninhydrin solution was made by including 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 arrangement was moreover arranged by including 0, 1, 5, 20, 50, 100, 150, 200 and 300 µg per 2 mL of 3% sulfosalicylic acid in test tubes for preparing a standard curve. Then 2 mL each of glacial acetic acid and acid ninhydrin solution was included in the test tubes containing sample and standard Proline solution. Test tubes were then warmed for 15 minutes in dry block radiator keeping up the 96˚C - 100˚C temperature and the reaction was ended in an ice bath. Optical densities of the solutions (test and standard solution) were measured at 520 nm wavelength utilizing a UV-visible spectrophotometer. Amount of Proline was determined from a standard curve.

2.4. Chlorophyll Content Determination of Rice Seedling

By the method described by [28] Chlorophyll content of rice seedling was determined. On the other hand, total chlorophyll was determined using the formulae given by [29].

Concentration of chlorophyll-a (Chl-a), chlorophyll-b (Chl-b), total chlorophyll and total carotenoid was measured by using following formula:

Chl-a = 12.21 A_{663} − 2.81A_{646} (μg·ml^{-1} of plant extract or mg·g^{-1} fresh weight)

Chl-b = 20.13 A_{646} − 5.03 A_{663}

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

Total carotenoid = 17.76 (A_{646}) + 7.34 (A_{663})

However, data were collected on Proline content, chlorophyll content (chlorophyll a and b) and total carotenoid content of the seedling. The collected data were analyzed statistically by using the statistical computer package program, MSTAT-C [30].

3. Results and Discussion

3.1. Proline Synthesis

In stress tolerance in plants, Proline is regarded to have multiple roles. Proline plays a vital role in plant defense mechanisms against both biotic and abiotic stresses and considered as a key factor in the metabolism and development of higher plants [31] [32]. The changes of proline synthesis by the selected cultivars at the different experimental times are shown in **Table 2**. At 20 days after seeding (DAS), the highest proline synthesis was found in V2 (0.813 mg·g^{-1}) while

Table 2. Mean proline (mg·g^{-1}) synthesized by rice seedlings at different DAS.

Varieties	Proline (mg·g^{-1}) synthesized by rice seedlings		
	20 DAS	24 DAS	28 DAS
V1 (BR-2)	0.497 b ± 0.25	0.876 bc ± 0.06	0.5863 b ± 0.13
V2 (BR-16)	0.813 a ± 0.52	0.931 ab ± 0.06	1.228 a ± 0.36
V3 (Pariza)	0.473 b ± 0.11	1.110 a ± 0.23	0.913 ab ± 0.22
V4 (Minicate)	0.714 ab ± 0.74	0.877 bc ± 0.14	0.5223 b ± 0.12
V5 (BRRI dhan50)	0.456 b ± 0.24	0.787 c ± 0.09	0.6669 b ± 0.31
LSD (5%)	0.632	0.254	0.4335

Mean followed by the same letter(s) did not differ significantly at 5% level by DMRT and ± values are for standard deviation.

the lowest value was observed in V5 (0.456 mg·g^{-1}). The second highest proline was observed in V4 followed by V1 and V3, respectively. At 24 DAS, the V3 variety synthesized the maximum amount of proline (1.110 mg·g^{-1}) followed by V2, the V4, and V1 while the minimum value was observed in V5 (0.797 mg·g^{-1}). At 28 DAS, V2 synthesized the highest proline (1.228 mg·g^{-1}) while the lowest proline content was observed in V4 (0.5233 mg·g^{-1}). The second highest proline value was found in V3 followed by V5 and V1.

In this study, the amount of proline synthesis of different rice cultivars showed a greatly variable at different times of the experimental period. The result showed that the rice seedlings tended to accumulate the higher proline up to (1.229 mg·g^{-1}) in V2 rice cultivar at 28 DAS and the lowest proline (0.446 mg·g^{-1}) in V5 rice cultivar 20 DAS while the temperature was below 13˚C. Among the cultivars, the V2 cultivar showed more potential to low temperature by overproducing proline. [33] stated that the proline content was increased rice leaves when the plants were subjected to cold acclimation treatment.

3.2. Chlorophyll

One of the major components of photosynthesis is Chlorophyll content additionally an important physiological characteristic closely related to the photosynthetic ability of rice.

3.2.1. Chlorophyll-a Content

Chlorophyll-a is the primary photosynthetic pigment. From the wavelengths of violet-blue and orange-red light, it absorbs most energy [34]. It plays an important role in the plant's survivability and also for the yield of crops. So the maximum chlorophyll-a content in leaf revealed that the cultivar may perform better as their physiological growth and may give maximum yield. The variability of chlorophyll-a content in rice seedling is shown in **Table 3**. At 20 DAS, the highest chlorophyll-a content was found in V2 seedlings (3.554 mg·g^{-1}) while the lowest chlorophyll-a content was observed in V5 seedlings (1.138 mg·g^{-1}). At 24 DAS, the V2 variety was observed to have the highest chlorophyll-a (2.824 mg·g^{-1}) and the lowest chlorophyll-a value was observed in V3 (1.044 mg·g^{-1}). At

Table 3. The Chlorophyll-a content (mg·g^{-1}) different rice seedlings at different DAS.

Varieties	Chlorophyll-a (mg·g^{-1}) synthesized by rice seedlings		
	20 DAS	24 DAS	28 DAS
V1 (BR-2)	1.921 bc ± 0.62	1.123 c ± 0.44	2.550 b ± 0.41
V2 (BR-16)	3.554 a ± 0.18	2.824 a ± 0.29	3.575 a ± 0.51
V3 (Pariza)	2.432 b ± 0.20	1.044 c ± 0.16	2.455 b ± 0.17
V4 (Minicate)	1.371 cd ± 0.35	1.879 b ± 0.16	1.741 c ± 0.05
V5 (BRRI dhan50)	1.138 d ± 0.28	1.534 bc ± 0.14	1.637 c ± 0.57
LSD (5%)	0.6674	0.4371	0.7380

Mean followed by the same letter(s) did not differ significantly at 5% level by DMRT and ± values are for standard deviation.

28 DAS, V2 cultivar contained highest chlorophyll-a (3.575 mg·g^{-1}). The V4 cultivar represented the lowest chlorophyll-a content (1.637 mg·g^{-1}) at 28 DAS.

The biosynthesis of chlorophyll-a in V2 considered the superior among the cultivars studied during the experimental period. Similar variation in chlorophyll-a content in different rice cultivars at low temperatures during the seedling growth stage was revealed by [33] [35]. A few studies have demonstrated that chlorophyll substance is emphatically related with the photosynthetic rate [36].

3.2.2. Chlorophyll-b Content

Chlorophyll-b is the accessory color that collects energy and passes it on to chlorophyll-a. It moreover directs the size of antenna and is more absorbable than chlorophyll-a. Hence, the amount of chlorophyll-b content in rice plant has a significant impact on the production of photosynthate. In this study, a significant variation in chlorophyll-b synthesis was evident in the seedlings of selected rice cultivars during the study period. The chlorophyll-b content is shown in Table 4. At 20 DAS, the highest chlorophyll-b content was found in V2 (1.369 mg·g^{-1}) while the lowest chlorophyll-b content was observed in V5 (0.837 mg·g^{-1}). The V2 cultivar showed more superiority to synthesize chlorophyll-b at 24 DAS (1.527 mg·g^{-1}) and at 28 DAS (1.118 mg·g^{-1}), respectively, when the temperature was below 13˚C. The V1 cultivar produced the lowest chlorophyll-b (0.866 mg·g^{-1}) at 24 DAS. At 28 DAS, The V5 variety showed the lowest chlorophyll-b (0.811 mg·g^{-1}) content while the prevailing air temperature was 11˚C.

Likewise chlorophyll-a, the V2 rice seedlings showed more superiority to those of other tested here. The tested rice cultivars responded differently to the same prevailing low air temperature due might be to their genetic variation and their difference in defense mechanism. There's a strong relationship between temperature and plant chlorophyll synthesis. Any deviation from the optimum condition leads to the development of chlorophyllase which is responsible for breaking down chlorophyll pigments [37].

3.2.3. Chlorophyll-a/b Ratio

The chlorophyll-a/b is directly related to temperature. Its content in a plant

Table 4. The Chlorophyll-b content (mg·g^{-1}) different rice seedlings at different DAS.

Varieties	Chlorophyll-b (mg·g^{-1}) synthesized by rice cultivars		
	20 DAS	24 DAS	28 DAS
V1 (BR-2)	0.811 b ± 0.04	0.866 b ± 0.14	0.925 ab ± 0.12
V2 (BR-16)	1.369 a ± 0.40	1.527 a ± 0.18	2.118 a ± 0.08
V3 (Pariza)	1.175 ab ± 0.23	0.951 b ± 0.15	0.980 ab ± 0.15
V4 (Minicate)	0.841 b ± 0.08	0.895 b ± 0.07	0.982 ab ± 0.11
V5 (BRRI dhan50)	0.837 b ± 0.11	0.996 b ± 0.39	0.811 b ± 0.12
LSD (5%)	0.3849	0.4252	0.2176

Mean followed by the same letter(s) did not differ significantly at 5% level by DMRT and ± values are for standard deviation.

varies concerning temperature change [38]. The chlorophyll-a/b ratio plays a vital role in plant acclimation. The variation of chlorophyll-a/b content is shown in Table 5. At 20 DAS, when the day lowest temperature was 13˚C, the highest chlorophyll-a/b ratio was found in V2 (2.797) while the lowest value was observed in V5 (1.396). The chlorophyll-a/b ratio of all cultivars at 24 DAS are statistically similar but V4 (2.078) and V2 (1.791) had the highest value among them, respectively. The lowest chlorophyll-a/b ratio at 24 DAS were in V3 (1.327). The performance of V2 was found superior (3.675) at 28 DAS while the lowest chlorophyll-a/b was found in V4 rice seedlings (1.880).

The performance of V3 followed by V4 was found as superior throughout the study period. In susceptible rice genotypes the low temperature stress significantly reduces chlorophyll concentration [23].

3.2.4. Total Chlorophyll Content

Chlorophylls are the foremost widely distributed plant pigments responsible for the characteristics of green color fruits and vegetables [39]. Chlorophylls are known to be easily degraded by conditions such as dilute acids, heat, light and oxygen [40]. In this study, the total chlorophyll content of selected rice cultivars was varied significantly during the experimental period which is shown in Table 6. At 20 DAS, the highest total chlorophyll content was found in V2 (4.813

Table 5. Variation in chlorophyll-a/b ratio in different rice seedlings at different DAS.

Varieties	Chlorophyll-a/b ratio in different rice cultivars		
	20 DAS	24 DAS	28 DAS
V1 (BR-2)	2.123 ab ± 0.71	1.439 a ± 0.53	2.911 ab ± 0.51
V2 (BR-16)	2.797 a ± 0.75	1.791 a ± 0.34	3.675 a ± 0.69
V3 (Pariza)	2.135 ab ± 0.55	1.327 a ± 0.16	2.582 abc ± 0.24
V4 (Minicate)	1.663 ab ± 0.55	2.078 a ± 0.37	1.880 c ± 0.21
V5 (BRRI dhan50)	1.396 b ± 0.51	1.685 a ± 0.53	2.153 bc ± 0.71
LSD (5%)	1.128	0.7229	0.9511

Mean followed by the same letter(s) did not differ significantly at 5% level by DMRT and ± values are for standard deviation.

Table 6. Total chlorophyll content in leaves of different rice seedlings at different DAS.

Varieties	Total chlorophyll ($mg \cdot g^{-1}$) synthesized in different rice cultivars		
	20 DAS	24 DAS	28 DAS
V1 (BR-2)	2.923 c ± 0.56	2.112 b ± 0.37	3.608 b ± 0.49
V2 (BR-16)	4.813 a ± 0.30	4.348 a ± 0.25	5.051 a ± 0.51
V3 (Pariza)	3.626 b ± 0.05	2.168 b ± 0.22	3.608 b ± 0.29
V4 (Minicate)	2.212 d ± 0.35	2.641 b ± 0.13	2.716 bc ± 0.14
V5 (BRRI dhan50)	1.951 d ± 0.21	2.431 b ± 0.55	2.805 c ± 0.54
LSD (5%)	0.6065	0.6112	0.7598

Mean followed by the same letter(s) did not differ significantly at 5% level by DMRT and ± values are for standard deviation.

mg·g^{-1}) while the lowest total chlorophyll synthesis was observed in V5 (1.951 mg·g^{-1}). At 24 and 28 DAS, the total chlorophyll content of V2 cultivar was 4.348 mg·g^{-1} and 5.051 mg·g^{-1}, respectively. The total chlorophyll content of V5 rice seedling at 24 DAS and 28 DAS were 2.431 mg·g^{-1} and 2.805 mg·g^{-1}, respectively when the environmental temperature was below 12˚C.

The performance of V2 showed better that of V4 and V5 regarding total chlorophyll pigment synthesis. [33] stated that some tolerant rice cultivar produced more chlorophyll during low temperature period.

3.2.5. Total Carotenoid Content

In this study, a significant difference in carotenoid content was found amongst the seedlings of selected rice cultivars during the study period.

The variation of carotenoid content has been shown in **Table 7**. The highest carotenoid content was found in V2 (1.0077 mg·g^{-1}) while the lowest carotenoid synthesis was observed in V1 (0.528 mg·g^{-1}) at 20 DAS. The V2 rice seedlings also contained the highest carotenoid content at 24 and 28 DAS while the environmental temperature was below 12˚C. The study showed that the highest carotenoid content was happen in V2 (1.211 mg·g^{-1}) and lowest value was observed in V1 (0.530 mg·g^{-1}) at 28 DAS and 20 DAS, respectively. The lowest total carotenoid content was found at 24 DAS (0.702 mg·g^{-1}) and 28 DAS (0.716 mg·g^{-1}) in V3 and V4 rice seedling. The performance of V2 was found as superior throughout the experimental period.

Carotenoids are multifunctional compounds serving as structural components of light-harvesting complexes, accessory colors for light collecting, substrates for abscisic acid synthesis, components of photoprotection and scavengers of singlet oxygen [41].

4. Conclusion

Based on the above parameters studied, it can be concluded that the V2 (BR-16) performed better among the studied varieties and might be selected for cultivation in an environment where temperature prevails below 15˚C.

Table 7. Amount of total carotenoid content (mg·g^{-1}) in different rice seedlings at different DAS.

Varieties	Total carotenoid content (mg·g^{-1}) in different rice cultivars		
	20 DAS	24 DAS	28 DAS
V1 (BR-2)	0.528 b ± 0.12	0.716 a ± 0.09	0.811 bc ± 0.07
V2 (BR-16)	1.0077 a ± 0.07	0.855 a ± 0.45	1.213 a ± 0.14
V3 (Pariza)	1.010 a ± 0.05	0.702 a ± 0.16	0.805 bc ± 0.13
V4 (Minicate)	0.6661 b ± 0.15	0.811 a ± 0.08	0.716c ± 0.09
V5 (BRRI dhan50)	0.548 b ± 0.08	0.714 a ± 0.08	0.830 b ± 0.12
LSD (5%)	0.1822	0.4245	0.1921

Mean followed by the same letter(s) did not differ significantly at 5% level by DMRT and ± values are for standard deviation.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Appendix

Table A1. Air temperature at regular interval during the experimental period.

Date	Temperature (˚C)			
	12:00 AM	06.00 AM	12.00 PM	06.00 PM
21 December 2016	10	11	18	15
22 December 2016	11	10	19	16
23 December 2016	11	10	19	14
24 December 2016	11	10	21	17
25 December 2016	11	10	14	12
26 December 2016	11	10	12	13
27 December 2016	9	8	17	13
28 December 2016	9	8	21	18
29 December 2016	11	9	21	18
30 December 2016	8	7	21	18
31 December 2016	10	8	24	22
01 January 2017	9	8	24	21
02 January 2017	11	9	25	22
03 January 2017	9	10	26	22
04 January 2017	11	10	24	22
05 January 2017	9	9	22	21
06 January 2017	8	6	22	19
07 January 2017	7	7	18	13
08 January 2017	8	6	13	10
09 January 2017	5	4	14	13
10 January 2017	6	5	18	15
11 January 2017	6	5	21	16
12 January 2017	6	5	23	21
13 January 2017	8	7	24	21
14 January 2017	8	7	24	21
15 January 2017	9	8	24	22
16 January 2017	9	8	24	22
17 January 2017	11	9	26	23
18 January 2017	11	10	26	22
19 January 2017	13	11	25	22
20 January 2017	12	13	24	20
21 January 2017	11	9	24	21
22 January 2017	11	9	25	22
23 January 2017	10	9	21	17
24 January 2017	11	9	18	17
25 January 2017	9	8	17	14
26 January 2017	8	9	18	13
27 January 2017	9	8	19	16
28 January 2017	11	9	22	19
29 January 2017	10	11	23	22
30 January 2017	11	11	26	23
31 January 2017	11	11	26	23
01 February 2017	11	11	25	24
02 February 2017	12	12	27	25
03 February 2017	12	11	28	24