

**COLD TOLERANT MECHANISM OF SOME SELECTED RICE
SEEDLINGS AT LOW TEMPERATURE ENVIRONMENT**

A THESIS
BY

Md. Abul Bashar
Student No. 1105101
Session: 2011-2012
Thesis Semester: July-December 2012



MASTER OF SCIENCE (M.S.)
IN
AGRICULTURAL CHEMISTRY



DEPARTMENT OF AGRICULTURAL CHEMISTRY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY
DINAJPUR

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Submitted to the Department of Agricultural Chemistry
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July-December 2012

*Dedicated
To My
Beloved Parents*



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ABSTRACT

A field experiment was carried out to investigate the physio-morphological characteristics of seedlings four rice cultivars for screening cold survival during low temperature in Northwest of Bangladesh. The test rice cultivars were V1, NERICA-10; V2, IR- 40 (GSR-IRRI- 40); V3, Lafaya and V4, Gochi, respectively. The leaf proline, chlorophyll content, relative water content (RWC), protein, starch, seedling shoot length, shoot weight and root length, root weight, total biomass, and seed germination rate were investigated. The V1 (NERICA-10) and V3 (Lafaya) seedling synthesized the higher leaf proline at low temperature than those of other tested cultivars. The highest chlorophyll-a and -b was in V1 (NERICA-10). The V3 cultivar (Lafaya) had the highest percentage of relative water content (92.93%). The V4 variety showed the highest content of protein percentage (13.35%). The highest percent starch content was found in V3 (29.82%). The V3 plants had the highest shoot weight at all times of experiment in 2012 irrespective of fresh and dried condition. This study infers that V1 (NERICA-10) and V3 (Lafaya) varieties showed the better survival potentiality during cold temperature by over-synthesizing proline, chlorophyll and other physio-morphological parameters.

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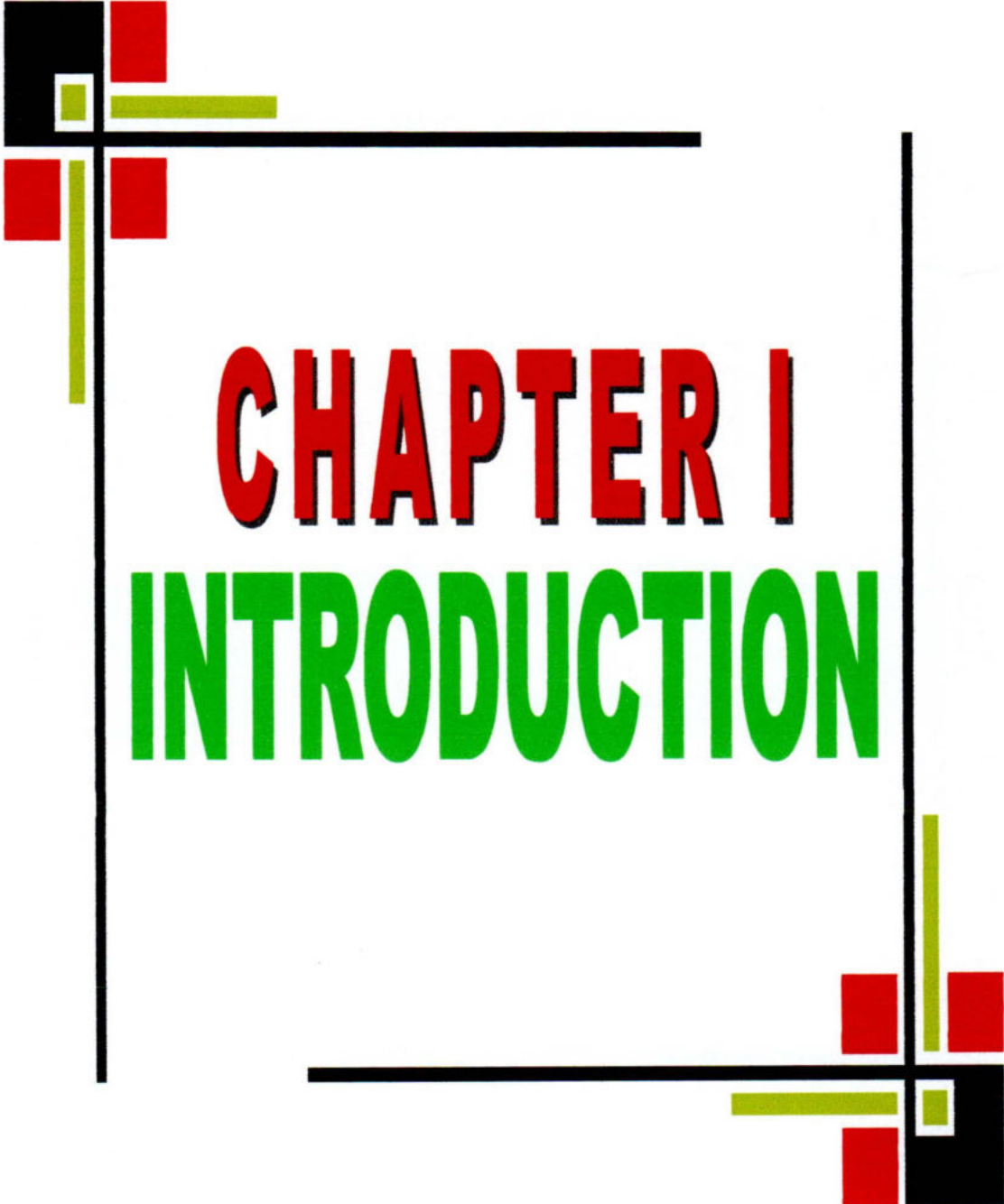
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CHAPTER I

INTRODUCTION

INTRODUCTION

Rice is one of the major food crops of the world. Worldwide rice is cultivated in 150 million hectares, which is more than 10% of the earth's arable land. It is also the staple food crops of nearly half of the total population of the world and is the main source of calories of almost 40% of the world population. It is the most important crop in Asia as a whole comprises to about 92% of the world rice harvest (IRRI, 1995). Bangladesh is an agro-based country where agriculture is the single largest sector and the mainstay of the country's economy. Bangladeshi agriculture is dominated by intensive rice cultivation. Among the crops grown in Bangladesh rice covering an area of 11.06 million hectares and producing 38.13 million tons of rice annually (FAO, 2002) and about 76% of the total population live in rural areas are directly or indirectly engaged in a wide range of agricultural activities (BBS, 2011). The agricultural sector plays a very important role in the economy of the country accounting for 23% of total GDP (BBS, 2010). Besides, rice production continues to be one of the important sources of livelihood accounting for 76% people's average calorie intake and 66% of protein intake (BBS, 2011). The major of this crop with regard to nourishment and livelihood implies that stagnant rice production would be devastating to food security and poverty alleviation. Rice provides 35% to 80% of the calories consumed by 3.3 billion people in Asia .So a sustainable increase in rice production will be reduced hunger and poverty and contribute to environmental conservation and better life for present and future generation.

Both biotic and abiotic stresses are generally understood as the reaction of a biological system to extreme environmental factors that, depending on their intensity and duration, may cause significant changes in the system (Nilsen and Orcutt, 1996; Godbold, 1998). Stressors or stress factors are defined as extreme

environmental conditions that induce functional changes in plants to such an extent that stress of the organism develops, resulting in inhibited growth, reduced bio-production, physiological acclimatization, adaptation of species or some combination of these changes (Grime, 1993; Nilsen and Orcutt, 1996). Abiotic stress in fact is the principal cause of crop failure worldwide, dipping average yields for most major crops by more than 50% (Eshghi et al., 2010). Plant growth and productivity is adversely affected by nature's wrath in the form of various abiotic and biotic stress factors. Plants are frequently exposed to a plethora of stress conditions such as low temperature, salt, drought, flooding, heat, oxidative stress and heavy metal toxicity. Various anthropogenic activities have accentuated the existing stress factors. Plants also face challenges from pathogens including bacteria, fungi, and viruses as well as from herbivores. All these stress factors are a menace for plants and prevent them from reaching their full genetic potential and limit the crop productivity worldwide.

Abiotic stresses cause losses worth hundreds of million dollars each year due to reduction in crop productivity and crop failure. These stresses cause water reduction and osmotic changes in the cellular milieu, suppress the activities of cellular molecules, and can result in reduced growth and extensive losses in agricultural production (Xiong et al., 2002). In response to these stress factors, various genes are unregulated, which can mitigate the effect of stress and lead to adjustment of the cellular milieu and plant tolerance. Among the abiotic stresses, temperature stress has tremendous impact on lowering the crop production. Each plant has its unique set of temperature requirements, which are optimum for its proper growth and development. A set of temperature conditions, which are optimum for one plant may be stressful for another plant. Low temperature constitutes one of the major hazards to agriculture and is an important factor that limits the survival, productivity and geographical distribution of plants in large area of the world (Boyer, 1982). Exposure to low

non-lethal temperature usually induct a variety of biochemical, physiological and enzymatic change in plant, which can result in an acclimation response that is characterized by a greater ability to resist injury or survive an otherwise lethal low temperature stress (Howarth and Ougham, 1993; Hughes et al., 1996).

From time immemorial, Bangladesh had been a cradle of rice culture. In Bengali, the local language, the words “rice” and “food” were often used synonymously. With at least 2.3 million people added each year to its population, the demand for rice was constantly rising. Population growth would be higher in regions characterized by pervasive poverty and malnutrition such as Bangladesh, where per capita grain consumption is expected to increase greatly. The challenge to the scientific community is how to help maintain a continuous increase in food supplies despite limited natural resources and declining arable land and water supplies in a manner that protected the soil, water, and biotic resource base from which all food must come. Meeting this daunting challenge requires continuing investment in agricultural research and delivery. The task is especially challenging: to spearhead a “green-green revolution” in rice, the staple food on which nearly two-and-half billion people already depended. Boro rice cultivation with increased yield now a possible one of the ways and means to fulfill the additional food requirement. But there is major problem of producing boro rice seedlings due to cold injury. About 25-30% of boro rice seedlings are damaged every year because of low temperature in mid December to mid January in greater Dinajpur and Rangpur Districts. Considering this case, cold tolerant rice variety for raising seedling in time and loss of seed due to damaging can meet up the problem. Farmers in this region may cultivate the Boro rice at proper time easily. Therefore a suitable research works is a burning issue for this region for screening suitable variety for cold acclimation.

Rice is a low temperature-sensitive crop especially during seedling, tillering, panicle development and flowering stages, which can easily be injured by cold

treatment. Most of the high-yielding and high quality varieties cannot be used in direct sowing because of low germination rate at low temperature. 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. 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 raising and frequently it is appearing repeatedly. Designing new strategies to improve cold tolerance in crop is essential in northwest Bangladesh where winter is comparatively longer.

Objectives

The research objectives are-

1. To investigate the physiological and morphological characteristics of four rice cultivars at seedlings stage cultured in low temperature.
2. To investigate the proline synthesis, chlorophyll content and water relation in four boro rice seedlings.
3. To screen the variety(ies) of fast seedling growth during cold temperature for the region to face upcoming rice production challenge.
4. To recommend suitable cold tolerant rice variety(ies) based of physiological and morphological parameters for boro season in the Northwest of Bangladesh for future food security.

CHAPTER II
REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Rice is chilling sensitive with an optimal temperature for growth and development between 20°C to 30°C. Specific stages of germination and early seedling growth are particularly sensitive to fluctuations in environmental temperature below the optimum range (Adair, 1968). Low temperature stress causes either dramatic reduction or failure of germination, depending on genotype. Furthermore, cellular injuries caused by low temperature stress often result in poor seedling vigor, which leads to negative effects on seedling survival, growth and development (Peterson *et al.*, 1978) and ultimately reduces the total rice production. However, plant responses to cold stress in terms of cellular and biochemical changes have been intensively investigated (Thomashow, 1999; Hasegawa *et al.*, 2000). Rice plants show a number of responses to the prevailing low temperature stress, which may be different in different species. A range of traits related to seedling growth and establishment under cold temperature have been characterized (Tiryaki and Andrews, 2001; Satya and Saha, 2010).

Nevertheless, these responses are at whole plant, organ, tissue, cellular and sub-cellular levels. They produce some special metabolites and bioactive compounds along with its structural and molecular level changes in response of the low temperature stress. Thus, response of rice plant to low temperature stress can be broadly grouped as the whole plant response, and physiological response.

2.1 Cold stress and Proline

Sarker *et al.* (2005) revealed that proline biosynthesis enhanced many times due to water stress in eggplant. Kulchhum (2011) stated that proline has multiple roles in stress tolerance in plants. It is difficult to claim whether the increase of proline content is eventually associated with differences in chilling tolerance of plants from the cultivated cultivars. The chilled plants from all cultivars accumulated similar amounts of proline in their leaves in response to low temperatures for 14 days or longer.

Catabolism of proline in stress free conditions generates reducing equivalents especially ATP to repair stress-induced damage (Hare and Cress 1997; Hare *et al.*, 1998). However, numerous researchers have found a positive relation between the accumulation of proline and low temperature stress tolerance in rice plant (Ito *et al.*, 2006; Huang *et al.*, 2009).

Hare *et al.* (1998) and Nanjo *et al.* (1999) described that many plants accumulate free proline in response to the imposition of environmental stresses such as drought, high salinity, and low temperature. Amino acids including proline are considered as key factors in metabolism and development of higher plants. Moreover, amino acids act as signaling molecules, controlling their own metabolism and the expression of a variety of genes in response to stress condition (Kiyosue *et al.*, 1996; Nielsen *et al.*, 1998).

2.2 Drought stress and Proline

Ashraf *et al.* (1994) reported that proline is an important osmolyte to adjust the plant under drought/saline conditions. There are, however, reasons to believe that proline accumulation may play a role in the salinity tolerance. Firstly, it is an osmolyte accumulated under stress in almost all the plant species. Secondly,

a high proline concentration has been described in organs which naturally have low water contents such as seed and inflorescence.

As stated by Anjum *et al.* (2011), proline accumulation is the first response of plants exposed to water-deficit stress in order to reduce injury to cells. Progressive drought stress induced a considerable accumulation of proline in water stressed maize plants.

The proline content increases as the drought stress progressed and reached a peak as recorded after 10 days stress, and then decreased under severe water stress as observed after 15 days of stress (Shakeel *et al.*, 2011).

Bates (1973) found that plants under water or salt stress, proline content increased more than other amino acids and this effect have been used as a biochemical marker to select varieties aiming to resist to such conditions. The increase in proline content is the most remarkable parameter in rice grown under salt stress conditions (Roy *et al.*, 1992).

Singh *et al.* (2000) observed that proline is a non-protein amino acid formed in most tissues subjected to water stress and together with soluble sugars is readily metabolized following recovery from drought.

Ashraf and Folad (2007) have demonstrated that proline accumulation increases the stress tolerance of plants. Drought stress increased the accumulation of proline significantly both in leaves and in grains but the increase was more marked at the soft dough stage.

Chu *et al.* (1978) reported that most chilling-sensitive plants, which also accumulated free proline during chilling stress, did not acquire improved cold tolerance. Free proline accumulation may have potential for alleviating chilling injury in chilling-sensitive plants. Free proline accumulation during stress plays a role in osmotic adjustment, stabilization of sub-cellular structures, membranes

and proteins of organelles, scavenging of free radicals, buffering the cellular redox potential (Wahid, 2007).

2.3 Cold stress and Protein

Many authors reported that the stress associated proteins play important role in plant response to environmental stress condition (Singla *et al.*, 1998; Vij and Tyagi, 2006). Amudha and Balasubramani (2011) reported that osmoprotectants are proteins that probably function in stress tolerance. They are water channel proteins involved in movement of water through membranes, the enzymes required for the biosynthesis of various osmoprotectants like sugars, proline, and glycine-betaine.

Cheng *et al.* (1993) described that changes in protein expression, accumulation, and synthesis have been observed in many plant species as a result of plant exposure to drought stress during growth. Both quantitative and qualitative changes to proteins were detected during drought stress.

Close *et al.* (1989) and Close (1996) indicated that proteins synthesized in response to drought stress are called dehydrins. Dehydrin proteins are also produced in response to various other environmental stresses, such as salt and cold stress, and have been characterized as hydrophilic, heat-stable, free of cysteine and tryptophan, responsive to ABA, and rich in lysine.

Dehydrin proteins accumulate along with other LEA proteins in response to a particular stress and have been proposed to play an important role in membrane protein stability and osmotic adjustment.

Lorimer (2001) and Kosakivska (2008) stated that the up regulation of stress proteins, which occurs against a background of depressive changes in polypeptide formation, relative to normal environmental conditions, is one of the main components of the adaptive response.

According to Dose (1980), proteins are compounds of fundamental importance for all functions in the cell. It is well known that alteration of gene expression is always involved in preparing plants for an existence under stress. Protein variation is an essential part of plant response to environmental stress as well as for adaptation to environmental conditions (Vierstra, 1993; Hieng *et al.*, 2004).

Close (1996) reported that one way of plants to tolerate abiotic stresses to some degree is by biosynthesis of so called stress proteins. Among them are dehydrins that accumulate in plants in response to ABA, low temperature and any other environmental influence that has a dehydration component, such as drought, salinity or extracellular freezing.

2.4 Cold stress and starch

In plants, starch serves as an important carbohydrate storage molecule. The breakdown of starch ensures retaining essential life processes in plants during periods of non-photosynthetic activity, especially in darkness or during seed germination. Soluble carbohydrates play a pivotal role in plant survival, the development from germination to leaf greening and the development of leaves, as well as in signaling and membrane stabilization in response to abiotic stresses.

(Kang *et al.*, 2002). Kang *et al.* (2002) also described the inhibition of cotyledon greening and true leaf development with sucrose levels greater than 4% concentration on growth medium. However, the accumulation of soluble carbohydrates in plants has been widely reported as a response to salinity or drought or extreme temperature, despite a significant decrease in net CO₂ assimilation rate (Murakeozy *et al.*, 2003).

Many connections have been made between sucrose and cold acclimation and osmotic stress. Tabaei-Aghdaei *et al.* (2003) determined that the mRNA

expressed in cells of plants supplied with sucrose resembled that of cold treated cells. Not only does sucrose induce cold acclimation responses in plants, but freezing tolerance of plants is also dependent on sucrose concentration. Freeze tolerance has been shown to increase with increasing sucrose concentration (Tabaei-Aghdaei *et al.*, 2003). The mechanism by which sucrose contributes to freezing tolerance is only partially understood; however, Uemura and Steponkus (2003) described *Arabidopsis* seedlings with increased freeze tolerance when exogenously supplied with different sucrose concentrations. Low concentrations of sucrose decrease expansion-induced lysis while higher concentrations reduced the loss of osmotic responsiveness to the cold.

In some herbaceous plants, including wheat (Perras and Sarhan, 1984), cloudberry (Kaurin *et al.*, 1981), and spinach (Guy *et al.*, 1992), changes in sugar content are correlated with those of freezing tolerance though the physiological role of sugar in the mechanism of freezing tolerance in plants is, therefore, still unclear.

Tinus *et al.* (1999) described that the relationship between cold hardiness and starch and sugar concentration of roots and needles may be explained by considering that the amount of starch and sugar present represents a balance between the rate of photosynthesis, consumption by respiration, and export to parts of the seedling that are growing. Because starch and sugar are readily interconvertible, the proportion that is present in plant tissues as sugar during acclimation and deacclimation is probably a function of the need for cryoprotection.

As stated by Levitt (1980), sugars are known to lower the freezing point and increase the intracellular osmotic potential. This would reduce the amount of dehydration during extracellular freezing. Premature defoliation of raspberry canes decreases both starch and sugar reserves and increases the susceptibility

of bud tissues to freeze injury (Doughty *et al.*, 1972). However, the qualitative changes in carbohydrates of raspberry canes and buds during cold acclimation have not been studied.

Quick *et al.* (1989) observed substantially reduced starch synthesis in osmotically stressed spinach leaves. Starch is the major carbohydrate store in plants and is important for energy and carbon skeleton supply (Ruggiero *et al.*, 2004). In addition to the frequent association of metabolite levels with the expression levels of genes involved in the synthesis of these metabolites, a strong correlation between different metabolic pathways, e.g. starch mobilization with raffinose and proline synthesis, was observed, suggesting a tight regulatory link between these pathways in response to salt stress (Kempa *et al.*, 2008).

2.5 Cold stress and chlorophyll

Farooq *et al.* (2009) revealed that chlorophyll is one of the major chloroplast component for photosynthesis, and relative chlorophyll content has a positive relationship with photosynthetic rate. The decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation. Photosynthetic pigments are important to plants mainly for harvesting light and production of reducing powers.

Kyparissis *et al.* (1995) and Zhang and Kirkham (1996) found that both the chlorophyll a and b are prone to soil dehydration. Decreased or unchanged chlorophyll level during drought stress has been reported in many species, depending on the duration and severity of drought.

van Huystee and Hodgins (1989) reported that illumination of etiolated seedlings of maize at low temperature resulted in reduced chlorophyll

accumulation, and impairment of chlorophyll biosynthesis was paralleled by an aberrant development of the thylakoid membranes (Hodgins and van Huystee, 1986). A slight photoconversion of Pchl_a to chlorophyll was possible at 12°C.

Glaszmann *et al.* (1990) reported that plants under environmental (peripheral) stresses lose their green chlorophyll tissues. It is known that environmental stresses in terms of chlorophyll degradation have similar effects on plants. Low temperature inhibits chlorophyll accumulations in actively growing leaves. In rice, cold tolerant lines, for example, japonica accumulates more chlorophyll under cold stress than do cold sensitive line, for example of indica rice.

Manirannan *et al.* (2007) detected a depression in Chl-a and Chl-b and total carotenoid in *Helianthus annuus* L. under water stress. But the chlorophyll content remained unchanged under drought as recorded by Jensen (1985) in onions.

The decrease of chlorophyll contents in rice leaves under saline conditions was reported by Iqbal *et al.* (2006). They observed some rice varieties where all genotypes, chlorophyll contents were decreasing. The decrease is significant in sensitive genotypes in comparison to tolerant.

Khosh and Ando (1995) indicated that in green plants, chlorophyll tissue in leaves under environmental stress in susceptible cultivar is decreased but with an increased resistance. Leaves in the susceptible cultivar have a darker green colour. Rapid loss of chlorophyll in cold-sensitive cultivars causes a decrease in photosynthetic activity. Several environmental factors causes chlorosis (yellowing) in plants. Chlorophyll is one of the basic pigments in plants, with its concentration reduction causing chlorosis, reduction in both growth and yield.

Akparobi (2009) found that the chlorophyll content is markedly decreased by exposure of rice to low temperatures. Chlorophyll synthesis is severely

depressed at low temperature in many chilling sensitive species. An apparent decrease in leaf chlorophyll content exposed to low temperatures has been suggested to be the results of photo-oxidation. The chlorophyll content is markedly decreased by exposure of rice to low temperatures.

2.6 Cold stress and root and shoot growth

Pote *et al.* (2006) revealed that supraoptimal temperature is a major factor limiting shoot and root growth of cool-season plant species. Growth of creeping bentgrass, a widely used cool-season turfgrass on golf courses, often declines during extended periods of supraoptimal temperatures. The optimal temperature for the growth of cool-season grass is broadly ranged from 18 to 24°C for shoot growth and 10 to 18°C for root growth.

Huang and Gao (2000) and Liu and Huang (2000) described that the production and viability of roots may play an important role in the adaptation of cool-season grasses to high soil temperatures.

Root number, length, and mass are commonly used to evaluate root growth. Root viability, expressed as the percentage of live root tissues, has been used to monitor root activity under heat stress. Many researchers have reported that these root parameters decreased with increasing temperatures for various grass species in controlled environment experiments (Liu and Huang, 2000) and field studies (Bonos and Murphy, 1999; Howard and Watschke, 1991; Kuroyanagi and Paulsen, 1988).

Kaya *et al.* (2008) observed that salt stress caused corresponding decrease in root length in all cultivars of chickpea and the longest roots were obtained from small seeds in each cultivar under all salt stress conditions.

Andaya and Mackill (2002) and Fujino *et al.* (2004) reported that fast shoot elongation growth is a favorable agronomic trait for seedling establishment and uniform crop stand.

Ranawake *et al.* (2008) found that cold tolerance at the post-germination stage in rice was studied using gained shoot length (GSL) as a new parameter, which was measured as increased shoot length during cold stress at 4°C followed by a 4-day recovery period at 35°C.

For rice plant (*oryza sativa* L.), temperature plays a significant role in vegetative growth as well as in reproduction (McDonald, 1971; Oldeman *et al.*, 1986; Yang, 1990; Yoshida, 1980). Chlorosis, reduced tillering, and reduced height occur in rice if extreme temperatures exist during the vegetative growth stage.

Korkmaz and Dufault (2001) indicated that cold stress decreased seedling shoot and root fresh and dry weights, leaf area, chlorophyll and carbohydrate contents but not seedling height. In 1998, all seedling growth variables decreased in response to longer durations of cold stress.

Plants cold stressed for up to 81 hours transpired more for one week after transplanting than those exposed to shorter periods of cold stress.

In a nutshell, almost every cellular process is altered during cold acclimation (Browse and Xin, 2001). Thus, a chilling sensitive maize variety is affected by reduced photosynthesis and decreased root growth while a chilling tolerant variety can maintain growth during low temperature periods as reported by Richner *et al.* (1996).

Brouwer (1983) concluded that most of the literature low-temperature effects on shoots and roots deals only with dry-matter accumulation. This may not reflect accurately the growth pattern, since a variable part of dry matter accumulation

corresponds to transient accumulation of non-structural carbohydrates (Brouwer, 1983; Stuiver *et al.*, 1995).

2.7 Cold stress and seed germination

Fujino *et al.* (2004) described that temperature between 20 to 35°C is optimum for seed germination and early seedling growth in rice, while temperature of 10°C was reported as the minimum critical temperature. Under such unfavourable low temperature conditions, fast shoot elongation growth is beneficial for uniform crop stand particularly in the direct seeded rice cultivation system in high altitudes in tropics and sub-tropics and also in temperate regions. Hence, developing rice cultivars with fast seedling growth under cold stress is an important target in these regions.

Yoshida (1980) reported that the minimum temperature for germination is 10°C and that the optimum temperature range is between 20 and 35°C. He also reported that low temperatures during the germination and seedling stages cause injury, which is exhibited by poor seed germination, delayed seedling emergence, stunting, and leaf discoloration.

Kabaki *et al.* (1982) and Takanashi *et al.* (1987) found that in rice plants growth rate and metabolism in rice are markedly inhibited even at temperatures above the chilling temperature in the range of 15-20°C but the mechanism for the effects of low temperature stress on growth and the accompanying metabolic changes remains unclear.

Bertin *et al.* (1996) suggested that a germination test at 10°C was the only valid test to evaluate chilling tolerance. The procedure involved germinating rice seedlings in flasks under 27/22°C day/night temperature with 12 hour day length for 7 days, transferring and growing the seedlings in pots under 82/72°C with 12 hour day length, then exposing the 2- leaf stage seedlings in a phytotron at 10°C

with 12 hour day length for 3, 6, or 9 days. Survival rates were then determined 10 days after the end of cold treatment and return to the greenhouse. This researcher also applied this procedure to 8-leaf stage seedlings to evaluate cold tolerance in the vegetative stage and found few significant correlations between results for these two stages, suggesting that different genes controlled cold tolerance in different stages.

Chen *et al.* (2006) concluded that most of the high-yielding and high quality varieties cannot be used in direct sowing because of low germination rate at low temperature (LTG). 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 mountain water is used for irrigation. In such areas, water temperature during sowing is below 15°C, whereas the optimum range for germination and early seedling growth of rice is 25 to 35°C. The delay in seedling emergence due to cold water 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.

Cruz and Milach (2004) reported that good performance during germination is important to guarantee fast establishment and uniform crop stand. In Asia, most rice cultivars belong to Indica subspecies, which present slow and not uniform germination under cold temperature, resulting in irregular emergence and low plant population (Souza, 1990). Since management practices cannot minimize the problem, genetic breeding for cold tolerance at the germination stage may contribute to a better crop establishment in early sowings.

2.8 Cold stress and biomass production

Akladios (2012) reported that the low temperature stress alone inhibited the growth of wheat seedlings as compared to control (22°C). Increase in growth

rate such as shoot length, root length, fresh and dry weights of seedlings after low temperature stress was higher in selenium-treated seedlings compared to the untreated ones (3 and 5°C). The lowest biomass production which occurred in wheat seedlings was observed at 3°C by soaking the seeds in selenium for 15 h before germination.

Nahar *et al.* 2007 reported that although, yield can possibly be reduced due to stress, they are not necessarily significant. Similarly, water stresses may show insignificant effect on height and dry matter production of plants.

Hue (1978) suggested that seedling growth is highly variable under different temperature regimes. At the vegetative stage, temperatures lower than 15°C reduce plant height, tillering, root growth and dry weight of the rice plant. Other studies suggest that Japonica rice varieties can tolerate low temperatures much better than Indica/Japonica varieties, with the latter requiring 2.5–3.0°C higher temperatures than do Japonica rice varieties for effective growth between germination and maturity.

Since the low temperature of flooded water greatly affects biomass and grain yield, several studies have been conducted to identify the underlying mechanisms (Satake, 1976; Shimono *et al.*, 2002). However, it is still unclear whether these mechanisms were caused by the integrated effects of low temperature on the root zone and shoot meristem near the soil surface or the effect on the root system (root temperature) alone, because in many studies flooded water was cooled in the paddy field accompanied with a decrease in air temperature. Leaf growth, which is important for dry matter production, is strongly affected by environmental factors such as temperature (Sato, 1972; Shimono *et al.*, 2002). The reduction of dry matter production by the low temperature of flooded water might be a direct effect of low temperature on the shoot growth.

Yadegari *et al.* (2007) reported that leaf fresh weights of cold-acclimated and non-acclimated soybean seedlings were reduced in chilling period. Leaf and root fresh weight of non-acclimated plants rapidly declined when exposed to chilling temperature and did not recover from the chilling treatment properly. Leaf and root fresh weight of cold-acclimated plants also declined significantly 24 h after exposure to chilling temperature.

Although the cold-acclimated plants did not completely recover from chilling injury compared with untreated control plants, the cold-acclimated plants showed a general increase in leaf and root weight compared with non-acclimated plants during the recovery period.

Mishra and Salokhi (2008) revealed that the SRI method revealed better nodal root development than the conventional method at the initial growth stage when soil nutrients were not limiting. The trend was similar for shoot length and dry matter production, indicating that drained soil favours shoot and root development. In the initial stage (12 DAS), seeding rates had no effect either on root or shoot growth under either soil conditions. Thus, a drained soil is one of the most important factors in SRI seedbed management to promote better root and shoot growth.

Bodapati (2005) stated that unlike seedling emergence, seedling dry matter production was dramatically reduced due to low temperature in most cultivars. Most interestingly, HSC-55 was one of the best performing cultivars, producing 18.5 % of dry matter at low temperature (18/13°C) compared to its potential at normal temperature (28/23°C). On the other hand, several of the tropical cultivars (eg., IR-36) performed very poorly at low temperature by producing only about 1% of their potential growth at normal temperature. The variation in cold tolerance (dry matter production) between the best performer, HSC-55 and the worst performer, IR 36 was 15.7 fold.

Tsukahara and Kozlowski (1986) concluded that growth was reduced much more by flooding than by low temperature. Interactive effects of flooding and temperature were shown on height growth, leaf initiation and expansion, and dry weight increment of leaves, stems and roots. The amount of growth reduction by flooding and low temperature was greater when based on analysis of dry weight increment of leaves, stems, and roots, than on their relative growth rates. The greater reduction of growth by flooding than by low temperature was associated with fewer and smaller leaves, more leaf injury, more stomatal closure, and greater decay of roots in flooded plants.

Farooq *et al.* (2009) reported that greater plant fresh and dry weights under water limited conditions are desirable characters. A common adverse effect of water stress on crop plants is the reduction in fresh and dry biomass production. Plant productivity under drought stress is strongly related to the processes of dry matter partitioning and temporal biomass distribution (Kage *et al.*, 2004). Diminished biomass due to water stress was observed in almost all genotypes of sunflower (Tahir and Mehid, 2001). However, some genotypes showed better stress tolerance than the others. Mild water stress affected the shoot dry weight, while shoot dry weight was greater than root dry weight loss under severe stress in sugar beet genotypes (Mohammadian *et al.*, 2005). A moderate stress tolerance in terms of shoot dry mass plants was noticed in rice (Lafitte *et al.*, 2007).

2.9 Cold stress and relative water content of leaf

Nayyar and Gupta (2006) reported that a decrease in the RWC in response to drought stress has been noted in wide variety of plants as when leaves are subjected to drought, 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.

Singh *et al.* (2000) observed significant differences in water potential among wheat genotypes under drought stress. Sinclair and Ludlow (1985) proposed that leaf RWC was a better indicator of water status than was water potential. Canopy temperature is also related to water stress. Singh *et al.* (2000) reported that the canopy temperature provided a good indication of the plant water potential of wheat when comparing environments with varying degrees of water stress.

Grossnickle (1992) stated that shoot water relations parameters are often measured to characterize the resistance of a species to drought. Some physiological factors that are related to drought resistance are also related to freezing temperature resistance, including tissue relative water content, membrane permeability and adjustment of cell solute concentration. Several studies on woody plants have implied that changes in freezing tolerance and shoot water relations parameters are related. Changes in both osmotic potential and cellular relative water content parallel changes in freezing tolerance (Maruyama 1988, Valentini *et al.* 1990).

Pattanagul and Madore (1999) reported that although the stressed plants showed wilting at the time of sampling, the relative water content of the stressed plants had not yet reached the lethal level, because the plants quickly recovered to full turgor after re-watering (data not shown).

Relative water contents of both control and stressed plants were unchanged throughout the whole daytime period from 6 am to 6 pm.

Suriya-arunroj *et al.* (2004) conducted a study to determine the association between RWC and salinity stress tolerance in rice and to investigate the

possibility of using RWC as screening criteria for breeders to incorporate tolerance to stress. The salt tolerant cultivars, could therefore, be selected based on their ability to maintain high relative water content during the initial phase of stress.

2.10 Justification of the Present Research

It has been pointed out that cold temperature, a common environmental stress in the temperate regions, affects several biochemical, physiological and metabolic functions in plants (Levitt, 1980; Wang, 1982; Murata and Yamada, 1984; Roughan, 1985; Guy, 1990).

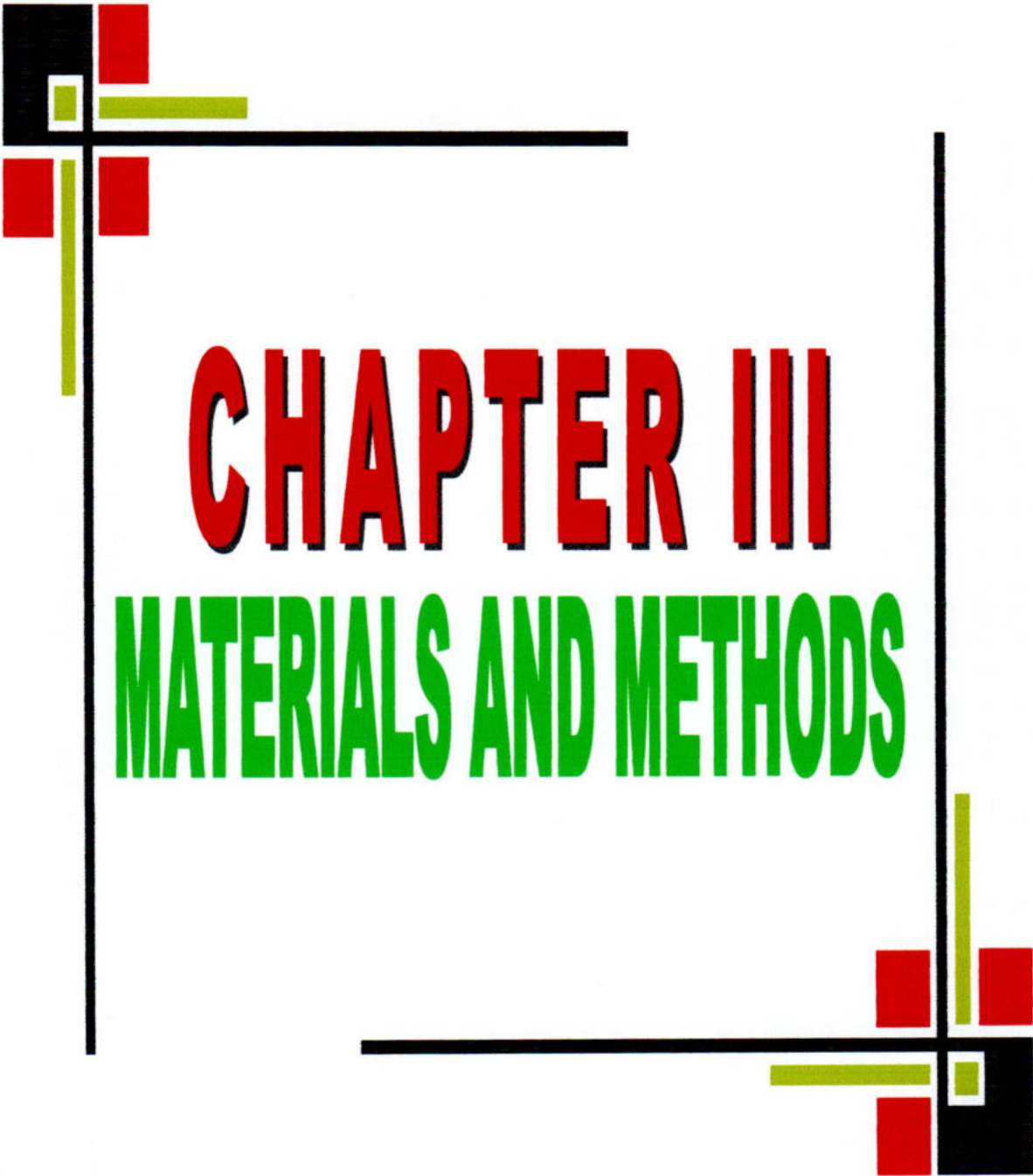
Graham and Patterson (1982) observed that many plants of tropical origin cannot tolerate cold and can be damaged even at moderate temperatures that are below 20°C.

Chinnusamy *et al.* (2007) stated that cold stress adversely affect plant growth and development and significantly constraints the spatial distribution of plants and agricultural productivity.

Akladios (2012) suggested that cultivated rice (*Oryza sativa* L.) is sensitive to temperatures below 15 to 20°C. Under low temperature conditions, rice plants respond in several ways and their sensitivity to cold can be manifested at all stages of development. Given the wide-ranging effects of low temperature on rice and the serious impact on productivity, tolerance to this stress is of considerable importance to temperate rice growing regions of the world such as the USA.

Efforts to improve cold tolerance typically focus on the early and the reproductive stages of development, which have the greatest impact on production.

Work on cold tolerance mechanism based on physiological functions is limited in Bangladesh; while those studied in other countries although provide useful information, but that cannot be recommended or practiced without trial in our local condition. Therefore, it is essential to undertake research on cold tolerant mechanism for *boro* rice for saving seeds as well as for higher yield.



CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

A research work was conducted at the research field of the Department of Agricultural Chemistry, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh during the period of November, 2011 to January, 2012 where low environmental temperature stress during seed germination and seedling growth stage were studied using boro rice in order to screen out relatively low temperature stress tolerant boro rice cultivar(s) for facing upcoming rice production challenge.

3.1 Experimental site and soil

The research field of the Department of Agricultural Chemistry, HSTU, Dinajpur located between 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) categorized as Himalayan Piedmont Plain as classified by UNDP and FAO (1988). The experimental field was a medium high land having sandy loam soil with soil pH 6.0. As per initial soil test report, the experimental site soil contained 0.07% total nitrogen, 1.17% organic matter, 13.77 ppm available phosphorus, 0.225 meq/100g available potassium, 38.59 ppm available sulphur and 0.56 ppm boron.

3.2 Climate and weather

The research work was conducted during boro season (November, 2011 to March, 2012) which is also known as dry season or Rabi season. The experimental area possesses subtropical climatic condition. The air temperature was relatively low at the beginning of the season and was increasing with advancement of the season toward Kharif with occasional gusty winds.

No rainfall was detected during experimental period. The meteorological information during the experimental period related to air temperature at a regular interval are represented in Appendix 1.

3.3 Planting materials

This research work was conducted using four selected rice cultivars taken from both local and high yielding varieties (HYV). Seeds of these selected rice cultivars were collected from different part of the country before initiation of research work. The name of the chosen varieties along with their production nature is represented in Table 1.

Table 1. Variety number, Name and Nature of selected rice cultivars

Variety number	Name of rice cultivar	Nature of rice cultivar
V1	NERICA-10	HYV
V2	IR- 40 (GSR-IRRI- 40)	HYV
V3	Lafaya	Local
V4	Gochi	Local

3.4 Treatment array and experimental design

The experiment was implemented for studying the effect of low temperature on different rice cultivars and to screen out relatively low temperature tolerate cultivar(s) from them. The experiment was designed following Randomized Complete Block Design (RCBD) technique with three replication.

The whole area was divided into three blocks and each block was divided into 4 (four) units maintaining plot dimension to 2 m ×2 m. The plot to plot distance was 30 cm. The four selected rice cultivars were regarded as treatments in this study assigned with three replications (Figure 1).

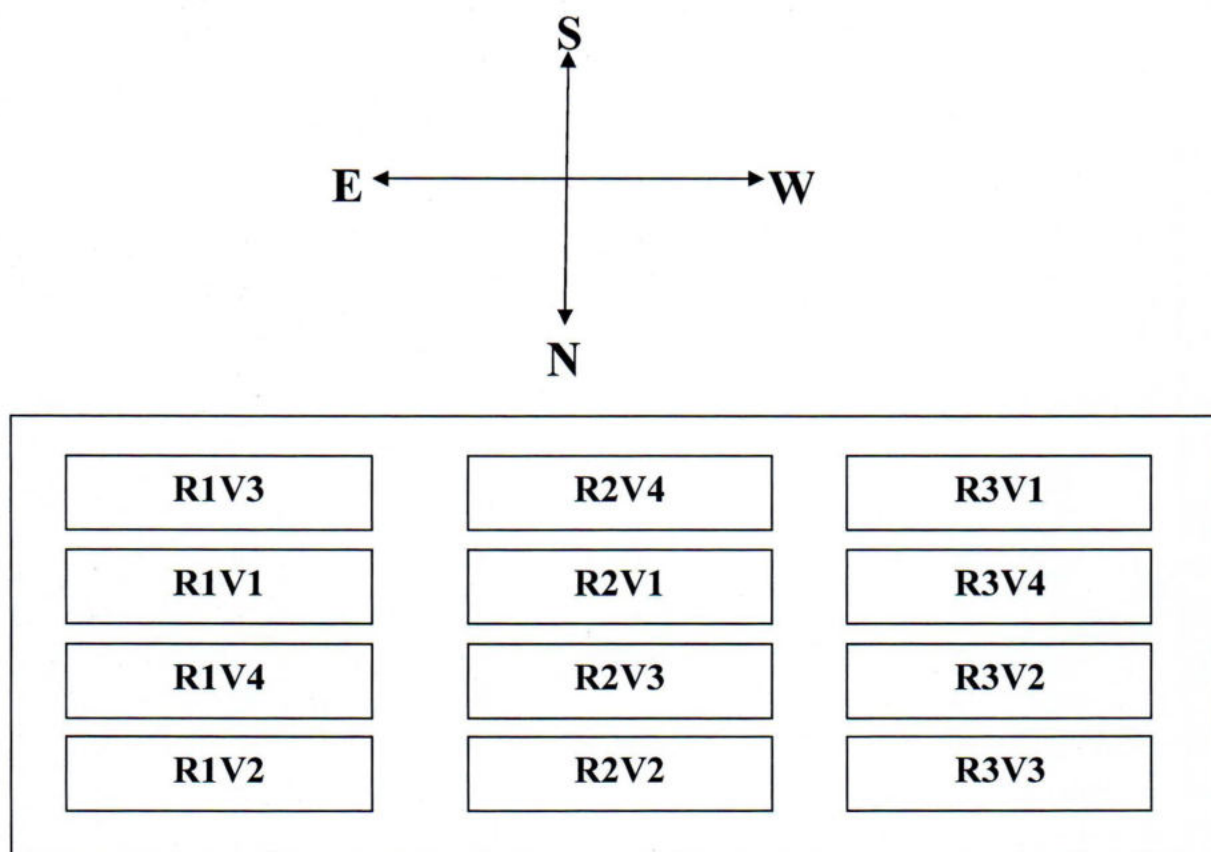


Figure 1. Layout of experimental design for growing rice seedling

3.5 Land preparation and fertilizer application

The experimental land was first ploughed with a tractor. The land was then ploughed and cross-ploughed three times with Power-tiller followed by laddering to obtain the good tilth.

The corners of the experimental field were spaded well and larger clods were broken into smaller pieces. All the stubbles and weeds like motha (*Cyperus rotundus*), chapra (*Eleusine indica*), bermuda grass (*Cynodon dactylon*) etc. were removed from the field carefully. The land was leveled well with addition of water and made ready for the propose of preparing wet seedbed. The each experimental plot was fertilized with chemical fertilizers based on the initial soil test report.

Most of the urea fertilizer was applied as basal dose during the final land preparation. Rest of urea was top dressed after 20 days of seeding at the seedbed. Triple Super Phosphate (TSP) and Muriate of Potash (MoP) were applied by following recommended dose (SRDI, 2009) for rice seedlings as stated in Table 2.

Table 2. Recommended dose of fertilizers applied in research field

Fertilizers	Dose (kg ha ⁻¹)	Quantity plot ⁻¹
Urea	220	88 g
TSP	200	80 g
MoP	200	80 g

3.6 Seed sowing, weeding and irrigation

The seeds of selected four 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. To make the experimental plot weed free, weeding was done at 20 and 27 days after sowing (DAS).

3.7 Data collection

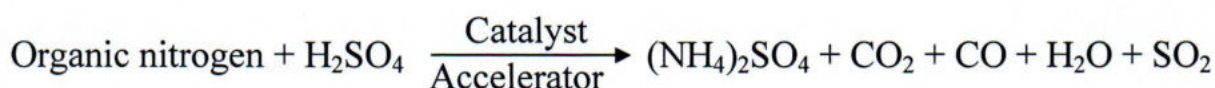
The data regarding various relevant parameters were collected accordingly during the experimental period. The parameters studied are-

- a. Morphological
- b. Physiological
- c. Biochemical

3.7.1 Determination of protein content of rice seedling

The determination of protein from rice seedlings was done by following Kjeldahl method (Total N₂ estimation method). The rice seedlings were collected carefully from the seedbed. Then the aerial parts of seedling were oven dried at 60°C for 72 hours. After that the dried samples were ground by blender. The total working procedure can be divided into three steps like below-

Digestion: The previously weighed sample material was digested with boiling concentrated H₂SO₄ to which catalyst such as “Digestion Mixture” (CuSO₄ : K₂SO₄ = 1:5) was added to raise the boiling point.



Distillation: In alkaline condition NH₃ was liberated from (NH₄)₂SO₄. Distillation was adapted to ensure the complete liberation of ammonia and trapping of the same in boric acid solution.



Collection of distillate and titration: The liberated ammonia was collected in 2% boric acid solution containing 2-3 drops of mixed indicator.

On completion, the distillate was titrated with standard HCl solution.



Working procedure:

One gram of previously prepared rice seedling sample was weighed accurately and was taken in the digestion flask (Kjeldahl flask). About 3-4 g of digestion mixture ($\text{CuSO}_4 : \text{K}_2\text{SO}_4 = 1:5$) was added to it. 20 mL of conc. H_2SO_4 was added into the flask. The flask was placed on digestion chamber and heated until the solution became clear. The flask was cooled slowly and 150 mL distilled water was added to it. Some glass beads and zinc granules were added to avoid bumping. 100 mL of 40% NaOH solution was poured into the flask slowly holding the flask about 45° angle and immediately the flask was connected to a distillation set. The liberated ammonia was collected in a conical flask which was previously placed under the condenser jet containing 25 mL 2% boric acid solution and 2-3 drops of mixed indicator (methyl red and methyl blue). At least 125 mL distillate was collected into the conical flask. Distillate was then titrated with 0.2 N HCl. The protein content of rice seedling was then calculated by following the below formulae.

$$\text{Percentage of nitrogen} = \frac{(T_S - T_B) \times \text{Normality of acid} \times (0.014) \times 100}{\text{Weight of sample (g)}}$$

Where,

T_S = Titre value of the sample

T_B = Titre value of the blank

0.014 = Milliequivalent of nitrogen

Percentage protein = % N $\times$ CF

Where,

CF = Conversion factor (5.5 for plant sample)

3.7.2 Determination of starch content of rice seedling

The starch content of rice seedling was determined by Fehling's solution method. In this procedure, the starch of sample was simplified to glucose and total glucose content was measured. The total glucose content was then used to calculate the amount of starch present accordingly. Reagent used in this test are as follows-

Fehling's solution 1: Fehling's solution 1 was prepared by dissolving 34.64 g of A.R. Grade crystalline copper sulphate in 500 mL water.

Fehling's solution 2: Fehling's solution 2 was made by dissolving 173.0 g of Rochelle salt (Sodium Potassium Tartarate) and 125 g of sodium hydroxide in water. It was then cooled and diluted to 500 mL in a volumetric flask.

Standard glucose solution: To prepare standard glucose solution, 10.0 g of pure glucose was dissolved in 1000 mL distilled water. When Fehling's solution was required, Fehling's solution 1 and Fehling's solution 2 were mixed thoroughly in a dry flask. As Fehling's solution deteriorates slowly on keeping, the solution mixture was prepared just when needed.

Working procedure:

At first, 250 mg of previously ground rice seedling sample was taken in a conical flask. About 50 mL of distilled water was added to it. The flask was then kept for 30 minutes to remove all impurities. It was then filtered with filter paper (Whatman No. 1) and crude was taken in another conical flask. 50 mL of 10% HCl was added to it and heated it for about 4 hours for breaking down starch to simple sugar. It was then cooled to room temperature and filtered with filter paper. Then the filtrate was taken in another dry conical flask and neutralized with 10% NaOH solution. Then the volume was adjusted to 100 ml by adding distilled water using volumetric flask.

It was then prepared for titration. Again, 5 mL of Fehling's solution was taken in a conical flask and diluted with 15-20 mL distilled water. It was then heated to boiling and standard glucose solution as added to it from the burette drop by drop until the color of the solution was about to disappear. The 2-3 drops of methylene blue indicator was added for determining appropriate ending point. Titration procedure was continued until the color of the methylene blue just disappeared. Same procedure was followed for the unknown glucose solution obtained from the sample.

Calculation:

For standard glucose solution,

$$\begin{aligned} 5 \text{ mL Fehling's solution} &= X \text{ mL standard glucose solution} \\ &= X \times 10 \text{ mg glucose} \end{aligned}$$

For unknown glucose solution,

$$5 \text{ mL Fehling solution} = Y \text{ mL unknown glucose solution}$$

$$\text{So, } Y \text{ ml unknown solution} = X \times 10 \text{ mg glucose}$$

$$\text{Or, 1 mL unknown glucose solution contains} = \frac{X \times 10 \text{ mg glucose}}{Y}$$

$$\text{So, 100 mL unknown glucose solution contains} = \frac{X \times 10 \times 100 \text{ mg glucose}}{Y}$$

$$= Z \text{ mg glucose}$$

$$= Z \times 0.9 \text{ mg starch}$$

3.7.3 Determination of 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 described by Bates *et al.* (1973). About 40-50 mg of fresh leaf sample by weight was collected in an eppendorf tube containing 0.5 ml 3% sulfosalicylic acid and homogenized well using eppendorf stick. 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 speed. 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 speed 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. The 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.

The optical density of the solutions (sample and standard solution) were measured at 520 nm wave length using UV-visible spectrophotometer. The amount of proline was determined from a standard curve.

3.7.4 Determination of chlorophyll content of rice seedling

The chlorophyll content of rice seedling was determined by following method described by Arnon (1949). Total chlorophyll was determined using the formula given by Porra (2002). Exactly 0.1 g fresh leaf tissues of rice seedling was taken in a test tube containing 10 mL of 80% acetone. It was then shaken overnight using a electric horizontal shaker. The optical density or absorbance of the supernatant was measured by using UV-visible spectrophotometer at different wavelengths. As 80% acetone was used in this study, the absorbance was measured at 663 nm, 645 nm and 470 nm wavelength for chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoid content. The concentration of chlorophyll-a (Chl-a), chlorophyll-b (Chl-b), total chlorophyll and total carotenoid was measured by using following formulae.

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

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

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

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

3.7.5 Determination of root and shoot fresh weight

The fresh weight of root and shoot of rice seedling was determined at regular time interval. The fresh weight of shoot was measured immediately after collecting the rice seedlings from experimental plots using digital balance. Weight of three randomly selected rice seedlings were taken and averaged.

Root fresh weight was also determined immediately after collecting followed by washing to remove adhering soil particles. Here also the roots of three rice seedlings were taken and averaged.

3.7.6 Determination of root and shoot dry weight

The sample collected for fresh weight determination was used for measuring the dry weight of shoot and root of rice seedlings. The shoot and root portions of samples were oven dried at 60°C for 72 hours separately followed by drying at room temperature for 72 hours. After complete drying, the dried root and shoot of rice seedlings were weighed and averaged carefully.

3.7.7 Determination of percent dry weight of root and shoot

The percent dry weight of root and shoot was calculated from the data obtained from root and shoot fresh and dry weight by using the following formula.

$$\text{Percent dry weight} = \frac{\text{Dry weight}}{\text{Fresh weight}} \times 100$$

3.7.8 Determination of relative water content of leaf

The relative water content (RWC) of leaf was determined at different times during experimental period to evaluate the susceptibility or tolerance of rice cultivars to low temperature stress. The fresh weight of leaves was taken immediately after collecting sample from the experimental field. Then the leaves were dipped in distilled water for about 4 hours to ensure full turgid or saturation condition. The leaves were blotted carefully and then the weight of the fully saturated leaves was taken. Then the leaf samples were dried in oven for 72 hours maintaining the temperature at 60°C. After that the dry weight (fully dehydrated) of the leaves were taken carefully. All weights were taken using highly sensitive digital balance (with accuracy level of 0.0001 g).

The RWC measurement was carried out by following formula (Barr *et al.*, 1962).

$$\text{RWC (\%)} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

Where,

RWC = Relative water content

FW = Fresh weight of leaves

DW = Dry weight of leaves

TW = Saturation or turgid weight of leaves.

3.7.9 Determination of root and shoot length

The root and shoot length of rice seedlings was measured by using scale having millimeter (mm) divisions for accurate determination. The shoot length was determined by placing measuring scale at the transitional zone of root and shoot toward top of the leaves. For measuring root length, the whole seedling was uprooted with the help of khupri keeping the root zone undisturbed. Then the collected root zone was washed carefully with running water so that all roots remained intake. Then the measurement of root length was made by placing the scale at transitional zone of root and shoot towards root apex.

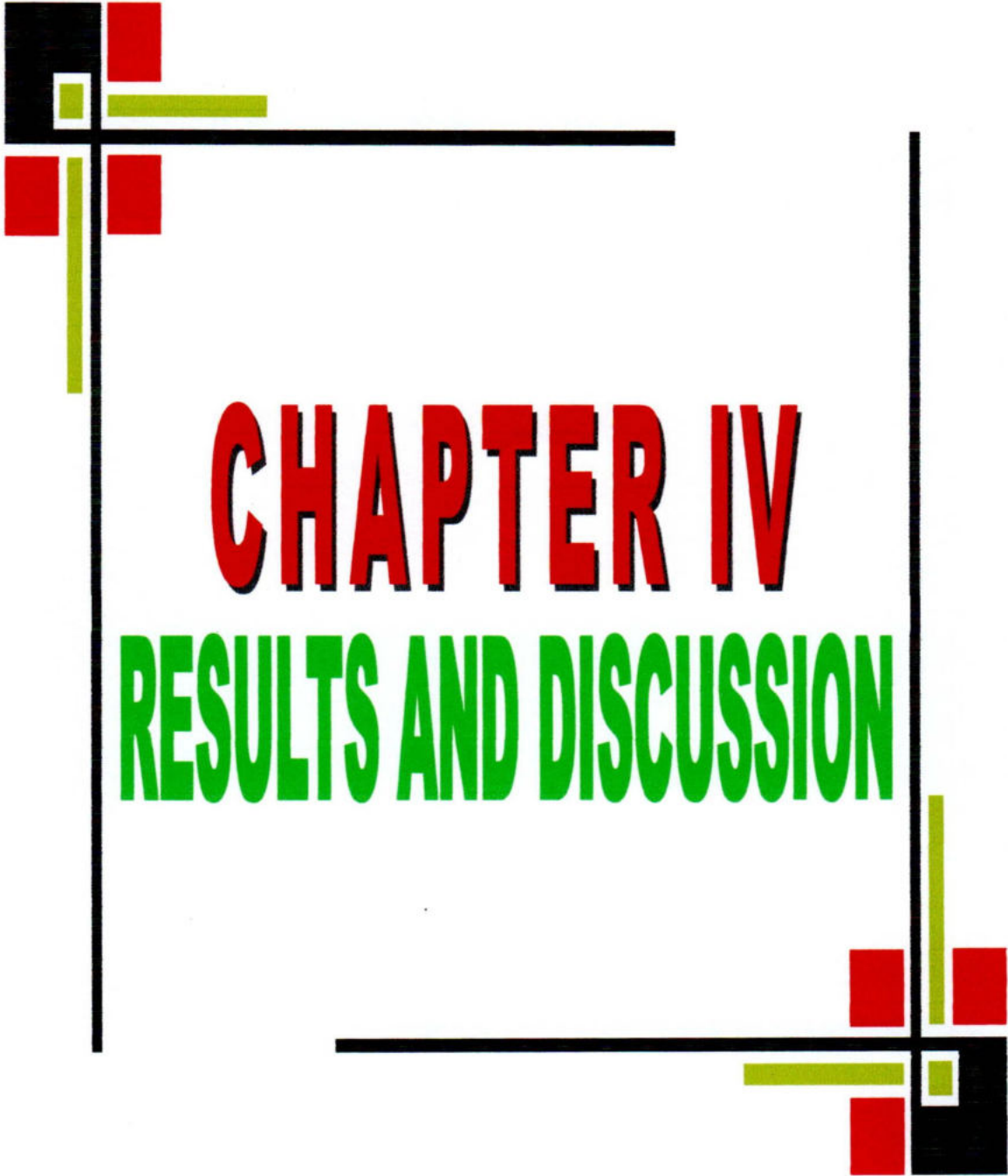
3.7.10 Determination of germination percentage

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

$$\text{Percent germination} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

3.8 Statistical analysis

The data for different parameters were compiled and tabulated accordingly. 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) if the ANOVA was significant.



CHAPTER IV

RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

Plants have a number of mechanisms to cope with stresses in their environment, which include such physical conditions as water (too much as well as drought), temperature (hot and cold), saline soils and oxygen deprivation, as well coping with biotic stresses such as predators and pathogens. As the exact defense mechanism of rice plants to low temperature stress is still unknown, various physiological and biochemical parameters were studied under this research work in order to find out best related responses. All parameters were studied in relation to temperature condition.

4.1 Proline

Over synthesis of proline in rice seedlings during low temperature environment was observed. The V1 (NERICA-10) plants showed the highest proline than those of other cultivars at 16 DAG. But the V2 plants synthesized higher proline than those of V1, V3 and V4 plants at 32 DAG. Proline is regarded to have multiple roles in stress tolerance in plants. Many plants accumulate free proline in response to the imposition of environmental stresses such as low temperature, drought and high salinity. Amino acids including proline are considered as key factors in metabolism and development of higher plants (Hare et al., 1998; Nanjo et al., 1999). Under environmental stress conditions such as salt, cold, and drought stresses, many plants accumulate compatible solutes such as proline and glycinebetaine. The synthesis of leaf proline in the different varieties of rice seedling during the experiment shown in the Table 3.

It is evident the rice seedlings tended to accumulate higher proline upto 1.539 mg g⁻¹ in V2 plants at 32 DAG while the lowest 0.51 mg g⁻¹ in V6 rice varieties at 20 DAG. At 32 DAG, markedly higher amount of proline was synthesized by V3 variety while the lowest amount was synthesized by V1 variety (Table 3). At 32 DAG, proline content in leaves decreased in all varieties but the V4 cultivar synthesized higher proline than those of other cultivars. Amongst four cultivars, seedlings of V3 synthesized the highest proline content most cases while the lowest in V4.

Table 3. Proline content in some selected land races of rice during seedling stage (mg g⁻¹)

Varieties	16 DAG	20 DAG	24 DAG	28 DAG	32DAG	36 DAG
V1	1.354 a	0.6563 a	0.7250 a	0.6673 a	0.963 a	0.797 a
V2	0.9310 a	0.3132 a	0.6080 a	0.7813 a	1.079 a	0.589a
V3	0.9277 a	0.4353 a	0.7557 a	0.8950 a	1.539a	0.445a
V4	1.217 a	0.4867 a	0.7343 a	0.8400 a	1.204a	0.846a
LSD(5%)	NS	NS	NS	NS	NS	NS

However, prolonged low temperature for 32 days generally increased the proline content. Accumulation of proline has been reported from increased proline biosynthesis, lower proline utilization, decreased degradation of proline and proteins (Delauney and Verma 1993). The level of proline accumulation varies from species to species and also among the plant organs, its highest levels found in flowers and seeds and lowest levels in roots. (Verbruggen *et al.*, 1993; Nathalie

and Christian 2008). Proline ameliorates the stress tolerance by preventing membrane and protein damage (Santoro *et al.*, 1992; Santarius 1992).

There are conflicting reports concerning the function of proline in the chilling resistance of rice plants. The higher level of free proline in cold-stressed plants has been suggested as a factor conferring chilling tolerance. In contrast, proline accumulation has also been considered as a symptom of injury rather than an indicator of low temperature tolerance (Hawrylak-Nowak *et al.*, 2010). Similar result was revealed by Kamata and Uemura (2004) as they concluded that prolonged low temperature for 14 to 21 days generally increased the proline content and total amino acids in the cultivated wheat as well as local varieties during cold acclimation. It is evident that rice seedlings produced relatively higher amount of proline under low temperature environment.

Total amino acids in the cultivated wheat as well as local varieties during cold acclimation revealed by Kamata and Uemura (2004). A positive correlation between the accumulation of proline and stress tolerance in different plants was revealed by Van-Rensburg *et al.*, 1993. Regarding proline content, leaves of V3, rice variety might be a low temperature tolerant cultivars, showed markedly greater amount of proline than those of others rice land races during low temperature. The non-chilled plants from the local rice land races accession contained a lower amount of proline but after prevailing low temperature.

Proline is considered as one of the most important osmolytes that accumulates in many microorganisms and plants subject to drought and salt stress along with cold stress (Smirnoff and Cumbes, 1989; Delauney and Verma, 1993; Van-Rensburg *et al.*, 1993; Kavi-Kishor *et al.*, 1995; Kamata and Uemura (2004) noticed the accumulation of proline and total amino acids along with sugars and glycinebetaine

in leaves of wheat plants during cold acclimation. The role of proline in chilling tolerance of plants may be explained as follows; under cold stress conditions, proline acts as a mediator of osmotic adjustment, a stabilizer of subcellular structures, a scavenger of free radicals, a buffer in cellular redox potential, and a major constituent of cell wall structural proteins that may provide mechanical support for cells (Hare et al., 1998; Nanjo et al., 1999). Moreover, proline degradation upon relief from stress might provide carbon, nitrogen and energy for plants to recover from stress (Hare and Cress, 1997). Hare et al. (1998) claimed that, if flux through the proline biosynthetic pathway is a more important determinant of stress tolerance than free proline content, this may account both for conflicting evidence that absolute proline levels are a reliable measure of stress tolerance and the observation that proline accumulation is frequently only evident after the onset of stress-induced injury.

This indicates that proline has multiple roles in stress tolerance in plants. However, it is difficult to claim whether the increase of proline content is eventually associated with differences in chilling tolerance of plants from the cultivated cultivars.

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 V3 compared to the HYV. Generally, rice seedlings tend to accumulate proline at below 10°C and a downward trend at above 10 °C 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

V3 variety was the highest among the sensitive varieties. 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.

4.2 Chlorophyll

Leaf chlorophyll content is one of the important physiological traits closely related to photosynthetic ability in rice (Teng et al., 2004). Undoubtedly, understanding the genetic mechanisms underlying the leaf chlorophyll content across different developmental stages of rice has significant implications for improving photosynthetic ability in rice. Chlorophyll content in leaves is one of the major components in photosynthesis.

4.2.1 Total chlorophyll content

There was a significant variation of total chlorophyll content among the tested rice seedlings. The total chlorophyll content was always higher in V1 plants, i.e., HYV NERICA-10. The lowest total chlorophyll content was recorded in the V2 plants which is also a HYV variety but during cold environment, the V2 plants synthesized the lowest amount of chlorophyll in their leaves (Table 4).

Table 4. The Total Chlorophyll content (mg g^{-1}) different rice seedlings at different DAG

Varieties	16 DAG	20 DAG	24 DAG	28 DAG	32DAG	36 DAG
V1	9.583 a	9.911 a	8.279 a	7.229 a	6.354 a	7.997 a
V2	4.653 d	5.434 b	5.594 b	4.253 b	5.632 a	5.047 a
V3	6.195 c	4.928 b	5.825 b	4.637 b	5.112 a	7.153 a
V4	8.399 b	6.966 b	5.004 b	4.590 b	5.126 a	5.120 a
LSD(5%)	1.160	2.544	1.818	1.716	NS	NS

However, chlorophylls are the most widely distributed plant pigments responsible for the characteristic green color of fruit and vegetables (Almela, 2000). It is important to plants because it is the substance that the chloroplast organelles in the cells that carry out photosynthesis. The major chlorophylls in different plants are chlorophyll-a, which has a methyl group at C-3 carbon, and chlorophyll-b, which a formyl group is bonded to the same carbon atom. Chlorophylls are known to be easily degraded by conditions such as dilute acids, heat, light and oxygen (Tonucci and Von-Elbe, 1992). An optimum temperature ranging from 25°C to 35°C is required for a good rate. At temperatures around 0°C the enzymes stop working and at very high temperatures the enzymes are denatured. In this study, the prevailing different level of low temperature during the experimental period might be the main cause of lowering and increasing the total chlorophyll level of selected rice cultivars. However, the variation of total chlorophyll content of rice seedlings was observed due to the genetical variation among the cultivars.

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). Decreased or unchanged chlorophyll level during drought stress has been reported in many species, depending on the duration and severity of drought (Kpyoarissis et al., 1995; Zhang and Kirkham, 1996).

Exposure of two olive cultivars to reduced irrigation led to lower Chl (a + b) contents. Loss of chlorophyll contents under water stress is considered a main cause of inactivation of photosynthesis. Furthermore, water deficit induced reduction in chlorophyll content has been ascribed to loss of chloroplast

membranes, excessive swelling, distortion of the lamellae vesiculation, and the appearance of lipid droplets. 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 (Table 4). Among the varieties studied, V2 produced the highest and might have cold tolerant potentiality in the study area.

4.2.2 Total Chlorophyll-a content

Table 5. The Chlorophyll-a content (mg g^{-1}) different rice seedlings at different DAG

Varieties	16 DAG	20 DAG	24 DAG	28 DAG	32DAG	36 DAG
V1	6.671 a	6.658 a	5.443 a	4.948 a	4.417 a	5.609 a
V2	3.061 d	3.617 b	3.716 b	2.930 b	4.071 a	3.452 a
V3	4.211 c	3.240 b	4.173 b	3.122 b	3.477 a	4.916 a
V4	5.690 b	4.501 b	3.379 b	3.192 b	3.662 a	3.587 a
LSD(5%)	0.7992	1.891	1.267	1.403	NS	NS

Table 6 shows the chlorophyll-a content during different times of experiment at low temperature environment. The highest chlorophyll-a content was obtained in V1 (NERICA-10) HYV cultivars (6.671 mg g^{-1}), and the lowest was in V2 (3.061 mg g^{-1}) varieties at 16 DAG while low air temperature was common. The V1

plants synthesized always higher during the experiment at cold environment. Among the local cultivars, the V4 plants (Gochi) showed the higher chlorophyll content and was statistically different than V3 plants (Lafaya) at 5% level of significance. The chlorophyll-a content in seedlings leaves of different varieties was significantly different at 5% level of probability (Table 5). The biosynthesis of chlorophyll-a in V1 plants considered the superior among the cultivars studied during the experiment. Similar variation in chlorophyll-a content in different rice cultivars during low temperature at seedling stage was revealed by Kulchhum (2011) and Haque (2012).

However, chlorophyll-a and b contained in leaves of higher plants are the main pigments of photosynthesis in the chloroplasts, and have important functions in the absorption and exploitation of the light energy, thereby influence photosynthetic efficiency (Pan and Dong, 1995). Some studies have demonstrated that chlorophyll content is positively correlated with photosynthetic rate (Araus *et al.*, 1997; Thomas *et al.*, 2005). Low temperature is an environmental signal that regulates the metabolic changes in the entire Chlorophyll synthesizing pathway. Prolonged low temperature may block the entire pathway of chlorophyll-a synthesis. Thus, the cultivar having more chlorophyll content during low temperature environmental condition is considered as more low temperature tolerant due to their higher photosynthesis ability. In the present study, V1 cultivar of rice seedling might have potentiality to withstand low temperature for their survival.



4.2.3 Total Chlorophyll-b content

Chlorophyll-b absorbs energy from wavelengths of green light at 640 nm. It is the accessory pigment that collects energy and passes it on to chlorophyll-a. It also regulates the size of antenna and is more absorbable than chlorophyll-a. Thus, chlorophyll-a is the primary photosynthetic pigment while chlorophyll-b is the accessory pigment that collects energy. Therefore, the amount of chlorophyll-b in plant leaves have a significant impact on the production of photosynthate.

The highest chlorophyll-b content was obtained in V1 cultivars during the experiment. The highest amount of chlorophyll-b was obtained at 20 DAG (3.253 mg g⁻¹) and is significantly higher than other cultivars at 5% level of significance, but the lowest was in V2 plants (1.688 mg g⁻¹). The V2, V3 and V4 rice cultivars showed almost similar chlorophyll-b content in their leaves, respectively. The V4 plants showed always lower quantity of chlorophyll-b but statistically similar with V2 and V3 plants. The chlorophyll 'b' content was variable (Table 6),

Table 6. The Chlorophyll-b content (mg g⁻¹) different rice seedlings at different DAG

Varieties	16 DAG	20 DAG	24 DAG	28 DAG	32DAG	36 DAG
V1	2.912 a	3.253 a	2.836 a	2.281 a	1.937 a	2.388 a
V2	1.591 b	1.817 b	1.878 b	1.323 b	1.561 a	1.594 a
V3	1.984 b	1.688 b	1.652 b	1.516 b	1.635 a	2.237 a
V4	2.709 a	2.465 ab	1.624 b	1.398 b	1.464 a	1.532 a
LSD(5%)	0.4094	0.7916	0.6381	0.3402	NS	NS

which seems to be attributed by genetically. Similar observation was also obtained by Haque (2012) and Kulchhum (2011).

4.2.4 Total carotenoid content

The highest total carotenoid content was obtained in V1 cultivars during the experiment. The highest amount of total carotenoid was obtained at 16 DAG (1.181 mg g⁻¹) and is significantly higher than V2 V3 and V4 cultivar at 5% level of significance. At 28, 32 and 36 DAG, the total carotenoid content in the leaves of the tested varieties was statistically similar. The lowest amount carotenoid was in V2 plants (0.587 mg g⁻¹). The V2, V3 and V4 rice cultivars showed marked variation in total carotenoid content, respectively. The total carotenoid content presented variable (Table 7) which seems to be attributed by genetically.

Table 7. The total carotenoid content (mg g⁻¹) different rice seedlings at different DAG

Varieties	16 DAG	20 DAG	24 DAG	28 DAG	32DAG	36 DAG
V1	1.181 a	1.119 a	0.869 a	0.914 a	1.013 a	1.084 a
V2	0.587 c	0.714 a	0.692 ab	0.667 a	1.051 a	0.828 a
V3	0.738 c	0.541 a	0.750 ab	0.555 a	0.828 a	1.090 a
V4	0.958 b	0.719 a	0.483 b	0.662 a	0.794 a	0.780 a
LSD(5%)	0.220	NS	0.283	NS	NS	NS

However, total carotenoids have additional roles and partially help the plants to withstand adversaries of drought (Farooq et al., 2009) and cold stress (Kulchhum, 2011).

4.2.5 Chlorophyll a/b ratio:

The chlorophyll-a/b is directly related to temperature. Its content in plant varies in relation to temperature change (Haldimann, 1999). Chlorophyll-a/b ratio plays a vital role in plant acclimation. Porra (2002) reported that the determination of Chl-a and b and of Chl-a/b ratios has also played an important role in investigations of how higher plants and algae adapt their photosynthetic apparatus during acclimation.

In this study, a significant difference in chlorophyll-a/b ratio was found among the seedlings of selected rice cultivars during the experiment at low temperature. The variation of chlorophyll-a/b content is shown in Table 8.

Table 8. The chlorophyll a/b ratio different rice seedlings at different DAG

Varieties	16 DAG	20 DAG	24 DAG	28 DAG	32DAG	36 DAG
V1	2.296 a	2.040 a	1.920 b	2.179 a	2.287 a	2.330 a
V2	1.921 b	1.986 a	1.969 b	2.193a	2.617 a	2.160 a
V3	2.129 ab	1.895 a	2.573 a	2.078 a	2.216 a	2.169 a
V4	2.105 ab	1.862 a	2.076 ab	2.289 a	2.506 a	2.336 a
LSD(5%)	0.2447	NS	0.5015	NS	NS	NS

At 16 DAS when the day of lowest temperature was 13°C, the highest chlorophyll-a/b ratio was found in V1 (2.296) while the lowest value was observed in V2

(1.921). At 20 DAS with air temperature below 10°C, the V1 variety also had highest chlorophyll-a/b (2.040) and the lowest chlorophyll-a/b value was observed in V4 (1.862). The chlorophyll-a/b ratio of all cultivars at 28, 32 and 36 DAG are statistically similar. The lowest chlorophyll-a/b ratio at 24 and 28 DAS were in V5 (1.590) and V6 (1.733), respectively.

4.3 Relative water content

Relative water content (RWC) of leaf is important characteristics that influence plant water relations. Relative water content is considered a measure of plant water status reflecting the metabolic activity in tissues and used as a most meaningful index for stress tolerance. In the experiment, four rice varieties were tested for cold stress mechanism. The relative water content in the seedling leaves during different DAG have presented in the Table 9.

Table 9. The relative water content of different rice seedlings at different DAG

Varieties	28 DAG	32DAG	36 DAG
V1	85.86 a	85.50 b	84.79 ab
V2	92.43 a	87.87 b	80.92 b
V3	92.93 a	89.97 ab	85.80 ab
V4	90.25 a	94.58 a	89.07 a
LSD(5%)	NS	5.566	6.894

Table 9 showed that the V3 local cultivar had the highest percentage of relative water content (92.93%), which is similar to other tested varieties at 28 DAG. The relative water content in the leaves of four cultivars was not significantly different

at 28 DAG. But at 32 and 36 DAG, V4 rice Cultivars had the higher RWC in comparison to others cultivars. The V1 plants had the lowest percentage of relative water content (85.50%) at 32 DAG. RWC of leaves was variable during the experiment but slightly decreased. RWC related to water uptake by the roots as well as water loss by transpiration.

A decrease in the relative water content (RWC) in response to drought stress has been noted in wide variety of plants as reported by, Sarker et al., (2005), Kulchhum (2011), and 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 cold and 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. In the present study, RWC was above the 80% which might not adverse effect on metabolism.

4.4 Protein content

Proteins are compounds of fundamental importance for all functions in the cell. It is well known that alteration of gene expression is always involved in preparing plants for an existence under stress. Protein variation is an essential part of plant response to environmental stress as well as for adaptation to environmental conditions (Vierstra, 1993; Hieng *et al.*, 2004). Various cultivars response to low temperature as well as other plant stress condition in different manner. In this

study, there was no significant variation in percent protein content of the seedlings of selected rice cultivars at 36 DAG was observed which is shown in Figure 2.

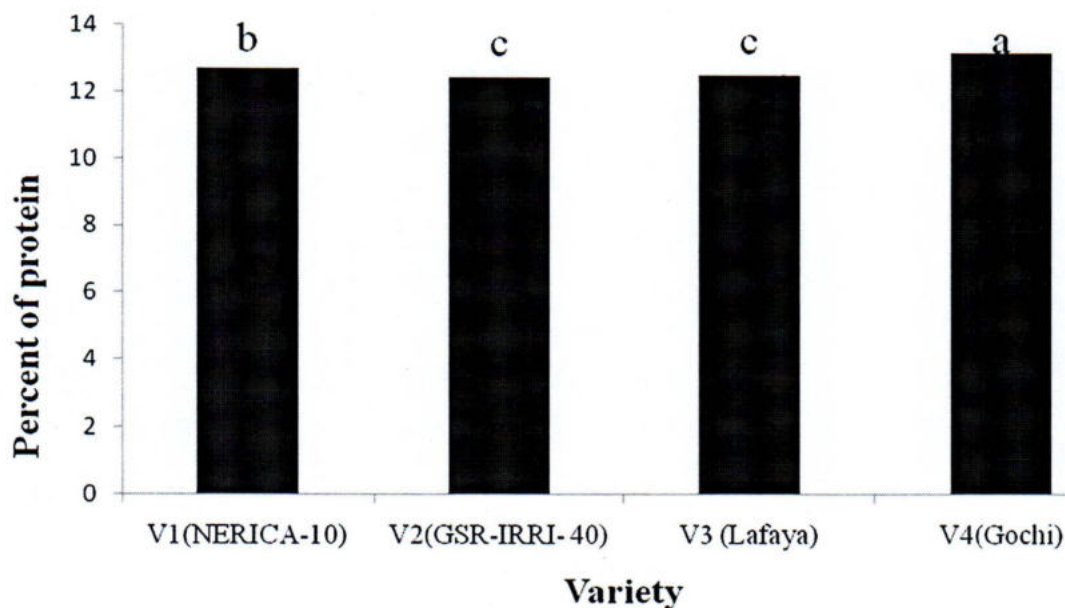


Figure 2. Percent protein content of rice cultivars at 36 DAS

At 36 DAG, The V4 variety showed the significantly highest content of protein percentage (13.21%) while the V2 contained the lowest percentage of protein (12.21%). The second highest value of percent protein content was found in V1 followed by V3 seedling. However, the percent protein content values of V2 and V3 rice cultivars are statistically similar. The variety having highest mean value is obviously low temperature tolerate in compare to other cultivars.

This result showed that total protein has protective role in cold-tolerant genotype subject to low temperature. Synthesis of specific proteins is an important mechanism involved in increasing cold tolerance (Koc *et al.*, 2010).

4.5 Seedling starch content

Many environmental stresses lead to major changes in carbohydrate metabolism. Under conditions such as low temperature, drought, desiccation or salt stress, soluble sugars are accumulated at high concentrations, and are thought to act as protective compatible solutes (Korn *et al.*, 2008). Soluble sugars have also been involved in other stress conditions, where they do not accumulate at the same levels as those resulting from drought or freezing stress, and thus do not act as compatible solutes. In this study, a significant variation in plant starch content in selected rice cultivars at 36 DAG was found which is shown in Figure 3. At 36 DAG, the highest percent starch content was found in V3 (29.82%) followed by V4, V2 and V1.

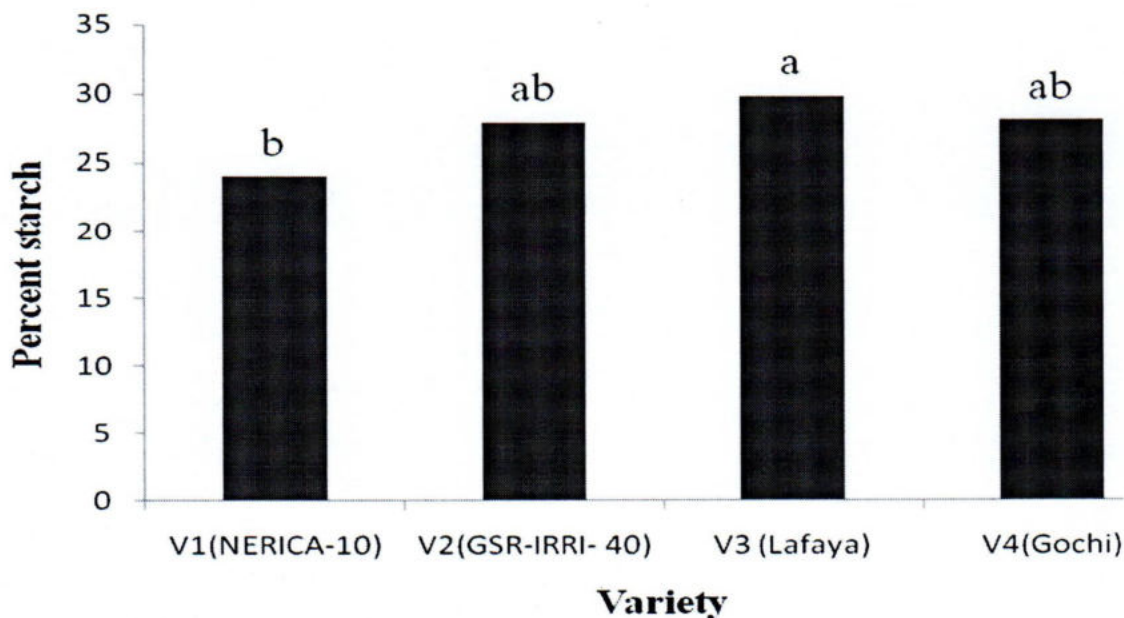


Figure 3: Percent starch content of rice cultivars at 36 DAS

The lowest percent starch content was recorded in V1 (23.99%). The best performer regarding percent starch content is V3 among cultivars. Various soluble carbohydrates are considered as protective compatible solutes functioning during plant stress condition. Cultivar having more mean percent starch content value is comparatively more low temperature stress tolerate than others. Similar result was observed by Rees *et al.* (1988).

4.6 Shoot length and weight

The effect of low temperature on shoot length at different times was significantly variable. The shoot length of all the cultivars increased in general at the low temperature environment. The growth rate regarding shoot length in seedling stage 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 10.

Table 10. Shoot length of seedlings of some selected rice varieties

Varieties	12 DAG	16 DAG	20 DAG	24 DAG	28 DAG	32 DAG	36 DAG
V1	31.22 a	41.47 b	56.44bc	56.78 b	57.89 b	67.78c	66.5 c
V2	34.44 a	41.46 b	48.67 c	52.89 b	51.33 b	61.11c	64.33c
V3	54.00 a	62.76 a	76.67 a	77.11 a	84.78 a	99.78 a	106.7a
V4	40.11 a	52.08ab	67.00ab	69.11ab	76.11a	87.44 b	88.44 b
LSD(5%)	NS	16.49	10.90	19.09	14.54	11.95	14.04

Table 10 showed all the shoot length was statistically similar at 12 DAG. The V3 plants showed the highest shoot length at all times of experiment. The V3 plant significantly better than those of other cultivars. In the present study local cultivar V3 showed higher growth than the HYV plants. The HYV, NERICA-10 and IRRI-40 cultivars shoot length growth was slower than local cultivars. 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 four rice cultivars while prevailing low temperature or cold stress 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 10). 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 10°C when the rice seedlings were stunted or growth stagnant occurred. However, there was an 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 (Table 10)

Table 10 shows the fresh and dry shoot weight of four rice varieties. The V3 plants had the highest shoot weight at all times of experiment in 2012 irrespective of fresh and dried condition. But the V2 seedling showed the lowest weight in dry and fresh condition. Dry matter accumulation in shoot is an important physiological parameter to add organic C in above ground part under any environmental stress. In the present study the V3 seedling accumulate dry matter in their shoot part. 12 DAG, shoot dry of V3 seedling was significantly greater than those of V2, V3 and

V4 seedlings. All the seedlings in different times of experiment, the highest shoot weight was recorded in V3, which was statistically different from other varieties. The V2 showed the lower fresh and dry shoot weight in comparison to others varieties. At low temperature all the cultivars showed slow growth in regard to shoot weight, which was supported by Haque (2012).

4.7 Root length and weight

Root length growth rate of rice seedlings was reduced by increasing the low temperature as well as in very low temperature prevails in the soil. Any stress that is cold, salinity or other environmental abnormality can hamper the growth of root of plants. The root length of different rice cultivars were recorded during experiment shown in the Figure 4

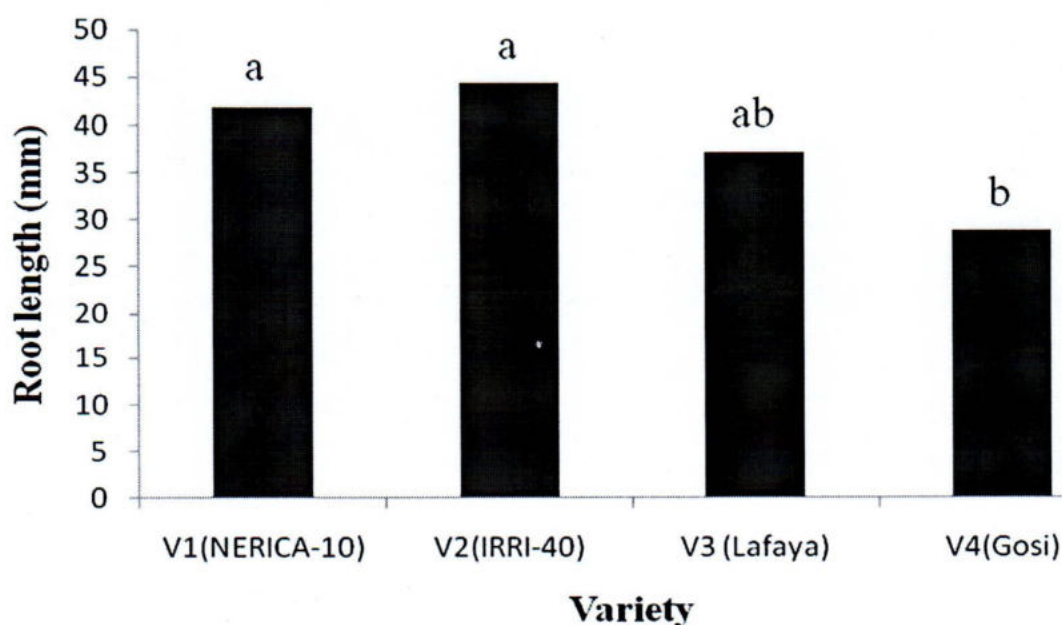


Figure 4 : Root length of seedlings of selected rice varieties at 36 DAS

Figure 4 shows the variable root length of four rice varieties tested the purpose. The V2 plants had the highest root length at all times of experiment in 2012. At 12 DAG, there was no significant difference among the cultivars. All the seedlings in 36 DAG, the highest root length was recorded in V2, which was similar to V1 and V3 followed by V4 seedlings. The V4 showed the lower root length growth in comparison to others varieties. At low temperature all the cultivars showed slow growth in regard to root length, however, V1 and V2 showed better performance. At 32 and 36 DAG. (Rahman et al., 2001). observed that root length was diminished by increasing chilling intensity. The gradual slow growth rate 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). Similar observation was found by Haque (2012). and Kulchhum (2011). 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. (2007) that maximum root length occurred at above 20°C, a opposite relation was observed between variations in root length and low temperature.

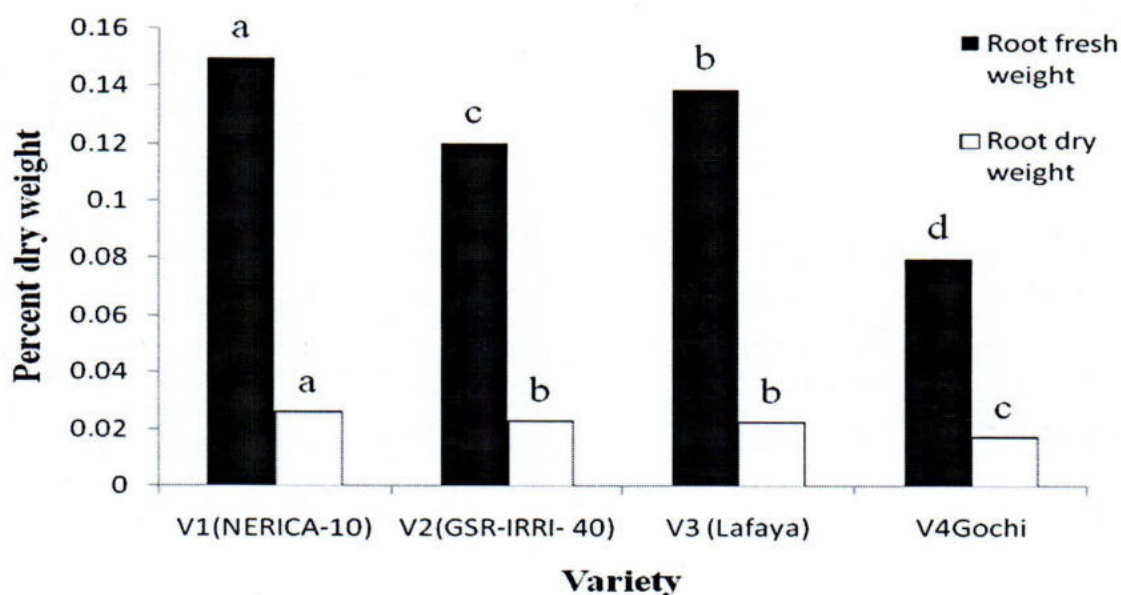


Figure 5: Root fresh and dry weight of rice seedling at 36 DAS

Figure 5 shows the fresh and dry root weight of four rice varieties. The V1 plants had the highest root weight at all times of experiment in 2012 irrespective of fresh and dried condition. But the V4 seedling showed the lowest weight in dry and fresh condition. Dry matter accumulation in root is an important physiological index to survive under any environmental stress. In the present study the V1 seedling accumulate dry matter in their root part. 12 DAG, root dry of V1 seedling was significantly greater than those of V2, V3 and V4 seedlings. All the seedlings in 36 DAG, the highest root weight was recorded in V1, which was statistically different from other varieties. The V4 showed the lower fresh and dry root weight in comparison to others varieties. At low temperature all the cultivars showed slow growth in regard to root weight, which was supported by Kulchhum 2011.

4.8 Seed germination

Figure 6 shows that the highest seedling emergence was noted in V1 and this was unaffected or minimum affected by cold stress and followed by V2, V3 and V4, respectively. The Figure 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.

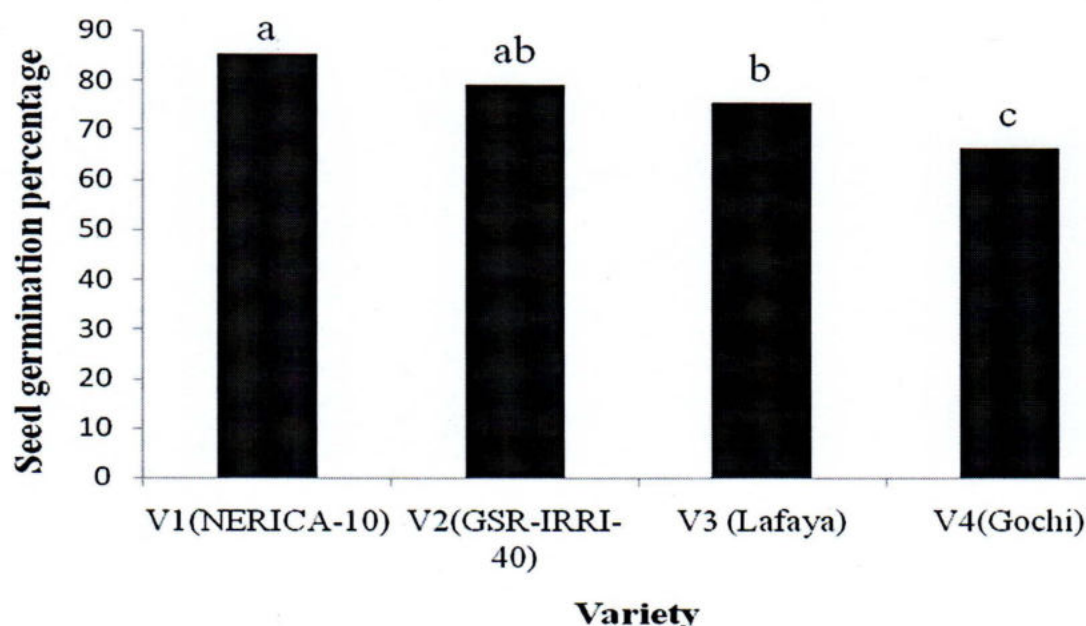


Figure 6: Seed germination percentage of rice cultivars at 10 DAG

Seed germination is an essential process in plant development to obtain optimal seedling numbers that results in higher seed 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 (Ashraf et al., 1994 and Kaya et al., 2008,). A significant reduction in seedling emergence was 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 (BSCB and BRRI, 2008). 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 V1 and this was less affected by cold stress (25/07°C).

4.9 Dry biomass production and allocation

Table 8 shows that total dry biomass of seedling in different rice cultivars at 36 DAG. Most interestingly, V3 plants showed the best performing cultivars amongst the tested varieties responding seedling mortality. Conversely, the cultivars V2 and V4 showed very poor at low temperature. Total plant weight tends to decrease by low temperature environment revealed by Kulchhum (2011).

Table 11. Total biomass of seedlings of different cultivars at 36 DAG

Variety	Total biomass
V1	0.063 b
V2	0.054 c
V3	0.066 a
V4	0.048 d
LSD (5%)	0.0019

CHAPTER V
SUMMARY AND CONCLUSION

CHAPTER V

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A field experiment was conducted in the field laboratory of the Department of Agricultural Chemistry, Hajee Mohammad Danesh Science and Technology University, Dinajpur during December 2011- January 2012. Four varieties of rice seedlings were used for investigating the physiological and morphological parameters related to cold survival mechanism of rice seedling during low temperature environment in Northwest Bangladesh.

The proline content of rice seedlings was no significantly influenced by the low temperature at different times of experiment. The V1 (NERICA-10) and V3 (Lafaya) seedling synthesized the higher amount proline at low temperature. . Amongst four cultivars, seedlings of V3 synthesized the highest proline content most cases while the lowest in V4. The higher proline synthesis in leaf cell acted as a stress reducer in seedling stage of rice. The chlorophyll is one of the major chloroplast component for photosynthesis, and relative chlorophyll content has a positive relationship with photosynthetic rate. The chlorophyll content is markedly decreased by exposure of rice to low temperature. The total chlorophyll content was always higher in V1 plants, ie., HYV NERICA-10. The lowest total chlorophyll content was recorded in the V2 plants which is also a HYV variety but during cold environment, the V2 plants synthesized the lowest amount of chlorophyll in their leaves (Table 5).

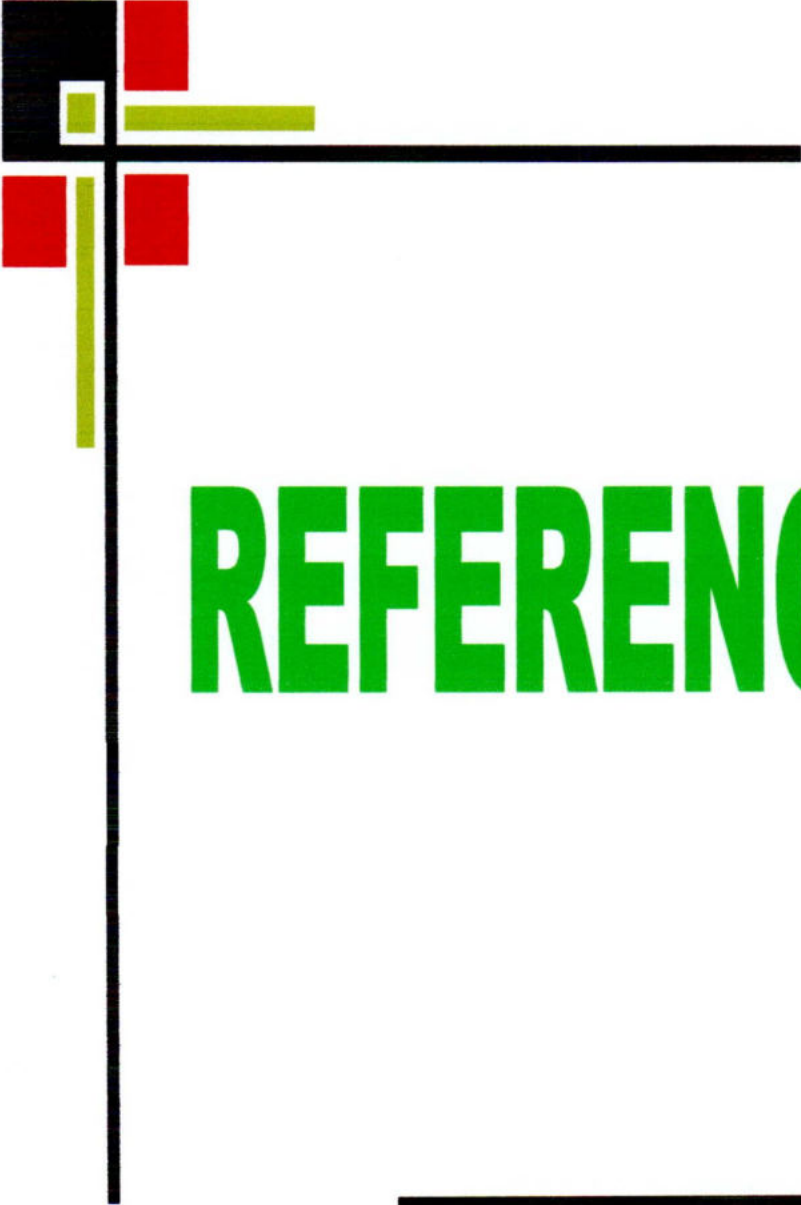
The highest chlorophyll-a content was obtained in V1 (NERICA-10) HYV cultivars (6.671 mg g^{-1}), and the lowest was in V2 (3.061 mg g^{-1}) varieties at 16 DAG while low air temperature was common. The V1 plants synthesized always higher during the experiment at cold environment. Among the local cultivars, the V4 plants (Gochi) showed the higher chlorophyll content and was statistically different than V3 plants (Lafaya) at 5% level of significance. The highest chlorophyll-b content was obtained in V1 cultivars during the experiment. The highest total carotenoid content was obtained in V1 cultivars (1.181 mg g^{-1}) during the experiment. In this study, a significant difference in chlorophyll-a/b ratio was found among the seedlings of selected rice cultivars during the experiment at low temperature.

Relative water content (RWC) is the appropriate measure of plant water status in terms of the physiological consequence of cellular water deficit. The V3 cultivar (Lafaya) had the highest percentage of relative water content (92.93%). The V1 plants had the lowest percentage of relative water content (85.50%) at 32 DAG. RWC of leaves was variable during the experiment but slightly decreased. RWC related to water uptake by the roots as well as water loss by transpiration. The V4 variety showed the highest content of protein percentage (13.35%) while the V2 contained the lowest percentage of protein (12.45%).

At 36 DAG, the highest percent starch content was found in V3 (29.82%) followed by V4, V2 and V1. The lowest percent starch content was recorded in V1 (23.99%). The best performer regarding percent starch content is V3 among cultivars. In the present study local cultivar V3 showed higher growth than the HYV plants. The HYV, NERICA-10 and IR- 40 (GSR-IRRI- 40) cultivars shoot length growth was slower than local cultivars. The V3 plants had the highest shoot

weight at all times of experiment in 2012 irrespective of fresh and dried condition. But the V2 seedling showed the lowest weight in dry and fresh condition. The V2 plants had the highest root length at all times of experiment in 2012. At 12 DAG, there was no significant difference among the cultivars. The V1 plants had the highest root weight at all times of experiment in 2012 irrespective of fresh and dried condition. But the V4 seedling showed the lowest weight in dry and fresh condition. The V3 plants showed the best performing cultivars amongst the tested varieties responding seedling mortality. Conversely, the V2 and V4 cultivars showed very poor at low temperature.

To the conclude, the present study indicated that seedling growth and physiological activities of rice seedling was suppressed due to low temperature during short winter in Northwest Bangladesh. Among the rice cultivars studied, NERICA-10 (V1) and Lafaya (V3) showed better survival potentiality based on proline and chlorophyll biosynthesis, and some other morphological parameters. This study suggests that a comprehensive study deserves to know physiological and molecular mechanism on cold survival. Therefore it is suggested that NERICA-10 (V1) and Lafaya (V3) may be selected in breeding works to develop cold tolerant rice cultivation for raising seedling for *boro* rice cultivation in Northwest Bangladesh.



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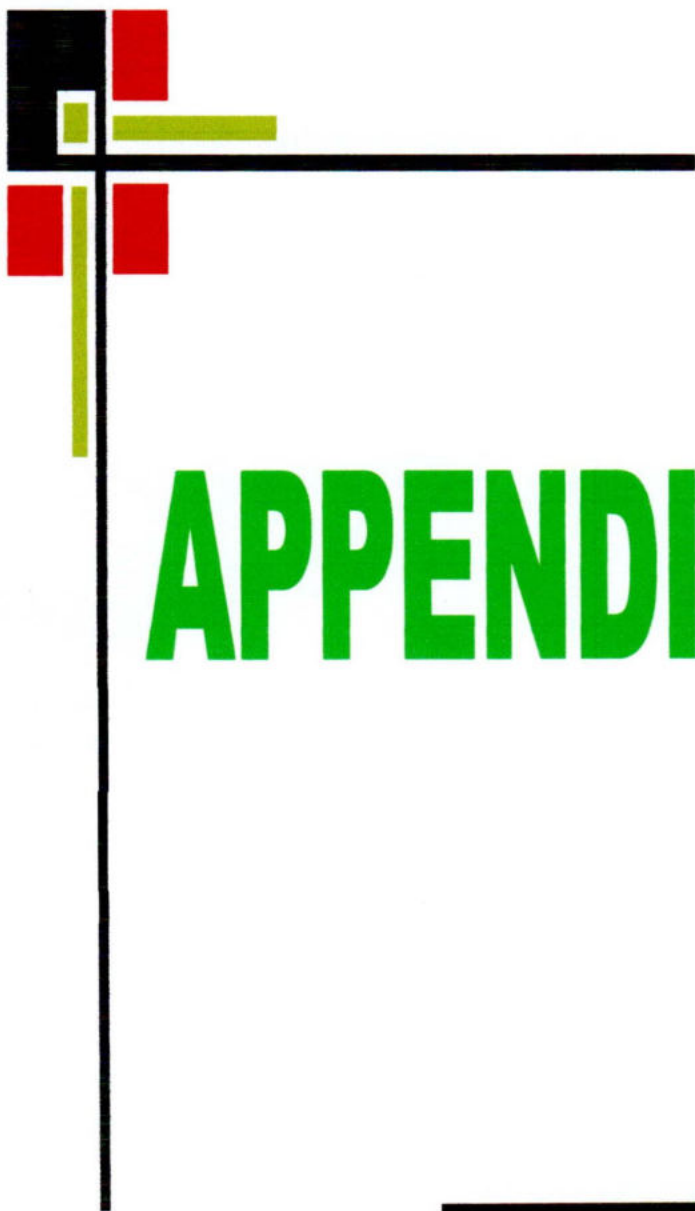
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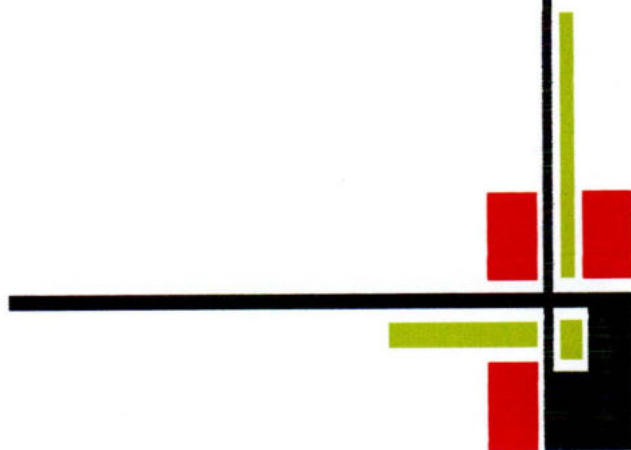
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APPENDICES



APPENDIX

Appendix 1. Air temperature during the experimental period at 6 hours interval

Date	Temperature (°C)			
	12:00 AM	06.00 AM	12.00 PM	06.00 PM
21 December 2011	11	10	15	13
22 December 2011	11	10	15	13
23 December 2011	9	7	10	11
24 December 2011	9	7	15	13
25 December 2011	10	9	19	17
26 December 2011	13	10	15	12
27 December 2011	14	11	19	15
28 December 2011	13	10	21	16
29 December 2011	12	11	22	15
30 December 2011	14	12	21	17
31 December 2011	15	12	22	18
01 January 2012	15	14	17	15
02 January 2012	14	13	15	13
03 January 2012	12	11	14	12
04 January 2012	10	10	13	12
05 January 2012	12	10	15	12
06 January 2012	14	12	18	14
07 January 2012	14	13	21	15
08 January 2012	12	12	20	14
09 January 2012	14	14	21	15
10 January 2012	15	13	22	14
11 January 2012	14	13	17	14
12 January 2012	13	12	15	13
13 January 2012	12	10	13	12
14 January 2012	11	9	14	13
15 January 2012	10	8	14	12
16 January 2012	12	8	14	13
17 January 2012	12	9	15	14
18 January 2012	13	12	16	14
19 January 2012	12	12	16	13
20 January 2012	13	12	18	15
21 January 2012	12	12	18	15
22 January 2012	12	12	18	15
23 January 2012	13	11	17	14
24 January 2012	13	12	18	16
25 January 2012	13	12	19	17

Date	Temperature (°C)			
	12:00 AM	06.00 AM	12.00 PM	06.00 PM
26 January 2012	14	11	19	16
27 January 2012	14	11	19	16
28 January 2012	14	11	19	19
29 January 2012	12	11	18	18
30 January 2012	13	11	18	19
31 January 2012	15	12	19	20
01 February 2012	14	12	20	17
02 January 2012	12	12	20	17
03 January 2012	12	11	21	18

