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Duration of low temperature changes physiological and biochemical attributes of rice seedling

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Abstract. The low temperature (LT) is detrimental to growth of rice seedling during boro season in Bangladesh. An experiment was conducted in growth chamber during June to October, 2021 using BRRI dhan29 as planting material. The aim of this experiment was to determine the effect of variable duration of LT on growth, physiological and biochemical traits of rice seedling and to determine the age of seedling that can tolerate cold effect. In this experiment 5, 10, 15 and 20 days old seedling (DOS) were exposed to 6 °C for 1, 2, 3 and 4 days. Seedlings were also grown under room temperature (25 °C, RT) which consider as control treatment. Seedlings were grown in plastic trays filled with mixture of soil and cowdung. The experiment was conducted following completely randomized design with 3 replications. Data on shoot length and weight, root length and weight, chlorophyll (Chl), carotenoids, malondialdehyde (MDA) and proline contain were determined after 5 days of temperature sock. The results revealed that the LT was injurious to younger rice seedling when they were exposed to LT for 1 to 2 days. The shoot and root length as well as their dry weight were reduced under low temperature. Further, the Chl and carotenoid content of younger rice seedlings degraded within 2 days of LT exposure. On the contrary, the proline and MDA content of rice seedlings increased to reduce the harmful effect of under LT. It could be concluded that the rice seedlings could tolerate the detrimental effect of LT when they attain at least 15 days.

Key words: cold injury, lipid peroxidation, photosynthetic pigment, stress.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important staple foods and extensively cultivated worldwide (Fairhurst & Dobermann, 2002; Shelton et al., 2002). More than half of the world's populations derives a significant proportion of their caloric intake from rice consumption. Asia is the major rice growing area where 90% of the worlds′ rice is grown (Rashid & Yasmeen, 2017). Bangladesh, a part of Asia, has a large agrarian base with 76% of total population living n the rural areas and 90% of the population directly related with agriculture. Agriculture contributes 14.1% of national GDP, where rice sector pays one-half (BRRI, 2021). Therefore, rice has a significant role in socio-economic condition of Bangladesh and it is imperative to increase rice production in order to meet the food demand in future.

Any stress condition either biotic or abiotic inhibits plant growth, reduced bio-production and changes physiological condition (Nilsen & Orcutt, 1996). The alteration in the plant system due to stress condition depends on the intensity and duration of the stress environment (Godbold, 1998). Different abiotic stress like drought, high and low temperature, salinity, submergence etc. cause an enormous loss each year worldwide due to reduction in crop productivity and crop failure. They cause water reduction and osmotic changes in the cellular level, suppress the activities of cellular molecules, and can result in reduced growth and extensive losses in agricultural production (Xiong et al., 2002). Abiotic stress in fact is the principal cause of crop failure worldwide, dipping average yield for most major crops by more than 50% (Eshghi et al., 2010). Among the stresses, temperature stress act as an abiotic stress factor that has a strong impact on the survival, growth, reproduction and distribution of plants in large area of the world (Boyer, 1982). Each plant has its unique set of temperature requirements, which are optimum for its proper growth and development. Every single plant is characterized by a certain genetically fixed level of resistance to LT, which reduces its metabolic activity. This level of resistance can vary among individual plants and species. The LT or cold is one of the major stresses that limit growth of rice seedling because rice is sensitive to chilling stress.

Rice is grown though out the year, but boro season (winter season) is the major rice growing season in Bangladesh. This season starts in the month of November and ends in April. Seedlings are grown in the seed bed during the month of November to December, when the air temperature remains below 20 °C. Therefore, rice seedling faces a low temperature (LT) stress in the seedling stage. The LT injury is a common problem during boro season in Bangladesh (BRRI, 2021) because air temperature goes below the critical temperature in many areas of the country (Rashid & Yasmeen, 2017). Therefore, seedlings of boro rice experienced very low temperature during the month of December and January. Germination rates becomes below and rice seedlings get yellowish color with stunted growth (Mukhopadhyay et al., 2004; Tuteja et al., 2012). It also causes a series of morpho-physiological and biochemical changes in young rice plant (Hasegawa et al., 2000). These changes also known as acclimation response (Hughes & Dunn, 1996) because the changes in molecular and cellular level occurs in the plant body to overcome and survive under stress condition.

The reduction in seedling growth of rice due to LT is one of the major problems in tropical and subtropical areas. However, 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). As the cold environment has numerous adverse effects on raising rice seedling as well as on rice production. In boro season, sever cold damages young rice seedlings every year in northern part of Bangladesh. This damage is largely due to acute dehydration associate with freezing. Farmers were not satisfied with the rice seedling due to poor growth and low quality because seedling mortality occurs after transplanting during boro season. Moreover, rice transplanting delayed due to cold weather during boro season. Overall, due to exposure to LT, the physiology of crop changes like total chlorophyll (Chl) content reduction, limitation of photosynthetic activity, and oxidative stress. LT causes irreversible injury in leaves, such as necrosis (Ye et al., 2009), chlorosis (Andaya & Mackill, 2003), reduction of crop survival rate, retard growth, and block the synthesis of proteins, lipids, and carbohydrates (Liu et al., 2013). Senberga et al. (2018) also reported that low temperature plays a vital role in seed germination of faba bean and root zone temperature needed at least 8 °C for germination and symbiotic development.

The objectives of this study were to determine the effect of variable duration of LT on growth, physiological and biochemical traits of rice seedling and to determine the age of seedling that can tolerate cold effect. Seedling height, dry matter production, pigment content, proline and malondialdehyde (MDA) contents were tested in this study.

MATERIAL AND METHODS

Experimental Site and Treatments

An experiment was conducted in growth chamber under laboratory condition of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur during June to October, 2021. BRRI dhan29 was used as planting material. In this experiment 5, 10, 15 and 20 days old seedling (DOS) were exposed to LT (6 °C) for 1, 2, 3 and 4 days. Seedlings were also grown under room temperature (25 °C, RT) which was considered as control treatment. The experiment was conducted following completely randomized design with 3 replications.

Seed Sowing

Seedlings were grown in plastic trays filled with mixture of soil and cow dung. The size of each tray was 48 × 48cm and contains 36 chambers. About 25 seeds were sown in each chamber. The ratio of soil and cowdung was 3:1. Before filling the trays, the mixture of soil was sieved to remove clods. At first, the trays were placed in a plain land. The one fourth of each chamber of trays was filled with clod free soil materials. The rice seeds were sown on the trays at five days intervals to get seedlings of 5, 10, 15 and 20 days old. The seeds were sown in a planned way so that each tray contains seedlings of 5, 10, 15, and 20 days old. After sowing, the seeds were covered with clod free soil mixture and irrigated to keep the seed moistened. The trays were irrigated at 2 times every day using plastic bottle.

Treatment Imposition

When the rice seedlings gained desirable age, the trays were transfer to growth chambers for 2 hours to impose temperature sock. After exposure to desirable temperature, the trays were removed from growth chamber to room temperature.

Data Collection

The data regarding various relevant parameters were collected accordingly during the experimental period. Data on shoot (SL) and root length (RL); shoot (SW) and root weight (RW), Chl, carotenoids, proline and MDA contents were determined after 5 days of temperature sock. Seedlings were collected from trays and washed with tap water.

After washing the seedlings, the were gently wiped with a tissue paper. The SL and RL of five seedlings were measured and averaged to get SL and RL of a rice seedling. The root and shoot of rice seedling were taken after oven dry at 80 °C for 72 hours. Strength of seedling was calculated from the following formula:

$$\text{Seedling strength (mg cm}^{-1}) = \frac{\text{Shoot weight (mg)}}{\text{Shoot length (cm)}} \tag{1}$$

The formulae for computing Chl a, b and total carotenoids were determined by the method of Yang et al. (1998). Chl content was determined on fresh weight (FW) basis extracting with 80% acetone by using double beam spectrophotometer. The acetone-water mixture (4:1) was used as a solvent. The absorbance maxima were read at 663.0 nm for Chl a, 645.0 nm for Chl b and 470.0 nm for carotenoids. Contents of Chl a, Chl b and total carotenoids were calculated from the following equations:

$$\text{Chl a (mg g}^{-1}\text{ FW)} = [12.7\ (\text{D663}) - 2.69\ (\text{D645})] \times [\text{V}/1{,}000 \times \text{W}] \tag{2}$$

$$\text{Chl b (mg g}^{-1}\text{ FW)} = [22.9\ (\text{D645}) - 4.68\ (\text{D663})] \times [\text{V}/1{,}000 \times \text{W}] \tag{3}$$

$$\text{Total Chl (mg g}^{-1}\text{ FW)} = [20.2\ (\text{D645}) + 8.02\ (\text{D663})] \times [\text{V}/1{,}000 \times \text{W}] \tag{4}$$

$$\text{Total carotenoids (mg g}^{-1}\text{ FW)} = \frac{[1{,}000\ (\text{D470}) - 2.27\ (\text{Chl a}) - 81.4\ (\text{Chl b})]}{227} \tag{5}$$

where, D (663,645,470) = Optical density of the Chl a, b and carotenoids extract at wavelength of 663, 645 and 470 nm, respectively. V = Final volume (mL) of the 80% acetone with Chl extract and W = Weight of fresh leaf sample in g. Proline content in leaf of rice seedling estimated. The 0.5 g of fresh weight of leaf was taken for proline estimation and subsequently proline was estimated according to (Bates et al., 1973).

Data Analysis

The data were analyzed using computer software CropStat 7.2 and the graphs were prepared using excel program. Treatment means were separated using *LSD* at 5% level of probability.

RESULTS AND DISCUSSION

Shoot Length

The growth of shoot depends on various environmental factors. Air temperature is one of them, which significantly controls the range of plant growth. Each plant has its optimum growth temperature but 18–21 °C temperature is considered as optimum for most of the temperate plants for subsequent growth and development (Junttila, 1986). The results of this experiment showed that the interaction of LT and seedling age (SA) had a significant effect on shoot length (SL) of rice seedlings (Table 1). The SL of 5 to

Table 1. Effect low temperature on shoot length (cm) of rice seedling

Seedling age (days)	Exposure to low temperature for				
	0 day (control)	1 day	2 days	3 days	4 days
5	13.7	12.3	12.0	12.6	11.3
10	14.2	14.8	14.7	12.4	13.7
15	14.4	14.5	14.6	14.9	13.6
20	15.8	13.8	14.6	14.8	13.8
*LSD*0.05					
SA	0.932				
TD	1.041				
SA × TD	2.073				
CV (%)	9.01				

SA = Seedling age and TD Temperature duration.

20 DOS of rice reduced significantly when they were treated with LT. Under LT, however, the reductions in SL of younger seedling (5 to 10 DAS) were more prominent than older one. The SL of 5 DOS was 13.7 cm when it was treated with 25 °C temperature (control), which reduced to 11.3 cm when LT exerted 4 days. Similarly, SL of 10 DOS reduced by 12% when they were experienced with LT upto 3 days. On the contrary, SL varied from 15.8 to 13.8 cm when 20 DOS were treated with LT upto 0 to 4 days. It revealed that aged seedlings were less affected by prolong LW. Zhao et al. (2020) stated that LT exhibited a significant inhibitory effect on growth of rice seedling. Chl content is an important index to evaluate photosynthesis capacity. Previous study showed that LT inhibited the activities of Chl biosynthesis enzymes (Nagata et al., 2007). Fyson & Sprent (1982) stated that seedling is exposed to temperature delayed nodulation in bean which is associated with the slower plant growth.

Shoot Weight

The production of percent shoots dry weight decreased in response to low environmental temperature. Low temperature severely reduces the dry weight content of plant (Hnilickova et al., 2002). In the present study, LT exerted a significant effect on shoot dry matter (SDM) of rice seedlings (Table 2). The SDM of 5 DOS varied from 6.4 to 4.3 mg plant^{-1} when treated with LT at 0 to d days. Similarly, the range of SDM was 5.33 to 7.61, 5.68 to 7.3, and 4.6 to 7.4 mg plant^{-1} in case of 10, 15 and 20, respectively when they are treated with LT at 0 to 4 days. This indicated that the prolong LT was detrimental to SDM of rice seedlings. Plant growth reduced under LT condition due to limited Chl content. The effect of LT for higher duration inhibited the greening process and seedling growth. Therefore, accumulation of SDM hampered significantly under LT. Ben-Haj-Salah & Tardieu (1995) also reported that LT inhibited the leaf cell division and elongation results lower SDM.

Table 2. Effect low temperature on shoot dry matter (mg plant^{-1}) of rice seedling

Seedling age (days)	Exposure to low temperature for				
	0 day (control)	1 day	2 days	3 days	4 days
5	6.4	5.5	5.1	5.0	4.3
10	7.6	6.1	5.7	6.7	5.3
15	7.2	5.5	5.8	5.9	5.7
20	7.4	6.1	6.1	7.1	4.6
LSD$_{0.05}$					
SA	1.756				
TD	2.195				
SA × TD	3.388				
CV (%)	7.0				

SA = Seedling age and TD Temperature duration.

Table 3. Effect low temperature on strength (mg cm^{-1}) of rice seedling

Seedling age (days)	Exposure to low temperature for				
	0 day (control)	1 day	2 days	3 days	4 days
5	0.47	0.45	0.42	0.40	0.38
10	0.54	0.41	0.38	0.54	0.39
15	0.50	0.38	0.40	0.39	0.42
20	0.47	0.44	0.42	0.48	0.33
LSD$_{0.05}$					
SA	0.295				
TD	0.334				
SA × TD	0.656				
CV (%)	8.0				

SA = Seedling age and TD Temperature duration.

Seedling Strength

Seedling strength was also decreased significantly due to LT treated trays (Table 3) in all aged seedling. In case of 5 DOS, the strength of seedlings was 0.47, 0.45, 0.42, 0.50 and 0.38 mg cm^{-1} when they were treated at 0, 1, 2, 3 and 4 days, respectively. However, the strength of seedlings reduced drastically in case younger seedling (5 DOS) than older one (20 DOS).

Root Length

For subsequent growth and development of plants, there should have optimum environmental temperature as the activation of various functional enzymes needs proper temperature along with other requirements. Proper root growth of maximum plant species requires 27–30 °C soil as well as environmental temperature (Drennan & Nobel, 1998). Any deviation from this optimum level of temperature leads to the decrease root growth. In this study, root length (RL) of rice seedling was significantly smaller when they were exposed to longer duration of LT (Table 4). In case of 5 DOS, the RL was 3.9 cm under control which reduced to 2.5 cm under 4 days treated trays. However, RL of 10 to 20 DOS was also reduced due increased duration of LT from 0 to 4 days. Roots are responsible to acquire water and nutrients to improve plant productivity (Klein et al., 2020). However, roots are the most sensitive part of the plant to temperature (Munyon et al., 2020). The results of these experiment also showed that the root system of rice seedling reduced significantly under LT. Senberga et al. (2018) also found that primary root length of faba bean also reduced significantly due to low temperature.

Table 4. Effect low temperature on root length (cm) of rice seedling

Seedling	Exposure to low temperature for				
age (days)	0 day (control)	1 day	2 days	3 days	4 days
5	4.0	3.5	2.8	2.7	2.5
10	3.8	3.3	3.1	2.7	2.7
15	2.7	2.7	2.5	2.8	2.8
20	2.5	2.3	2.4	2.8	2.7
$LSD_{0.05}$					
SA	0.624				
TD	0.682				
SA × TD	1.371				
CV (%)	30.4				

SA = Seedling age and TD Temperature duration.

Root Weight

Table 5. Effect low temperature on root dry matter (mg plant^{-1}) of rice seedling

Seedling	Exposure to low temperature for				
age (days)	0 day (control)	1 day	2 days	3 days	4 days
5	1.2	0.73	1.2	1.3	1.4
10	1.1	1.2	1.3	1.9	1.5
15	0.43	0.87	1.6	1.6	1.3
20	0.43	0.28	0.50	0.19	0.76
$LSD_{0.05}$					
SA	2.104				
TD	2.353				
SA × TD	4.711				
CV (%)	25.1				

SA = Seedling age and TD Temperature duration.

The effect of LT on dry matter partitioning between shoot and roots is difficult to predict because temperature may affect directly water and nutrient uptake as well as other physiological and biochemical processes inside the plant body. However, root dry matter (RDM) of rice seedlings significantly increased when they were exposed to LT from 0 to 4 days (Table 5). In case of 5 DOS, the RDM was 1.2 mg plant^{-1} under control which was 1.4 mg plant^{-1} under 4 days treatment. Similar trend was also true for 10 DOS. However, RDM of older rice seedling (15 to 20 DOS) reduced dramatically when they experienced with LT. Root weight ratio of different bean varieties were reduced under 4 °C (Senberga et al., 2018). Root development not only the indicator of plant growth, but also demonstrates the effect of microorganisms in the soil that the seedling is exposed to, as delayed nodulation in bean due to low temperature has been associated with the slower plant growth (Fyson & Sprent, 1982).

Chl a Content:

Chl content of rice seedling varied significantly when they are exposed to various duration of LT (Figs 1 to 3). The LT showed a sever effect on Chl a content with the increasing of duration. Chl a content of 5 to 20 DOS varied 1.8 to 2.2 mg g^{-1} fresh weight (FW) under control, while it was 0.7 to 1.04 mg g^{-1} FW under 4 days duration. Similarly, the range of Chl a was 1.2 to 2.0, 1.1 to 1.8 and 1.0 to 1.7 mg g^{-1} FW under 1, 2 and 3 days of LT exposure (Fig. 1). LT stress is one of the most important factors that limit photosynthetic activities of plants by reducing the pigment content in the plants. It has been reported that Chl a and b content decreased in plants when they were subjected to cold stress (Yadegari et al., 2007).

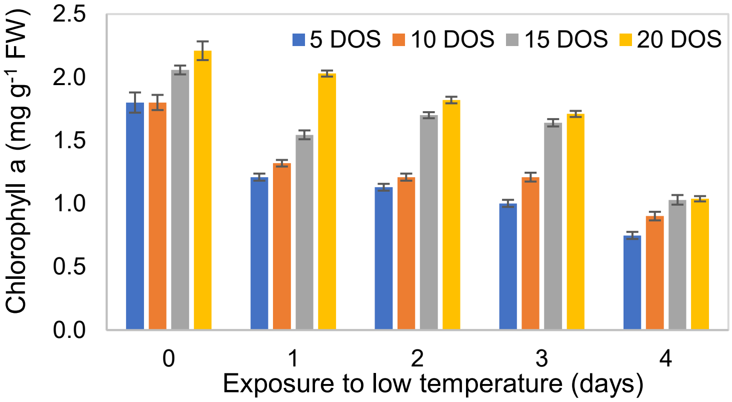

Figure 1. Effect low temperature on chlorophyll a content of rice seedling.
Var indicates mean value ± standard error.

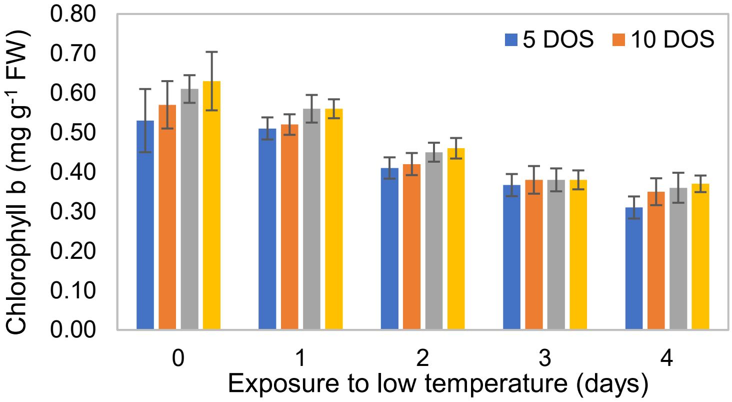

Figure 2. Effect low temperature on chlorophyll b content of rice seedling.
Var indicates mean value ± standard error.

Chl b Content

The concentration of Chl b in rice seedlings were also affected due to various duration of LT. The Chl b content ranged from 0.53 to 0.63 mg g^{-1} FW under control and 0.51 to 0.56 mg g^{-1} FW under 1 day exposure to LT (Fig. 2). Similarly, the content of Chl b in rice plant reduces gradually when they were exposed to longer duration of LT. The decrease in the Chl b content under LT stress might be due to suppression of Chl biosynthesis, which probably by inhibiting the activities the activities of Chl biosynthesis enzymes (Nagata et al., 2007).

Total Chl Content:

The LT showed a sever effect on total Chl content of rice seedling. Under LT, the rice seedlings became yellow due to degradation of Chl. The younger seedlings were more affected when the duration of LT increases. The total Chl content of control treatment varied 2.3 to 2.8 mg g^{-1} FW, while it was 1.1 to 1.4 mg g^{-1} FW under 4 days of LT (Fig. 3). It was observed that LT induced a significant decrease in the content of photosynthetic pigment fraction (Chl a and b) as a result of the content of total Chl content in the leaves. The result was also agreed with the findings of Habibi et al. (2011).

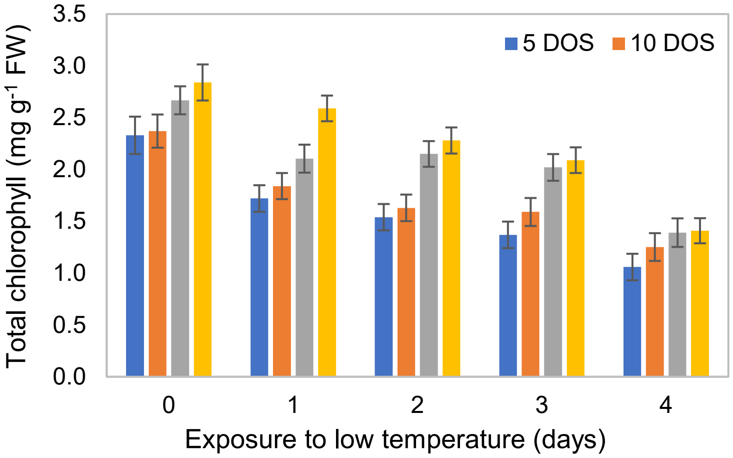

Figure 3. Effect low temperature on total chlorophyll content of rice seedling.
Var indicates mean value ± standard error.

Carotenoid Content

Carotenoid content of rice seedlings varied significantly due to various duration of LT (Fig. 4). The concentration of carotenoids in rice seedling drastically decreased when they were exposed to LT from 1 to 4 days. However, the 5 to 10 DOS affected severely regarding carotenoids content from 1 to 4 days LT treatment. Moreover, the carotenoids content of 5 DOS was 0.5 mg g^{-1} FW, while it was 0.20, 0.21, 0.20 and 0.091 mg g^{-1} FW at 1, 2, 3 and 4 days LT treatment, respectively. The content of this pigment of 10 to 20 DOS also reduced significantly under 1, 2, 3 and 4 days LT treatment as compared to control. The LT reduced carotenoids level in plants was also reported by Zhao et al. (2020).

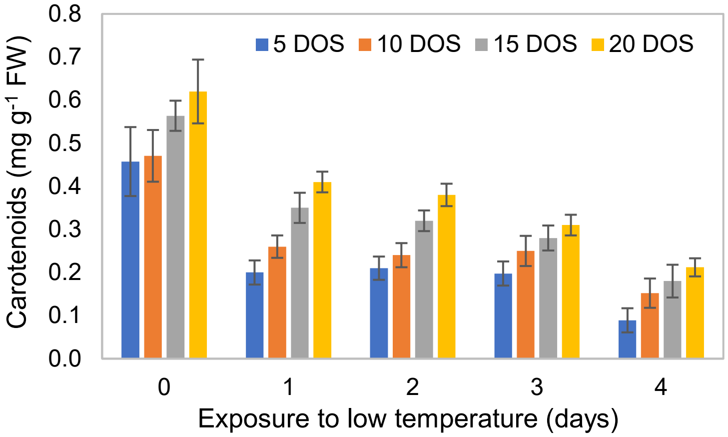

Figure 4. Effect low temperature on carotenoids content of rice seedling.
Var indicates mean value ± standard error.

Proline Content

The proline content dramatically increased in younger rice seedlings (15 to 20 DOS) when they exposed to LT for longer duration (Fig. 5). In case of 5 DOS, the proline content varied from 0.011 µg g^{-1} FW in control, which increased to 0.025, 0.026, 0.028 and 0.033 µg g^{-1} FW at 1, 2,3 and 4 days exposure to LT. Proline accumulation in plants is one of the most popular approach used by the plants to deal with LT stress. Proline is an osmolyte which helps plants to survive under LT through cold acclimation (Ritonga & Chen, 2020). In this experiment, it was observed that more proline accumulated in the younger seedlings. The result also supported by the findings of Habibi et al. (2011).

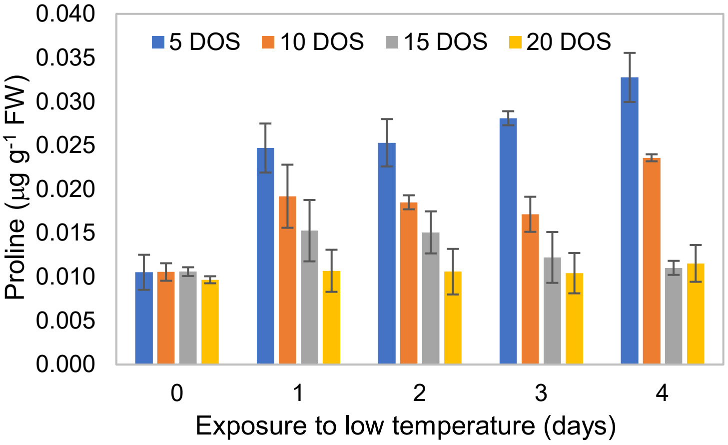

Figure 5. Effect low temperature on proline content of rice seedling.
Var indicates mean value ± standard error.

MDA Content

The MDA content dramatically increased in rice seedlings when they exposed to LT for longer duration (Fig. 6). However, in case 5 DOS, the proline content varied from 9.2 to 13.5 µ mol g^{-1} FW, while it ranged from 7.8 to 13.6 µ mol g^{-1} FW in case of 10 DOS under 1 to 4 days of LT. The amount of MDA increases in the rice seedlings when they were treated with longer duration of LT. This result indicated that the LT stress induced reactive oxygen species accumulation and caused higher amount of MDA in rice plant. The results also supported by the findings of Zhao et al. (2020).

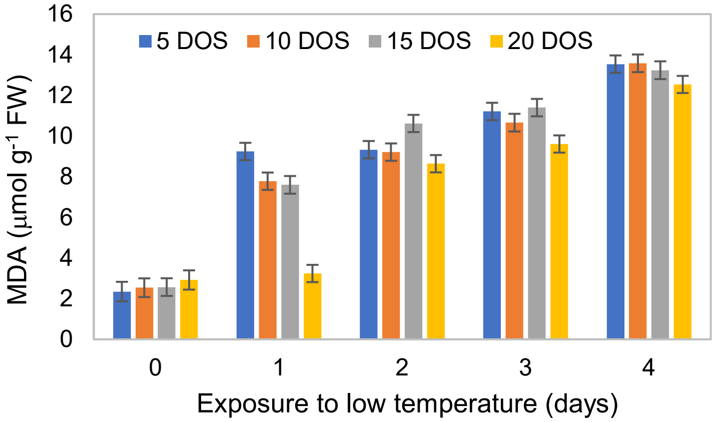

Figure 6. Effect low temperature on MDA content of rice seedling,
Var indicates mean value ± standard error.

CONCLUSIONS

The present study indicated that low temperature (LT) was injurious to younger rice seedling when they were exposed to LT for 1 to 2 days. The shoot and root length as well as their dry weight reduced, chlorophyll and carotenoid content of younger rice seedling within 2 days of LT exposure. On the contrary, the proline and MDA content of rice seedlings increased to reduce the harmful effect of under LT. Based on this study, it might be concluded that the rice seedlings could tolerate the detrimental effect of LT when they attain at least 15 days.

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