



## COLD TOLERANCE MECHANISM OF RICE CULTIVARS BASED ON PHYSIO-MORPHOLOGICAL CHARACTERISTICS

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### ABSTRACT

Plant growth and climate change are interrelated processes, both of which take place on a global scale. A field experiment was carried out to investigate the physio-morphological characteristics of seedlings of nine rice cultivars for screening based on cold tolerance during low temperature in Northwest of Bangladesh. The test rice cultivars were V1, Bashful; V2, Poshushail; V3, Gochi; V4, Taipee; V5, Bogra; V6, Lafaya; V7, Banglamoti (BRRI dhan50); V8, Jotapari and V9, BRRI dhan28, respectively. The leaf proline content, chlorophyll content, relative water content (RWC), seedling shoot and root length, total biomass, seed germination and seedling mortality rate were investigated. The V2 (Poshusail) and V9 (BRRI dhan28) seedling synthesized the higher leaf proline at low temperature than those of other tested cultivars. The highest chlorophyll-a and -b were found in V1 (Bashful, 1.75 and 1.42 mg g<sup>-1</sup> FW) which was statistically similar to V3 (Gochi). The highest percentage of RWC in rice leaf (95%) was found in V5 cultivars. The highest shoot length was 11.8 cm at 35 DAG found in V5 and the lowest shoot length was found in V8 cultivar. The V8 plant showed significantly greater root dry weight and root length while the lowest was in V6 plant. The V6 cultivars produced higher root but the V2 had the highest shoot weight, germination percentage and lower mortality rate than those of others, respectively. This study infers that V2 (Poshusail- Habiganj VI) and V9 (BRRI dhan28) varieties showed the better survival potentiality during cold temperature by over-synthesizing proline, chlorophyll and other physio-morphological parameters.

**Key words:** Chlorophyll, cold tolerance, rice seedling, proline, root weight, shoot weight

### INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food for more than half of the world population. The global rice production is 550 million tons and 2.15 million tons is in Asia (Rao *et al.* 2007). It is one of the most important cereal crops, the basic food of more than 3 billion people and it accounts for 50-80% of their daily calories intake. Besides its immense economical importance, rice has become a model system for genomics because of its relatively small genome size of 440 mega base pairs in the gramineae family, and because of its closeness to other major cereal crops (Gale and Devos 1998). World population is increasing at an alarming rate and is expected to reach about six billion by the end of year 2050 (FAO 2000). On the other hand, food productivity is decreasing due to the effect of various abiotic stresses; therefore minimizing these losses is a major area of concern for all nations to cope with the increasing food requirements. Cold, salinity and

drought are among the abiotic stresses, which adversely affect plants growth and productivity; hence it is important to develop stress tolerant crops. In general, low temperature mainly results in mechanical constraint, whereas salinity and drought exerts its malicious effect mainly by disrupting the ionic and osmotic equilibrium of the cell. It is now well known that the stress signal is first perceived at the membrane level by the receptors and then transduced in the cell to switch on the stress responsive genes for mediating stress tolerance. Understanding the mechanism of stress tolerance along with a plethora of genes involved in stress signaling network is important for crop improvement. Morace *et al.* (2009) showed that low temperature inhibit rice growth and yield in Texas. Christie *et al.* (1991) stated that cold temperature, a common environmental stress in the temperate zone, affects numerous biochemical, physiological and metabolic functions in rice plants.

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Abiotic stresses adversely affect growth, productivity and trigger a series of morphological, physiological, biochemical and molecular changes in plants. Cold stress is a major environmental factor that limits the agricultural productivity of plants in northeast and northwest areas. Plants respond and adapt to this stress to survive under stress conditions at the molecular and cellular levels as well as at the physiological and biochemical levels. However, expression of a variety of genes is induced by different stresses in diverse plants (Sanghera *et al.* 2011). In many crop plants, it is known that seed germination and early seedling growth are the stages most sensitive to environmental stresses (Cook 1979). In the cultivated tomato, chilling temperatures within the range of 0 to 12°C result in a significant delay in the onset of germination, reduction of the germination rate and considerable variance of seedling growth events (Jones 1986; Foolad and Lin 1998). Chinnusamy *et al.* (2007) stated that cold stress adversely affects plant growth and development and significantly constraints the spatial distribution of plants and agricultural productivity.

The reduction in seedling growth of rice due to low temperature is one of the major problems in tropical and subtropical areas at high altitude as well as in areas where cold temperature is common in short winter. The delay in seedling emergence due to cold temperature greatly increases seedling mortality and causes serious decreases in yield and increases competition with weed. In many Asian countries, the direct seedling culture has become increasingly important in rice growing areas. Chilling stress is common during rice seedling period and frequently it is appearing repeatedly in Northwest Bangladesh. Designing new strategies to improve cold tolerance in crop is essential in Northwest Bangladesh where winter is comparatively longer. Thus, the objectives of this research were - i) to investigate the physiological and morphological characteristics of nine rice cultivars at seedlings stage cultured in low temperature, and ii) to screen rice cultivars of rapid seedling growth during cold environment for the Northwest Bangladesh.

## MATERIALS AND METHODS

A field experiment was conducted at the Agricultural Chemistry farm, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur during the period from November 2010 to January 2011. Seeds of nine rice varieties were used in the study. Seven varieties were local and remaining two were HYV varieties. The varieties were V1, Bashful; V2, Pashushail (Habigonj-VI), V3, Gochi; V4, Taipi; V5, Bogra; V6, Lafaya; V7, BRRRI dhan50 (Bashmoti);

V8, Jotapari and V9, BRRRI dhan28. The experiment was laid out in Randomized Complete Block Design (RCBD). The total number of treatment was 9 in the present experiment and each treatment was replicated three times. The seeds of selected 9 varieties were sown in seedbed made by hand plough on 10 November 2010. Weeding was done at 20 and 27 days after sowing (DAS).

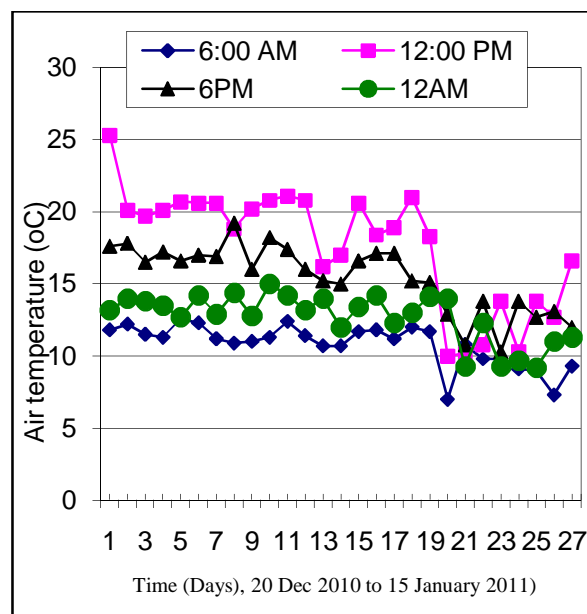


Figure 1. Daily temperature at different times recorded during experiment period.

**Determination of chlorophyll pigments in rice leaves:** Chlorophyll pigments in rice leaves were estimated following by Arnon (1949) and Lichtenthaler and Wellburn (1983). About 0.1 g fresh leaf tissues were taken in 10 mL acetone. It was shaken overnight. The chlorophyll-a and chlorophyll-b were determined using the supernatant by measuring absorbance with a UV-visible spectrophotometer at 663 nm wave length and 645 nm wave length. Total carotenoid was measured at 470 nm wave length. Concentration of chlorophyll-a, chlorophyll-b and total carotenoid were determined by the following formulae-

$$\text{Chl-a} = 12.21 A_{663} - 2.81 A_{646}$$

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

$$\text{Total carotenoid} = (1000A_{470} - 2.05 \text{Ca} - 114.8\text{Cb})/245$$

( $\mu\text{g ml of plant extract}^{-1}$  or  $\text{mg/g fresh weight}$ )

**Determination of proline:** Fresh leaf samples from rice seedlings at different times were used for the determination of proline. Fresh leaf sample of 40-50 mg by weight was collected in an eppendorf tube and homogenized in 3% (w/v) salicylic acid for the

estimation. Proline content was estimated colorimetrically by the acid ninhydrin method following Bates *et al.* (1973).

**Dry weight of rice seedlings:** Four-week-old whole plants including shoots and roots from 9 different cultivars were collected and freshly weighed. These plants were dried in the room temperature for 72 hours, followed by drying in an oven at 65°C for 72 hours, and then weighed again after the complete drying. The percentage of dry weight was calculated from the following equation:

$$\% \text{Dry weight} = (\text{Dry weight} / \text{Fresh weight}) \times 100$$

**Relative water content (RWC) of leaf:** The RWC was measured on the youngest emerging leaf to ensure uniformity across all the plants. Leaves were harvested directly into 15 mL-ependorf tube and place on ice to prevent any further water loss and then weight to determine fresh weight (FW). The leaves were placed in a beaker containing pure water for 24 hours at room temperature to allow for rehydration. Following rehydration, the leaves were blotted and weighed for turgid weight (TW). The leaves were dried at 65°C for 72 hours and weighed for dry weight (DW).

Relative water content (RWC) of leaf was measured by the following formula:

$$\text{RWC} (\%) = [(W - DW) / (TW - DW)] \times 100$$

Where, W = sample fresh weight, TW=sample turgid weight and DW = sample dry weight.

**Determination of root, shoot and total dry matter of rice seedlings:** The root and shoot length of seedlings were ascertained by measuring meter scale placed from ground level to top of the leaves. For measuring root length, the whole seedling was collected from the soil by careful destruction method using a *Khurpi*. Then collected seedling roots were washed carefully using running tap water to remove soil and other debris. Similarly, shoot of seedling weight was taken from the same plants used for root weight. The amount of biomass production was recorded per seedling in each plot collected by destructive method containing roots, stems and leaves. The plants were washed clearly to remove dust and soil mass. Four-week-old whole plants including shoots and roots from 9 rice cultivars were collected and freshly weighed. These plants were dried in the room temperature for 72 hours, dried in an oven at 60°C for one week, and then weighed again after the complete dry.

**Statistical analysis:** The data for different parameters were compiled and tabulated in proper form. The obtained data on different parameters under the experiment were statistically analyzed to obtain the level of significance using MSTAT-C computer program developed by Russel (1986). For treatments being significant, the treatments means were compared by LSD followed by Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984).

## RESULTS AND DISCUSSION

**Leaf proline content:** The result showed that the rice seedlings tended to accumulate highest proline upto 1.51 mg g<sup>-1</sup> in V2 rice land races while lowest 0.51 mg g<sup>-1</sup> in V6 rice varieties at 20 DAG. At 35 DAG, significantly higher amount of proline was synthesized by V2 variety while the lowest amount was synthesized by V5 variety (Table 1). The second highest proline content was in V4 cultivar followed by V9, V1, V5, V8, V3, V7 and V6, respectively. At 25 DAG, proline content in leaves decreased in all varieties but V2 cultivar synthesized higher proline. Amongst nine cultivars, seedlings of V2 always synthesized highest proline content while the lowest in V5. On the other hand, the V6 and V5 varieties synthesized lower quantity of proline during the experimental period.

The result showed that there are two groups of cultivars on basis of their proline content. However, after 30 days, there was a slight increased proline content in all cultivars. Eventually, there was a slight increased in the proline content during 25 days to 35 days. Regarding the proline content in leaves of the local rice land races accession, the V5 and V6 plants contained a less amount of proline while V2, V4 and V1 contained higher amount proline than that of the HYV rice seedlings V7 and V9. During 20 DAG of seedling ages with air temperature 10°C, the proline content decreased. However, when the plants were suffered from low temperature for longer periods, the proline content increased again to reach about 1.46, 1.96, 1.05, 1.51, 0.34, 0.51, 0.74 0.81 and 0.83 mg g<sup>-1</sup> in V1, V2, V3, V4, V5, V6, V7, V8 and V9 at 35 DAG, respectively (Table 1). Comparing the proline content in the cultivated and local rice land races, the content in the local rice seedlings specially Haor cultivars V2 (Poshushail) and V1 ( Bashful) showed higher amount of proline content but the cultivars from Netrakona (V5 and V6 local land races) contained less amount of proline. BRRIdhan 28 contained third lowest quantity but was greater than that of the V5 and V6 rice seedlings. Upon chilling (prevailing low temperature 10-12°C) for 5 days, the

**Table 1.** Proline content in some selected land races of rice during seedling stage (mg g<sup>-1</sup>)

Varieties	20 DAG	25 DAG	30 DAG	35 DAG
V1	1.18b	0.93ab	0.84de	1.46b
V2	1.51a	1.07a	1.12abc	1.96a
V3	0.88c	0.54ef	0.82e	1.05c
V4	1.38a	0.87b	1.17ab	1.51b
V5	0.95c	0.16g	0.83e	0.34f
V6	0.51d	0.44f	0.86de	0.51ef
V7	0.64d	0.70cd	0.96cde	0.74de
V8	0.95c	0.62de	1.02bcd	0.81cd
V9	1.20b	0.83bc	1.23a	0.83cd
LSD	0.131	0.151	0.169	0.250
SE	0.032	0.037	0.041	0.006
CV%	3.36	9.49	7.33	10.43

**Table 2.** The Chlorophyll content (mg g<sup>-1</sup>) different rice seedlings at 30 DAG seedling stage

Cultivars	Chl-a	Chl-b	Total carotenoid	Total chlorophyll	Chl-a/b ratio
V1	1.75a	1.42a	0.42d	3.59a	1.23
V2	1.47b	1.4a	0.53ab	3.40a	1.05
V3	1.59ab	0.64c	0.49cd	2.72bc	2.48
V4	1.40b	0.53d	0.58bc	2.51c	2.64
V5	0.74d	0.62c	0.65a	2.01d	1.19
V6	0.85cd	0.44e	0.45cd	1.68e	1.93
V7	1.41b	0.84b	0.51bc	2.76b	1.68
V8	0.99c	0.65c	0.42d	2.06d	1.52
V9	0.91cd	0.54d	0.61a	2.06d	1.69
LSD	0.226	0.075	0.075	0.213	3.00
SE	0.055	0.018	0.018	0.052	3.00
CV%	7.57	4.70	3.49	3.55	11.22

content sharply changed in all cultivars but V2, V1 content sharply changed in all cultivars but V2, V1 and V4 cultivars contained a higher quantity of proline compared to the other local and cultivated races at same time. However, prolonged low temperature for 14 and 21 days generally increased the proline content and total amino acids in the cultivated wheat as well as local varieties during cold acclimation revealed by Kamata and Uemura (2004). The content of proline in rice seedlings was

differently altered with the increase in low temperature. There was only a slight increase in the content of proline at slow increase in air temperature in V2 compared to the HYV. Generally, rice seedlings tend to accumulate proline at above 10 and a downward trend beyond below 10 was observed. In addition, proline also plays a role as an osmoprotectant to adjust osmotic stability; it was expected that the accumulation of proline was associated with the percentage of water content.

However, no significant correlation among the physiological parameters and proline content was found. It seemed that cold stress provoked rice genotypes to different extents. The accumulation of proline in V2 variety was the highest in more sensitive variety. The result suggested that the increase in proline content might be associated with cold tolerance, but rather with the extent of damage encountered by chilling stress, as shown by the greater increase in the content of proline in the susceptible genotypes.

**Chlorophyll-a content:** Table 2 shows that the highest chlorophyll-a content was obtained in V1 (Bashful) local cultivars ( $1.75 \text{ mg g}^{-1}$ ) and the lowest was in V5 ( $0.74 \text{ mg g}^{-1}$ ) varieties. V2 (Poshushail) contained the second highest amount ( $1.47 \text{ mg g}^{-1}$ ) of chlorophyll-a and are statistically similar at 1% level of significance. The chlorophyll-a content in V3, V4, V6, V7, V8 and V9 were, 1.59, 1.40, 0.85, 1.41, 0.99 and  $0.91 \text{ mg g}^{-1}$ , respectively. The chlorophyll-a content in seedling leaves of different varieties was significantly different at 1% level of probability (Table 2).

**Chlorophyll-b content:** The highest chlorophyll-b content was obtained in V1 and V2 (Bashful and Poshushil) cultivars ( $1.42 \text{ mg g}^{-1}$  and  $1.40 \text{ mg g}^{-1}$ ) and are statistically similar at 1% level of significance and the lowest was in V6 and V9 ( $0.44 \text{ mg g}^{-1}$  and  $0.54 \text{ mg g}^{-1}$ ) treatment (Table 2). The V3, V4, V5, V7 and V8 had chlorophyll-b 0.64, 0.53, 0.62, 0.84 and  $0.65 \text{ mg g}^{-1}$ , respectively. The chlorophyll-b content was significantly influenced by different cultivars (Table 2), which seems to be attributed by genetically.

**Total carotenoid content:** Total carotenoid content was present in Table 2. The highest carotenoid content was  $0.65 \text{ mg g}^{-1}$  in V5 followed by V9 (Banglamoti) cultivars. The V1, V2, V3, V4, V6 and V8 had 0.42, 0.53, 0.49, 0.58, 0.45, 0.51 and  $0.42 \text{ mg g}^{-1}$  carotenoid, respectively. The carotenoid content was significantly varied in different cultivars. However, carotenoids have additional roles and partially help the plants to withstand adversaries of drought (Farooq *et al.* 2009).

**Chlorophyll a/b Ratio:** Varieties V1, V2, V7 and V3 performed better than other varieties at low temperatures for chlorophyll. The results of this study suggested that V1, V2, V7 and V3 had high chlorophyll content. These varieties should be made available to farmer for planting in these locations. Also, this study indicates that V2 (Poshushail) varieties with high chlorophyll-a contents will have high vitality and growth performance. Chlorophyll biosynthesis was significantly reduced in the seedling stages at 30 DAG.

The result showed that non-significant differences ( $P < 0.01$ ) in total chlorophyll contents observed among the nine varieties throughout the sampling periods. Chlorophyll is one of the major chloroplast components for photosynthesis, and relative chlorophyll content has a positive relationship with photosynthetic rate. The decrease in chlorophyll content under cold stress has been considered a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation. Both the chlorophyll a and b are prone to soil dehydration (Farooq *et al.* 2009). From a physiological perspective, leaf chlorophyll content is a parameter of significant interest in its own right. Studies by majority of chlorophyll loss in plants in response to water deficit occurs in the mesophyll cells with a lesser amount being lost from the bundle sheath cells. The present study indicated that several varieties produced greater amount chlorophyll-a which is significantly important for survival during cold temperature. Among the varieties studied, V2 produced the highest and might have cold tolerant potentiality in the study area.

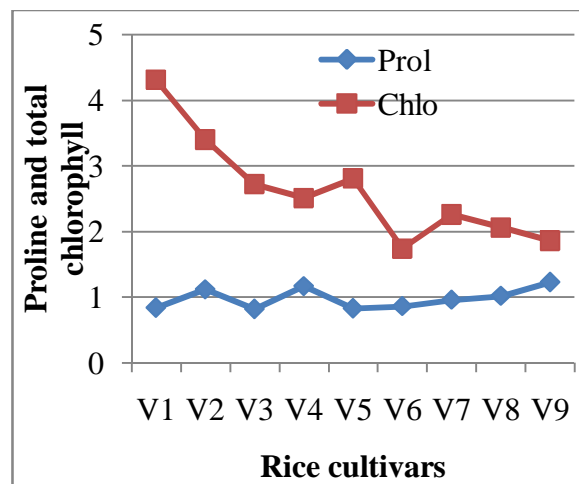


Figure 2. Relationship between proline and chlorophyll content of rice seedlings

Figure 2 showed that there is correlation amongst the cultivars. The V2, V4 and V7 showed higher chlorophyll content due to high amount of proline synthesized while V5 V6 and V8 produced lower quantity of chlorophyll due to low proline content. The amount of proline is increased with the increase of chlorophyll. Chlorophyll content was lower in variety V5 followed by V6 and V7 with the decrease of proline.

**Relative water content:** Leaf temperature and canopy temperature is important characteristics that influence plant water relations. Relative water content is considered as a measure of plant water

status, reflecting the metabolic activity in tissues and used as a most meaningful index for stress tolerance. In the experimental site, nine rice land races content relative water content in their leaves and roots at 30 DAG expressed in Figure 3.

Figure 3 showed that the V5 local cultivar had the highest percentage of relative water content (94.56%), which is similar to V6 and the lowest percentage of relative water (85.68% and 87.4%) content was found in the leaves of V9 and V8 at 30 DAG. V4, V3, V1, and V2 cultivars contained the lower amount of relative water content at 30 DAG in comparison to V5. The HYV BRRI dhan28 (V7) contained 88.57% relative water in their leaves which was in intermediate amount. RWC of leaves is higher in the initial stages of leaf development and declines as the dry matter accumulates and leaf matures. RWC related to water uptake by the roots as well as water loss by transpiration.

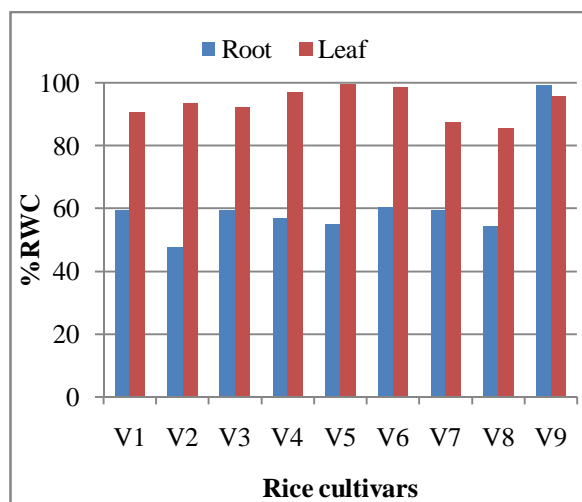


Figure 3. Relative water content (RWC) of leaf and root of rice seedlings

A decrease in the relative water content (RWC) in response to drought stress has been noted in wide variety of plants as reported by Nayyar and Gupta (2006) that when leaves are subjected to drought or in cold, leaves exhibit large reductions in RWC and water potential. Exposure of plants to drought stress substantially decreased the leaf water potential, relative water content and transpiration rate, with a concomitant increase in leaf temperature (Siddique *et al.* 2001).

In fact, although components of plant water relations are affected by reduced availability of water, stomatal opening and closing is more strongly affected. Moreover, change in leaf temperature may be an important factor in controlling leaf water status under cold temperature.

#### Relationship between RWC and proline content:

There was a negative correlation between proline content and relative water content in rice seedlings leaves but not statistically significant. Relative water content in rice leaves increased with the decreased of proline content and vice versa. Therefore, relative water content in plants is important factor for growth and development. It was also noted that the decrease in water content was accompanied by an increase in proline content in sensitive variety.

**Shoot Length and Root length:** The effect of low temperature on shoot length at different times was significantly variable. Shoot length of all the rice cultivars declined in general at all the different stress or abnormal environment condition. The reduction in seedling height of many crop plants grown under different stress environment is a common phenomenon (Javed and Khan 1995). The shoot length of different cultivars at different times of the experiment has been present in Figure 4.

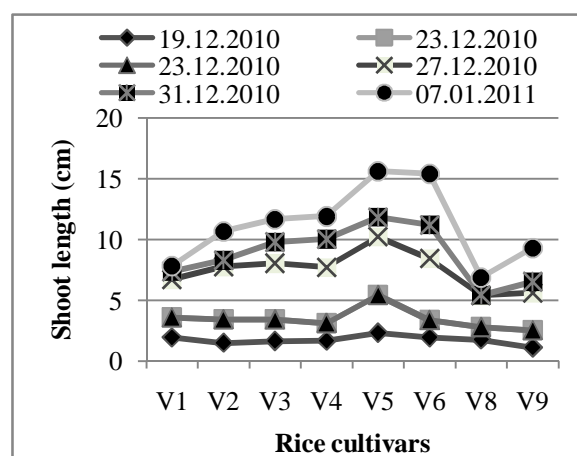


Figure 4. Shoot length of seedlings of some selected rice varieties

Figure 4 showed all the shoot length which was statistically similar at 15 DAG. The V5 and V6 showed the highest shoot length and V7 and V9 obtained the lowest shoot length at 35 DAG, V5 obtained the highest shoot length while V8 and V7 recorded the lowest shoot length during experiment time. Under low temperature, local cultivars V5 and V6 showed higher shoot growth. The differential response of shoot length might be due to the environmental factors and genotype.

The result showed that a significant variation on shoot length among the nine rice cultivars while low temperature or cold stress was prevailing at the experimental site. The analyzed data show that in terms of shoot height, rice seedlings responded differently to low temperature and levels. There was

a significant ( $P < 0.01$ ) increase in the shoot height at 20°C compared to the below 10°C (Figure 4). However, this marked increase in shoot length was not observed when the temperature remained below 15°C. The shoot growth decreased when low temperature tended to reduce below 12°C when the rice seedlings were stunted or growth stagnant occurred. However, there was a increased tendency in shoot height when temperature rose and reached above 16°C. Rice seedlings tended to increase their shoot height when they were exposed to high temperature (Figure 4). The present study revealed that V5 genotypes had the tallest and V8 had shortest shoot length at 35 DAG.

Root length of rice seedlings was reduced by increasing the low temperature as well as in very low temperature prevails in the soil. Any stress like cold, salinity or other environmental abnormality can hamper the growth of shoot and root of plants. The root length of different rice cultivars were recorded during experiment shown in the Figure 5.

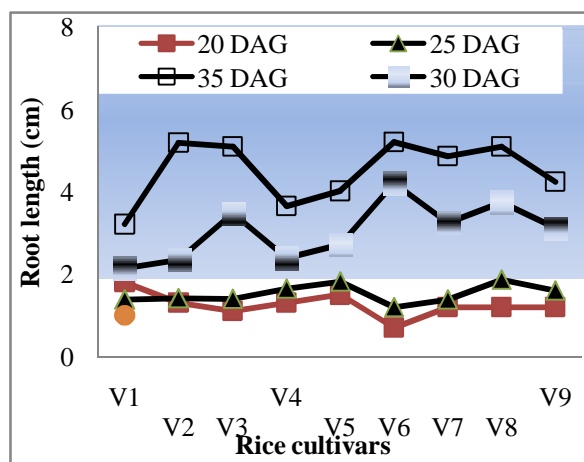


Figure 5. Root length of seedlings of selected rice varieties during low temperature

Figure 5 showed that, at 20 DAG, the highest root length was recorded in V1, V5 and followed by V2 and V4. At 25 DAG, highest root length was recorded in V8 and followed by V5, V4, V9, V2, respectively. The root length was more affected with increased cold levels compared to shoot and root were more prominent than shoot at each specific chilling stress level. At low temperature all the cultivars showed serious reduction in root length, however, V2 and V3 showed better performance. At 35 DAG, root growth decreased and/or completely arrested for all species. Soltani *et al.* (2002) observed that root length was diminished by increasing chilling concentration. The gradual decrease in root length with the increase in cold environment might probably be due to more inhibitory effect of chilling to root

growth compared to that of shoot growth (Rahman *et al.* 2001). Cold tolerance caused corresponding decrease in root length in all cultivars of rice and the longest roots were obtained from small seeds in each cultivar under all cold stress conditions (Kaya *et al.* 2008).

Similar result was observed and noted by Momayezi *et al.* (2009) that root length of all rice genotypes was reduced with increasing chilling concentration. Rice seedling growth was significantly hampered by cold stress while the air temperature remained below 15°C. These finding are consistent with those of Jamil *et al.* (2007) and Rodríguez *et al.* (1997) that maximum root length occurred at above 20°C, an opposite relation was observed between variations in root length and low temperature.

**Seed Germination and Mortality rate:** Table 3 shows that the highest seedling emergence was noted in V2 and this was unaffected or minimum affected by cold stress and followed by V6, V1, V8, V5 and V7, respectively. Table 3 showed that the lowest seedlings emergence was recorded in V4 cultivars during the experiment. The germination data were recorded in 10 DAG of seedlings aged.

**Table 3.** Seed germination and mortality rate of tested cultivars

Variety	Total seedling	Germination %	%Mortality 1	%Mortality 2
V1	568	81.97	19.03	12
V2	507	88.05	11.95	8
V3	330	79.36	20.64	10
V4	408	68.19	31.81	18
V5	385	76.88	23.12	19
V6	421	86.25	13.75	22
V7	329	75.87	2.13	28
V8	509	76.62	23.38	21
V9	346	75.35	22.65	11

Mortality 1= Seed Mortality; Mortality 2 = Seedling Mortality

Seed germination is an essential process in plant development to obtain optimal seedling numbers that results in higher crop yield. Germination and seedling growth declined with many abiotic factors such as chilling stress that are perhaps two of the most important grounded abiotic stress that limit number of seedling and seedling growth (Atak *et al.* 2006).

The result also showed that the highest mortality rate was recorded in V7 followed by V6 rice land race and followed by V5, V8, V9 and V1. The lowest mortality rate was recorded in V2 cultivar at the same time. The mortality rate was recorded while seedlings at 28 DAG. A significant reduction in seedling emergence was found due to low temperature. In case of pure seed, above 80 percent of seed should germinate in normal (25°C) temperature prevail in the environment. As the results from the low temperature were positively correlated ( $p < 0.01$ ) with the emergence of seedlings. The highest seedling emergence was noted in V2 and this was less affected by cold stress (25/07°C).

**Dry matter production and allocation:** Table 4 shows that total dry matter (biomass) of seedling in different rice cultivars at 35 DAG. Most interestingly, V2 plants showed the best performing cultivars amongst the tested varieties responding seedling mortality. Conversely, the cultivars V5 and V6 showed very poor at low temperature by producing only about 5-6% dry matter of their potential growth at normal temperature

**Table 4.** Shoot, root and total biomass of seedlings of different cultivars

Variety	Shoot dry wt. (g)	Root dry wt. (g)	Total Dry Matter (g)
V1	0.00444	0.04582	0.05026
V2	0.0083	0.046	0.054
V3	0.0068	0.072	0.078
V4	0.0062	0.058	0.064
V5	0.0062	0.0400	0.046
V6	0.0072	0.085	0.087
V7	0.005	0.0078	0.0083
V8	0.0044	0.0068	0.0112
V9	0.0043	0.0074	0.0117

Under low temperature stress, dry matter production at 30 DAG was the lowest in V7 plants. A similar trend to shoot and root weight at 30 DAG followed by V8 and V9 indicating that cold stress declines the seedling vigour. On the other hand total biomass was highest in variety V6. Unlike seedling emergence, seedling dry matter production was dramatically reduced due to low temperature in most cultivars.

The variation in dry matter production amongst the cultivar V6, V7, and V8 considered the better and the worst performer were V5 and V6. The main difference was in dry matter allocation between roots and shoot.

## CONCLUSION

In conclusion, the present study indicated that seedling growth and physiological activities of rice seedling was decreased due to low temperature during short winter in Bangladesh. Among the rice cultivars studied, poshushail and BRRI dhan28 showed better survival potentiality based on proline and chlorophyll biosynthesis, and some other morphological parameters. This study infers that further research is needed using physiological and molecular studies regarding cold survival mechanism.

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