# RESPONSE OF TRANSPLANTED AMAN RICE VARIETIES TO DIFFERENT SOIL MOISTURE LEVELS

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# CERTIFICATE

This is to certify that the thesis entitled, "**NATURE OF RESPONSE OF TRANSPLANTED AMAN RICE (*Oryza sativa* L) GENOTYPES UNDER VARIOUS SOIL MOISTURE CONDITIONS**" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in the **DEPARTMENT OF AGRICULTURAL BOTANY**, embodies the result of a piece of bona fide research work carried out by **Mohammad Mohidur Rahman** Registration No. 11-04689 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation, has duly been acknowledged.

**Dated:** ..................................

**Place: Dhaka, Bangladesh** (Dr. Kamal Uddin Ahamed)

**Professor**

**DEDICATED**

**TO MY**

**FAMILY AND SUPERVISOR**

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# RESPONSE OF TRANSPLANTED AMAN RICE VARIETIES TO DIFFERENT SOIL MOISTURE LEVELS

## MOHAMMAD MOHIDUR RAHMAN

### ABSTRACT

Due to irregular rainfall during *aman* season between July and November rice suffers from drought. Sometimes the farmers do not afford to provide irrigation in *boro* rice field; where providing irrigation in the *aman* rice field seldom happens leaving the best option to cultivate drought tolerant rice varieties in *aman* season. Considering the above conditions three pot culture experiments were carried out at Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period from July 2012 to November 2014 to study the performance of four transplanted *aman* rice genotypes (BRRI dhan56, BRRI dhan57, Binadhan-7 & BRRI dhan49) under three different soil moisture levels (100% field capacity moisture content or control, 70% of the control moisture and 40% of the control moisture). Another experiment under different PEG induced water stress conditions, was conducted to justify whether a lower soil moisture condition affects rice growth. The experiment was laid out in two factors Randomized Complete Block Design with four replications. The results indicated that plant height, tiller number, life duration, effective tiller, leaf area and total dry matter decreased under water stress. Increased dry matter investment to root in BRRI dhan49 helped to uptake more water under water stress. Relative water content, stomatal conductance and transpiration rate were found the highest at 100%FC condition and the lowest at 40%FC condition; but the reduction was comparatively lower in BRRI dhan56 and BRRI dhan49. BRRI dhan56 and BRRI dhan49 also performed comparatively better in PEG treatment. The grain yield and harvest index decreased with decreasing soil moisture in all the genotypes; BRRI dhan49 produced more than 90% relative grain yield under 70%FC treatment. Increased proline content in BRRI dhan56 and BRRI dhan49 helped them to give better yield under low soil moisture condition. The relative injury, chlorophyll content, soluble sugar content, starch content decreased with increasing water stress. The lower drought susceptible index (DSI) in BRRI dhan49 and BRRI dhan56 and higher in BRRI dhan57 and Binadhan-7 indicated that BRRI dhan49 and BRRI dhan56 were tolerant and BRRI dhan57 and Binadhan-7 were susceptible to water stress.

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# LIST OF ABBREVIATIONS

| ABBREVIATION | FULL WORD |
|---|---|
| AEZ | Agro-Ecological Zone |
| ANOVA | Analysis of Variance |
| AGR | Absolute Grain Growth Rate |
| @ | At the rate of |
| ATP | Adenosine Tri Phosphate |
| BRRI | Bangladesh Rice Research Institute |
| BY | Biological Yield |
| cm | Centimeter |
| cv. | Cultivar(s) |
| CV | Coefficient of Variation |
| DAA | Days after anthesis |
| DMRT | Duncan’s Multiple Range Test |
| e.g. | *exempi gratia* (for example) |
| et al. | *et alibi* (and others) |
| EY | Economic Yield |
| etc. | *et cetra* (and so on) |
| FC | Field capacity |
| g | Gram |
| HI | Harvest index |
| HYR | High yielding rice |
| $H_2O_2$ | Hydrogen per oxide |
| i.e. | *id est* (that is) |
| IRRI | International Rice Research Institute |
| kg | Kilogram |
| LA | Leaf area |
| LSD | Least significant difference |
| LWR | Leaf weight ratio |

| ABBREVIATION | FULL WORD |
| --- | --- |
| mg | miligram |
| MP | Muriate of potash |
| NAR | Net assimilation rate |
| PEG | Polyethylene glycol |
| pH | Hydrogen ion concentration |
| RCBD | Randomized complete block design |
| RGR | Relative Growth Rate |
| ROS | Reactive oxygen species |
| SAU | Sher-e-Bangla Agricultural University |
| SRDI | Soil Resources and Development Institute |
| SPAD | Soil Plant Analysis Development |
| SLW | Specific leaf weight |
| TDM | Total dry matter |
| TSP | Triple super phosphate |
| $t\ ha^{-1}$ | Ton per hectare |
| Viz. | *Videlicet* (namely) |

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I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

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# CHAPTER 1

# INTRODUCTION

In Bangladesh, rice is the key staple crop and there is no alternative of increasing rice production to feed the ever increasing population of Bangladesh. The second largest production of rice in Bangladesh comes from *aman* season after *boro*. Now a days, drought is one of the major problems in *aman* season due to prevailing climatic changes. Transplanted *aman* is the second rice crop in a year, grown in the wet *kharif II* season, between July and November; it may be strongly affected by drought spells (Selvaraj *et al*., 2006). Abrupt ending of monsoon in September can create severe water stress on the transplanted *aman* rice (up to maximum tillering stage) and rainfall may meet the crop water demand. After October, rainfall is not sufficient for potential yield of rice and most of the *aman* rice remains at the flowering and grain filling stage at that period. If water is not supplied on those farms rice yield will be reduced drastically (Sattar and Parvin, 2009). The changing pattern of CRDs (Continuous Rainless Days) play a considerable negative role on transplanted *aman* production and might have an effect on water requirement in near future during this period (Rashid, 2008). The intensity of rainfall in growing period of transplanted *aman* increased but frequency and distribution pattern changed which might occur to ensure the fulfillment of water requirement demand for a certain time but not for a definite time interval which is very essential to harvest good yield for any type of agriculture crop (Basak, 2011). Rising levels of $CO_2$ and other GHGs have been recommended as causes of variations of rainfall that are characterized as climate change. There are strong evidences that climate change will change the rainfall pattern and consequently more frequent droughts are happened. During the *kharif* season, it causes significant destruction to the transplanted *Aman* crop in approximately 2.32 million hectare every year (Dey *et al*., 2011).

Thus it is necessary to take appropriate actions to mitigate the effect of drought in order to increase the yield of *aman* rice. Mitigation of drought can be done by-(i) supplemental irrigation, (ii) other management practices, such as changes of sowing time, fertilizer management etc. and (iii) cultivating drought tolerant varieties. It has become very expensive to irrigate b*oro* rice field and sometimes the farmers are unable to provide irrigation in *boro* rice field. In such a situation, providing irrigation in the *aman* rice field is another difficult task. Usually *aman* rice is a rainfed crop in Bangladesh. If there is no rain in proper time, the irrigation becomes necessary. It is also quite impossible to conserve soil moisture in such a big cultivable land through mulching. So, it is the best option to cultivate drought tolerant rice varieties in *aman* season.

Drought is one of the major causes for low crop yield worldwide, reducing average yields by 50% or more (Wang *et al*, 2004). In Bangladesh, the drought prone areas are mainly located in the north-western and northern regions and spread over an area of 5.46 million hectare in the districts of Chapai Nawabganj, Naogaon, Rajshahi, Natore, Rangpur, Dinajpur, Joypurhat, Pabna and Bogra. Among the regions of the north-western Barind tract is specially drought prone. Droughts are associated with the late arrival or an early withdrawal of monsoon rains and also due to intermittent dry spells coinciding with critical stages of transplanted *aman* rice. Droughts in May and June can destroy broadcast *aman*, *aus* and jute. Inadequate rains in July can delay transplantation of *aman* in high Barind areas, while droughts in September and October reduce yields of both broadcast and transplanted *aman* and delay the sowing of pulses and potatoes. *Boro* rice, wheat and other crops grown in the dry season are also periodically affected by drought. Due to drought severity, crop loss ranges between 20 and 60 percent or even more for transplanted *aman* and other rice varieties. The agricultural drought, linked to soil moisture scarcity, occurs at different stages of crop growth, development and reproduction. A strong drought can cause greater than 40% damage to broadcast *aus*. During the *kharif* season, it causes significant destruction to the transplanted *aman* crop in approximately 2.32 million hectare every year. In the *rabi* season, about 1.2 million hectare of agricultural land face droughts of different magnitudes (Dey *et al*., 2011).

*Aman* rice is generally cultivated under rainfed condition during June-December. Normally, this crop suffers from moisture stress when the rainfall ceases by the first week of October. It passes through reproductive stages (panicle initiation, booting, flowering and grain filling) in October and November. The total rainfall in these two months is very irregular and often inadequate in Bangladesh which fails to meet the evapotranspirational demand of *aman* rice, consequently water stress develops and affects translocation of assimilates and grain development in rice. Sometimes, due to lack of rainfall, the upland transplanted *aman* rice suffers from moisture stress during vegetative stage also. The *aman* rice crop is vulnerable to drought due to uncertain rain and its uneven distribution. During 2014, total *aman* production area was 5.53 million hectare of land including transplanted and broadcast *aman* in Bangladesh. The yield levels of *aman* genotypes are much lower than other countries and drought is one of the major causes for this yield reduction. In such a situation we should be more conscious about the drought tolerance as well as the water requirement of our traditional high yielding *aman* rice varieties.

A lot of research works have been done on drought tolerance of rice worldwide and were published in renowned journals. But in Bangladesh, research on drought tolerance as well as the soil water requirement of *aman* rice is little. The previous drought researches of *aman* rice were confined on breeding for drought tolerance and the effect of drought on *aman* rice only. Research on the identification of drought tolerant characters and determining the level of drought tolerance of different *aman* rice is new one.

Drought tolerance can broadly be achieved through three major mechanisms: (1) Drought escape due to early completion of the life cycle; in such cases, early flowering varieties can escape terminal drought. (2) Dehydration avoidance which enables the plant to uptake or conserve more water to avoid dehydration; this is achieved through traits related to root architecture, stomatal control and transpiration efficiency. (3) Dehydration tolerance which is achieved through traits such as cell membrane stability, osmotic adjustment, stem reserve mobilization and stability of flowering process (Dixit *et al*., 2014). Drought tolerance in rice is a complex trait collectively determined by numerous component traits (Verulkar and Verma, 2014). It is strongly believed that understanding of a physiological and molecular basis may help target the key traits that limit yield. Such approach may complement conventional breeding programs and hasten yield improvement (Cattivelli *et al*., 2008). In order to develop drought tolerant varieties, a thorough understanding of the various morphological, biochemicals, physiological and anatomical characters that govern the yield of rice under water stress condition are prerequisites. There is lack of information about these characters of our traditional high yielding *aman* varieties. On the other hand, information about the response of *aman* rice genotypes under different soil moisture levels is also very little.

The physiological potentiality of drought tolerance of different *aman* rice varieties should be studied so that tolerant varieties may be identified. Alternatively, yield improvement in water-limited environments could be achieved by identifying the traits contributing to drought resistance and selecting for those traits in breeding programs. Selection on the basis of a single parameter could not provide the true picture and different parameters should be studied for identifying drought tolerant genotypes as well as the drought tolerant characteristics of *aman* rice. BRRI (Bangladesh Rice Research Institute) released two *aman* varieties (BRRI dhan56, BRRI dhan57) for the cultivation in the drought-prone areas (mainly for barind tracts and north western areas) in Bangladesh. BINA (Bangladesh Institute of Nuclear Agriculture) also released a *aman* variety (Binadhan-7) for the cultivation in drought-prone areas. But the drought tolerant characteristics of those varieties not yet been studied in detail. We need to identify the drought tolerant characters for further improvement of these genotypes and to incorporate these tolerant genes to our traditional high yielding *aman* rice varieties. Furthermore, it is also important to know the response of those varieties under different soil moisture levels in order to know the drought tolerant level of those varieties for the cultivation in different drought-prone areas of Bangladesh; because the traditional practice of abundant water environment for rice cultivation needs to be reexamined as water is becoming increasingly scarce. As the demand for effective management of water resource increase, future rice production will therefore depend heavily on developing and adopting strategies and practices that will use efficient irrigation schemes (Guerra *et al.*, 1998). Through this way the drought effect on the *aman* rice could be reduced and got better harvest. But, little research has been done on these aspects. So, the ultimate goal of the present study was to find out the drought tolerant characters as well as the tolerant levels of the varieties studied. The findings of the present experiment may facilitate designing suitable screening criteria for breeding program. Three pot culture experiments and one experiment with nutrient solution through hydroponics system were conducted in order to achieve that goal; accordingly, different morpho-physiological, anatomical and biochemical parameters were recorded to understand the response of different *aman* rice varieties to different levels of soil moisture stress.

**Objectives**

1. To study the response of different *aman* rice varieties to different soil moisture levels considering their morpho-physiological, biochemical and anatomical parameters,
2. To identify the characters that contribute to drought tolerance, and

3. To identify the strongly drought tolerant rice genotypes (among the tested varieties) suitable for cultivation in highly drought-prone areas.

# CHAPTER 2

# REVIEW OF LITERATURE

## 2.1. Rice under limited soil moisture condition

Plants capture solar energy and atmospheric carbon dioxide ($CO_2$) through photosynthesis, which is the primary component of crop yield, and needs to be increased considerably to meet the growing global demand for food. Environmental stresses are increasing with climate change, adversely affect photosynthetic carbon metabolism (PCM) and limit yield of cereals such as rice (*Oryza sativa*) that feeds half of the world population (Ambavaram *et al*., 2014). Drought is the most important limiting factor affecting rice production. Passioura (2007) stated that drought has become a problem for rice developing countries all over the world. Currently, the situation has become more severe by climate change (Kawasaki and Herath, 2011; Prasad *et al*., 2012). Rice is a semi-aquatic plant that grows normally in flooded conditions. However, almost 50% of the paddy field system does not have sufficient water for irrigation. Therefore, a serious drought problem can affect rice production and quality (Maisura *et al*., 2014). To ensure that food supplies keep pace with population growth, a complete understanding of the processes involved in crop growth and development is required. The optimization of plant performance and crop sustainability under variable environmental stress conditions will be dependent on the degree to which plant vegetative and reproductive growth patterns can be regulated. Plant growth is a function of complex interplay between sources and sink limitations of the two main organs of a plant, the root system and the shoot. The permanent or temporary water deficit severely hampers the plant growth and development more than any other environmental factor. Water stress trigger a wide range of plant responses, ranging from altered gene expression and cellular metabolism to changes in growth and productivity.

### 2.1.1. Effect on rice morphology

Huang *et al.* (2014) observed that rice (*Oryza sativa*. L) is more sensitive to drought stress than other cereals, and large genotypic variation in drought tolerance (DT) exists within the cultivated rice gene pool and its wild relatives. Harris *et al*. (2002) revealed that the first and foremost effect of drought is impaired germination and poor stand establishment. Cell growth is considered one of the most drought-sensitive physiological processes due to the reduction in turgor pressure. Growth is the result of daughter-cell production by meristematic cell divisions and subsequent massive expansion of the young cells. Under severe water deficiency condition, the cell elongation of higher plants can be inhibited by interruption of water flow from the xylem to the surrounding elongating cells (Nonami, 1998). Hussain *et al*. (2008) stated that drought caused impaired mitosis; cell elongation and expansion resulted in reduced growth and yield traits. They also stated that the water deficits reduce the number of leaves per plant and individual leaf size, leaf longevity by decreasing the soil's water potential. Leaf area expansion depends on leaf turgor, temperature and assimilates supply for growth. It was reported that Drought-induced reduction in leaf area is due to suppression of leaf expansion through reduction in photosynthesis (Rucker *et al*., 1995). It was also reported that the common adverse effect of water stress on crop plants is the reduction in fresh and dry biomass production (Zhao *et al*., 2006).

#### 2.1.1.1. Leaf area under water stress

Zubaer *et al.* (2007) stated that the interaction effect of different moisture levels and rice genotype of leaf area per hill at all growth stages was significant. They also reported that at booting stage, the highest leaf area was found at 100% FC in different rice genotypes and the leaf area was reduced with the reduction of moisture levels but the degree reduction was not similar for all genotypes. It was reported that the effect of drought stress on leaf area at flowering and maturity stages was more or less similar as booting stage. They also found that the flowering stage was more critical than other stages. It was also reported that the reduced soil moisture levels produced lower leaf area; might be due to inhibition of cell division of meristematic tissue under water starved condition (Aggarwall and Kodundal, 1988 and Hossain, 2001).

#### 2.1.1.2. Leaf rolling under water stress

The leaf rolling under water stress condition was observed by Zulkarnain *et al*. (2009) and found that the sensitive rice varieties showed higher leaf rolling score and the tolerant cultivars showed lower leaf rolling. It was also reported that after a long time drought condition the leaves of all rice cultivars (tolerant and sensitive) were rolled at midday.

#### 2.1.1.3. Root under water stress

Roots are the place where plants first encounter drought stress, it is likely that roots may be able to sense and respond to the stress condition. It was stated that significant progress has been made in understanding root growth under drought stress (Sharp *et al*., 2004). However, there has been no genetically defined drought-adaptive response in root development. Xiong *et al*. (2006) stated that Arabidopsis seedlings can give rise to a significant amount of lateral roots within 1 week on the control plate, whereas the elongation of lateral roots is significantly inhibited under drought treatment. Zulkarnain *et al*. (2009) observed that the root depth of rice was reduced when the soil subjected to drought condition (water stress) for different rice varieties. They also observed that under well watered treatment, root depth was higher than under water stress condition for different rice varieties. Under well watered treatment, the root depth of drought tolerant and drought susceptible genotypes were comparable with a minor difference. The susceptible variety indicated lower root depth under water stress treatment and the tolerant variety indicated a higher root depth than the other varieties under the water stress treatment. Therefore, under water deficit conditions, it is assumed that osmotic adjustment in the root occurs before that in the leaf, to enhance turgor pressure for continued root growth and absorption of water and nutrients. Thus, osmotic adjustment in the root is expected to delay the onset of water deficit in the shoots, which reduces the activity of stomatal conductance and photosynthetic activity. It was also reported that the changes of soil moisture not only significantly affect the spatial distribution of crop roots and the efficiency of nutrition and water adsorption (Sarani *et al*., 2014).

#### 2.1.1.4. Root-shoot ratio under water stress

The root shoot ratio is reduced when the soil is subjected to drought condition (water stress) for different rice varieties. It is reported that under the well watered treatment, the root shoot ratio was higher than under water stress treatment for different rice varieties. Under the well watered treatment, drought tolerant and drought susceptible genotypes do not show much difference in the root shoot ratio. However, under water stress treatment drought tolerant genotypes show high root shoot ratio than the other varieties under the water stress treatment.

#### 2.1.1.5. Total dry matter content under water stress

It was found that different moisture levels and rice genotypes interacted significantly for producing total dry matter per hill (Zubaer *et al*., 2007). They also found that total dry matter per hill was maximum at 100% FC followed by 70%FC and it was lowest at 40% FC in different rice genotype at all growing stages. So, total dry matter production is decreased with decreasing soil moisture levels. Decreased total dry matter under lower soil moisture might be due to inhibited photosynthesis. The results confirm with Hossain (2001). But the degree of reduction was different in different genotypes.

### 2.1.2. Effect on rice physiology

#### 2.1.2.1. Relative water contents of leaf under water stress

Relative water content (RWC), leaf water potential, stomatal resistance, rate of transpiration, leaf temperature and canopy temperature are 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 dehydration tolerance. RWC of leaves is higher in the initial stages of leaf development and declines as the dry matter accumulates and leaf matures. RWC related to water uptake by the roots as well as water loss by transpiration. A decrease in the relative water content (RWC) in response to drought stress has been noted in wide variety of plants as reported by Nayyar and Gupta (2006) that when leaves are subjected to drought, leaves exhibit large reductions in RWC and water potential. It was also reported that the exposure of plants to drought stress substantially decreased the leaf water potential, relative water content and transpiration rate, with a concomitant increase in leaf temperature (Siddique *et al*., 2001).

It was reported that although components of plant water relations are affected by reduced availability of water, stomatal opening and closing is more strongly affected. Moreover, change in leaf temperature may be an important factor in controlling leaf water status under drought stress. Drought-tolerant species maintain water-use efficiency by reducing the water loss. However, in the events where plant growth was hindered to a greater extent, water-use efficiency was also reduced significantly. Zulkarnain *et al*. (2009) also observed that the relative water contents of different rice varieties were similar under the well-watered condition on all the measurement occasions. However, RWC declines progressively in stressed plots with the development of severe water deficit. The decline in the RWC is more rapid in susceptible rice genotypes than in the tolerant varieties. They also reported that the tolerant has relatively higher water content than the other varieties, even after 10 days of exposure to soil drying. They also found that all the varieties had similar and lowest values of the RWC at the end of the soil drying cycle. Sinclair and Ludlow (1985) proposed that RWC is a better measure for plant's water status than thermodynamic state variables (water potential, turgor potential and solute potential). In an experiment, Bayoumi *et al*. (2008) determined the RWC of some wheat genotypes to give indication on the plant water status under drought condition and found that RWC decreased with water stress in all the tested genotypes. Similar observations have been reported in Common Bean by Korir *et al*. (2006). The genotypic variation in RWC may be attributed to differences in the ability of the genotypes to absorb more water from the soil and or the ability to control water loss through the stomata.

**2.1.2.2. Root signaling under drought stress**

An extensive root system is advantageous to support plant growth during the early crop growth stage and extract water from shallow soil layers that is otherwise easily lost by evaporation. There are controversial evidences on effect of drought stress on root growth. Wu and Cosgrove (2000) stated that in General, when water availability is limited, the root: shoot ratio of plants increases because roots are less sensitive than shoots to growth inhibition by low water potentials. They also reported that under drought stress conditions roots induce a signal cascade to the shoots via xylem causing physiological changes eventually determining the level of adaptation to the stress. It was revealed that the abscisic acid (ABA), cytokinins, ethylene, malate and other unidentified factors have been implicated in the root–shoot signaling. This drought-induced root-to-leaf signalling through the transpiration stream results in stomatal closure, which is an important adaptation to limited water supply in the field. ABA promotes the efflux of $K^+$ ions from the guard cells, which results in the loss of turgor pressure leading to stomata closure. Dehydration of plants has been shown to cause ABA level increase up to 50-fold due to loss of cell turgor or cell membrane perturbation (Guerrero and Mullet, 1986).

### *2.1.2.3. Stomatal conductance under water stress*

It was reported that the stomatal conductance decreased in different rice varieties of rice as the intensity of water deficit increased with the time of soil drying. It was found that stomatal conductance of drought susceptible rice varieties declined more rapidly than in other varieties. After long day drought period all varieties showed a considerable decrease in stomata conductance. The tolerant one exhibited a higher stomatal conductance under stress than the other varieties. It was also stated that the stomatal conductance is dependent on some factors that are also altered by drought; among which are external factors such as soil water availability, vapor pressure deficit, and so on, and internal factors such as abscisic acid (ABA), xylem conductivity, leaf water status, and so on. Under drought a chemical signal is produced by ABA in plant roots, which is synthesized in the roots in response to soil drying. This signal is transported from the root to leaf through the xylem by the transpiration stream; this is thought to induce stomatal closure (Wilkinson and Davies, 2008).

### 2.1.2.4. Photosynthesis under water stress

Allen and Ort (2001) reported that the environmental stresses have a direct impact on the photosynthetic apparatus, essentially by disrupting all major components of photosynthesis including the thylakoid electron transport, the carbon reduction cycle and the stomatal control of the $CO_2$ supply, together with an increased accumulation of carbohydrates, peroxidative destruction of lipids and disturbance of water balance. It was also reported that the ability of crop plants to acclimate to different environments is directly or indirectly associated with their ability to acclimate at the level of photosynthesis, which in turn affects biochemical and physiological processes and, consequently, the growth and yield of the whole plant (Chandra, 2003). Menconi *et al*. (1995) observed that the drought stress severely hampered the gas exchange parameters of crop plants and this could be due to decrease in leaf expansion, impaired photosynthetic machinery, premature leaf senescence, oxidation of chloroplast lipids and changes in structure of pigments and proteins. Lauteri *et al.* (2014) stated that drought stress induced reductions in yield and rates of gas exchange across in different varieties of *O. sativa* in their study. Many studies have shown the decreased photosynthetic activity under drought stress due to stomatal or non-stomatal mechanisms (Ahmadi, 1998; Del Blanco *et al*., 2000; Samarah *et al*., 2009). Stomata are the entrance of water loss and $CO_2$ absorbability and stomatal closure is one of the first responses to drought stress which result in declined rate of photosynthesis. It was stated that the stomatal closure deprives the leaves of $CO_2$ and photosynthetic carbon assimilation is decreased in favor of photorespiration. Considering the past literature as well as the current information on drought-induced photosynthetic responses, it is evident that stomata close progressively with increased drought stress. It is well known that leaf water status always interacts with stomatal conductance and a good correlation between leaf water potential and stomatal conductance always exists, even under drought stress. It is now clear that there is a drought-induced root-to-leaf signaling, which is promoted by soil drying through the transpiration stream, resulting in stomatal closure which ultimately affects the photosynthesis. The "non-stomatal" mechanisms include changes in chlorophyll synthesis, functional and structural changes in chloroplasts, and disturbances in processes of accumulation, transport, and distribution of assimilates. It was also reported that the physiological analysis of *O. sativa* has great potential in identifying those photosynthetic and leaf gas-exchange characteristics that confer attributes such as high yield and tolerance to drought (Lauteri *et al*., 2014).

#### 2.1.2.5. Osmolyte accumulation

Rhodes and Samaras (1994) stated that plants accumulate different types of organic and inorganic solutes in the cytosol to lower osmotic potential thereby maintaining cell turgor. Under drought, the maintenance of leaf turgor may also be achieved by the way of osmotic adjustment in response to the accumulation of proline, sucrose, soluble carbohydrates, glycinebetaine, and other solutes in cytoplasm improving water uptake from drying soil. The process of accumulation of such solutes under drought stress is known as osmotic adjustment which strongly depends on the rate of plant water stress. Among these solutes, proline is the most widely studied because of its considerable importance in the stress tolerance. Proline accumulation is the first response of plants exposed to water-deficit stress in order to reduce injury to cells. It was reported that the proline can act as a signaling molecule to modulate mitochondrial functions, influence cell proliferation or cell death and trigger specific gene expression, which can be essential for plant recovery from stress (Szabados and Savoure´, 2009). Accumulation of proline under stress in many plant species has been correlated with stress tolerance, and its concentration has been shown to be generally higher in stress-tolerant than in stress-sensitive plants. It also revealed that proline influences the protein solvation and preserves the quarternary structure of complex proteins, maintains membrane integrity under dehydration stress and reduces oxidation of lipid membranes or photoinhibition (Demiral and Turkan, 2004). Furthermore, Ashraf and Foolad, (2007) stated that proline also contributes to stabilizing sub-cellular structures, scavenging free radicals, and buffering cellular redox potential under stress conditions. Osmolytes have some important role in plant responses to water stress and resistance. They also indicated that the accumulation of compatible solutes in plants causes resistance to various stresses such as drought, high temperature and high salinity. Compatible solutes are divided into three major groups - amino acids (e.g. proline), polyamins and quaternary amines (e.g. glycinebetaine, dimethylsulfoniopropionate), polyol (e.g. mannitol, trehalose) and sugars like sucrose and oligosacharids. Free proline is believed to play a key role in cytoplasmic tolerance in many species and, therefore, in the resistance of the whole plant to severe drought. Sugars can play a role in osmoregulation under a drought condition in many plants. Many studies also indicated that solute accumulation under water stress contributes to inhibition of shoot growth. It is clear because compatible solute synthesis and accumulation need high energy level.

### 2.1.3. Effect on rice biochemistry

#### 2.1.3.1. Chlorophyll contents under water stress

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 drought stress has been considered a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation. Farooq *et al.* (2009) reported that the photosynthetic pigments are important to plants mainly for harvesting light and production of reducing powers. Both the chlorophyll a and b are prone to soil dehydration. It was also stated that the decreased or unchanged chlorophyll level during drought stress has been reported in many species, depending on the duration and severity of drought (Kyparissis *et al.,* 1995; Zhang and Kirkham, 1996). It was indicated that the loss of chlorophyll contents under water stress is considered a main cause of inactivation of photosynthesis. Furthermore, it was also described that the 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 (Kaiser *et al*., 1981). They also observed that the low concentrations of photosynthetic pigments can directly limit photosynthetic potential and hence primary production. Thus the 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. It was reported that the chlorophyll content was decreased with decreasing the irrigation water and this decrease was correlated with relative water content in leaves (Munne-Bosch and Alegre, 2000). Although studies in relation to cultivation techniques in rosemary have been realized, the agronomic and physiological responses to irrigation are scarce (Nicola´s *et al*., 2008). There have been few studies performed. Munne-Bosch and Alegre (1999) stated that the chlorophyll loss is a negative consequence of water stress; however, it has been considered as an adaptive feature in plants grown under water deficit. In addition, the negative effect of deficit irrigation was reflected in decreasing the chlorophyll content of rosemary leaves. On the other hand, some authors found an opposite trend since chlorophyll increased by deficit irrigation. Khayatnezhad *et al*. (2011) and Alaei (2011) reported that drought stress condition increased the leaf chlorophyll content in wheat genotypes. It was also reported that the exact effect of deficit irrigation may vary according to the intensity of the water stress imposed (Cameron *et al*., 1999).

**2.1.3.2. Proline content under water stress**

Many studied indicated that the accumulation of proline was affected by water regimes and rice varieties and the accumulation of proline was higher under water stress treatment than the well watered treatment for different rice varieties. The level of increase in the proline concentration in response to water stress varied among the rice varieties. Production and accumulation of free amino acids, especially proline by plant tissue under water deficit conditions is an adaptive response. Proline has been proposed to act as a compatible solute that adjusts the osmotic potential in the cytoplasm (Caballero *et al*., 2005) and its content can be used as a physiological marker in relation to osmotic stress (Sarani *et al*., 2014).

Maisura *et al.* (2014) reported that the interaction between drought and variety has a significant effect on proline accumulation at the ages of 11 and 13 weeks after transplant of rice. It was found that the proline accumulation is an early response when a plant is experiencing a water deficit, in which it decreases cell damage (Anjum *et al*., 2011). They also reported that the proline accumulation did not only happen in a tolerant variety, but also on susceptible varieties. However, it was revealed that the drought-tolerant varieties can accumulate proline for a longer period of time than susceptible varieties (Saruhan *et al.*, 2006). Sharma and Dietz (2006) stated that proline plays an important role as an osmoprotectant, an energy sink to regulate redox potential, and as a radical hydroxyl scavenger. It was also stated that the proline protects macromolecules from denaturization and also reduces acidity in the cell (Kishor *et al.,* 2005) and acts as an antioxidant (Vendruscolo *et al*., 2007). According to Szabados and Savoure (2009) proline can act as a molecular signal to modulate mitochondrial function, affect cell proliferation or cell death, and initiate certain gene expression that is important role in protecting plants from stress.

#### 2.1.3.3. Sugar and starch content under water stress

Sugar accumulation in rice varieties is severely affected by drought. Total sugar accumulation is one mechanism for a tolerant plant in facing drought. Kishor *et al*., (2005) stated that the total sugar accumulation also plays a role in substrate hydrolysis in a biosynthetic process, producing energy and acts as a sensor and signal. It also functions as a typical osmoprotectant to maintain cell stability and maintain turgor pressure. It was reported that during drought stress, total sugar accumulation increases especially in the stem (Hu *et al*., 2006). Total sugar accumulation in the organ of a tolerant variety is more effective because of the high membrane stability and low water loss than susceptible varieties (Valentovic *et al*., 2006).

Maisura *et al.* (2014) reported that the varieties that are relatively tolerant to drought, clearly demonstrate that total sugar accumulation is significantly different in both the control and drought treatment during anthesis phase and harvesting stage. This is due to most sugar and starch in the stem being actively relocated to seeds. Mc Dowell and Sevanto (2010) reported that drought can block carbohydrate transport, usage, and mobilization. If severe drought happens, then the plant will die if it reaches its critical point. Therefore, a starch content variable that was observed during the stage of anthesis and harvest in drought treatment can give information on how varieties experience blocked carbohydrate transport caused by water inefficiency in susceptible varieties. Kumar *et al*. (2014) found that physiological and biochemical traits, viz. leaf area index (LAI), relative water content (RWC), membrane stability index (MSI), total soluble sugar and starch were significantly reduced by drought stress at reproductive stage

**2.1.3.4. Reactive oxygen species (ROS) content under water stress**

The generation of reactive oxygen species (ROS) is one of the earliest biochemical responses of eukaryotic cells to biotic and abiotic stresses. It was reported that the production of ROS in plants, known as the oxidative burst, is an early event of plant defense response to water-stress and acts as a secondary massager to trigger subsequent defense reaction in plants. ROS, which include oxygen ions, free radicals and peroxides, form as a natural byproduct of the normal metabolism of oxygen and have important role in cell signaling. However, it was found that during environmental stress such as drought, ROS levels increase dramatically resulting in oxidative damage to proteins, DNA and lipids (Apel and Hirt, 2004). Being highly reactive, ROS can seriously damage plants by increasing lipid peroxidation, protein degradation, DNA fragmentation and ultimately cell death.

Farooq *et al*. (2009) stated that the drought induces oxidative stress in plants by generation of reactive oxygen species (ROS). It was reported that the ROS such as $O^{2-}$, $H_2O_2$ and $OH^-$ radicals, can directly attack membrane lipids and increase lipid peroxidation (Mittler, 2002). Drought-induced overproduction of ROS increases the content of malondialdehyde (MDA). The content of MDA has been considered an indicator of oxidative damage (Moller *et al*., 2007). They also stated that MDA is considered as a suitable marker for membrane lipid peroxidation. A decrease in membrane stability reflects the extent of lipid peroxidation caused by ROS. Furthermore, lipid peroxidation is an indicator of the prevalence of free radical reaction in tissues. Moreover, oxygen uptake and loading on the tissues generate reactive oxygen species, particularly $H_2O_2$ that produced at very high rates by the glycollate oxidase reaction in the peroxisomes in photorespiration.

### 2.1.3.5. Antioxidant enzymes under water stress

It was reported that there is a defensive system in plants, that is to say, plants have an internal protective enzyme-catalyzed clean up system, which is fine and elaborate enough to avoid injuries of active oxygen, thus guaranteeing normal cellular function (Horváth *et al*., 2007). Moller *et al.* (2007) reported that the balance between ROS production and activities of antioxidative enzyme determines whether oxidative signaling and/or damage will occur. It was also reported that to minimize the affections of oxidative stress, plants have evolved a complex enzymatic and non-enzymatic antioxidant system, such as low-molecular mass antioxidants (glutathione, ascorbate, carotenoids) and ROS-scavenging enzymes (superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX) (Apel and Hirt, 2004). Non-enzymatic antioxidants cooperate to maintain the integrity of the photosynthetic membranes under oxidative stress. The enzymatic components may directly scavenge ROS or may act by producing a non-enzymatic antioxidant. $O^{2-}$ can be dismutated into $H_2O_2$ by SOD in the chloroplast, mitochondrion, cytoplasm and peroxisome. POD plays a key role in scavenging $H_2O_2$ which was produces through dismutation of $O^{2-}$ catalyzed by SOD. CAT is a main enzyme to eliminate $H_2O_2$ in the mitochondrion and microbody (Shigeoka *et al*., 2002) and thus help in ameliorating the detrimental effects of oxidative stress. It is found in peroxisomes, but considered indispensable for decomposing $H_2O_2$ during stress. Sharma and Dubey (2005) stated that maintaining a higher level of antioxidative enzyme activities may contribute to drought induction by increasing the capacity against oxidative damage. The capability of antioxidant enzymes to scavenge ROS and reduce the damaging effects may correlate with the drought resistance of plants. Free oxygen radicals, produced as the usual secondary consequence of environmental stresses, are very dangerous for cell components and must be precisely regulated. So, it is clear that all plants have developed several antioxidant systems, both enzymatic and non-enzymatic, to scavenge these toxic compounds. The degree of activities of antioxidant systems under drought stress is extremely variable. The defining factors include variation in plant species, in the cultivars of the same species, development and the metabolic state of the plant, and the duration and intensity of the stress, etc.

### 2.1.4. Effect on rice anatomy

Zhang *et al.* (2015) stated that in the majority of the plant species, water stress is linked to changes in leaf anatomy and ultrastructure. Shrinkage in the size of leaves, decrease in the number of stomata; thickening of leaf cell walls, cutinization of leaf surface, and underdevelopment of the conductive system- increase in the number of large vessels, submersion of stomata in succulent plants and in xerophytes, formation of tube leaves in cereals and induction of early senescence are the other reported morphological changes.

### 2.1.5. Effect on yield characters

Many yield-determining processes in plants respond to water stress. Yield integrates many of these processes in a complex way. Grain yield is the result of the expression and association of several plant growth components. Farooq *et al*. (2009) stated that the deficiency of water leads to severe decline in yield traits of crop plants probably by disrupting leaf gas exchange properties which not only limited the size of the source and sink tissues but the phloem loading, assimilate translocation and dry matter partitioning are also impaired. It was reported that drought stress inhibits the dry matter production largely through its inhibitory effects on leaf expansion, leaf development and consequently reduced light interception (Nam *et al.,* 1998). It was also reported that drought at flowering commonly results in barrenness. A major cause of this, though not the only one, was a reduction in assimilate flux to the developing ear below some threshold level necessary to sustain optimal grain growth (Yadav *et al*., 2004). Many authors revealed the drought-related reduction in yield and yield components of plants could be ascribed to stomatal closure in response to low soil water content, which decreased the intake of $CO_2$ and, as a result, photosynthesis decreased (Chaves, 1991; Cornic, 2000; Flexas *et al*., 2004). So, drought reduces plant growth and development, leading to hampered flower production and grain filling and thus smaller and fewer grains. A reduction in grain filling occurs due to a reduction in the assimilate partitioning and activities of sucrose and starch synthesis enzymes. Verulkar and Verma (2014) stated that the most robust and integrative selection criteria for drought tolerant are biomass accumulation and yield performance. Grain yield under defined stress condition should be the primary criterion for selection.

Maisura *et al.* (2014) found significant differences in grain yield per hill in rice varieties under control and drought conditions. Drought stress reduced grain yield of different rice varieties. Rice is a crop that is very sensitive to water shortage in the reproductive phase, with water shortage leading to a higher reduction in grain yield. Decrease in grain yield is due to reduced panicle formation and high sterility. Liu *et al.*, (2008) reported that water stress can abort pollination for up to 67 percent of total grain per panicle. Pollen cannot be on the surface of the flower because flowers fail to open due to drought. Grain yield is a multigenic factor, with susceptible varieties producing high grain yield under normal conditions, but it also shows a high percent reduction over control during drought. It was reported that the yield stability in tolerant varieties was due to specific adaptive feature that make them able to produce stable grain yield even under stress (Van Heerden and Laurie, 2008; Liu *et al*., 2008). Kumar *et al.* (2014) also found increase in percent spikelet sterility under depleting of soil moisture during the reproductive stage resulted in decreased grain yield under stress condition.

### 2.1.6. Drought tolerance and its mechanism

It was stated that the acclimation of plants to water deficit leads to adaptive changes in plant growth and physio-biochemical processes, such as changes in plant structure, growth rate, tissue osmotic potential and antioxidant defenses (Duan *et al*., 2007).

#### 2.1.6.1. Root and shoot traits for drought tolerance

Different root traits, that are related to drought tolerance are- (1) deeper thicker roots (2) greater root volume (3) greater root penitration ability (4) high dry weight (5) polling resistance (6) higher number of xylem vessels (7) presence of parenchyma, sclerenchyma and (8) high fresh weight.

Different shoot traits, that are related to drought tolerance are- (1) greenness of leaf (2) stomatal number and conductance (3) leaf drying (4) epicuticular wax (5) osmotic adjustment (6) leaf rolling (7) water use efficiency (8) membrane stability (9) proline content (10) relative water content (11) water use efficiency (12) sugar content (13) leaf temperature (14) spikelet sterility (15) harvest index (16) carbon descrimination (17) total dry matter (18) grain yield (19) delay flowering (20) transpiration under stress (21) photosynthesis under stress and (22) recovery after rewatering.

### 2.1.6.2. Leaf anatomy and drought Tolerance

Palisade mesophyll (PM) cells are a site for 90% of active photosynthesis of plant, increased height of palisade mesophyll positively increases the rate of photosynthesis. Thicker layer of collenchyma, larger phloem width, higher number of xylem poles per cross-section and compact parenchyma are tolerant characters.

KulKarni *et al*. (2008) stated that the intercellular spaces between palisade mesophyll cells increase the volume of the leaf which comes in contact with air leading to a higher loss of water from palisade mesophyll cells, increasing transpirational losses and ultimately resulting in reduced growth and rate of photosynthesis. As a result, cellular growth is also hampered, which mainly depends on the maintenance of turgor pressure. In addition to the increased height of palisade mesophyll cells, compactness also affects the rate of photosynthesis. Different results lead to a conclusion that the compactness of palisade mesophyll cells and reduced air spaces by the smaller area of spongy mesophyll cells can be an ideal trait for the highest photosynthetic ability of leaves. If palisade mesophyll cells are well developed, the transpiration rate lowers due to the compactness of the tissue. On the other hand, if loose spongy mesophylls are well developed (like in the case of drought susceptible genotypes), the rate of transpiration is greater, which ultimately leads to the reduced rate of photosynthesis.

### 2.1.6.3. Morpho-anatomical correlations for drought tolerance

Besides anatomical features some morphological aspects of roots affect drought tolerance. The drought tolerant genotypes exhibit larger roots along with higher density of secondary or tertiary roots. The basis for a difference between cultivated and normal genotypes was in the root growth habit. Resistant genotypes were observed to show deep and vertical growth habit whereas cultivated genotypes showed shallow growth and a lower number of roots. Courtois *et al*. (1996) studied variability in the root depth of upland rice, which is used as a potential donor for longer root traits. Lafine *et al*. (2001) reported highly significant genotypic variance for both maximum root depth and deep root weight comparing traditional Australian varieties and temperate Japonicas. Yadav *et al.* (1997) reported 0.77 broad-sense heritability for root length. The root depth and deep root weight are quantitatively controlled in a doubled haploid population from *Indica* × *Japonica* segregants.

KulKarni *et al*. (2008) revealed that the drought resistant genotypes exhibited longer and deeper roots whereas the root growth of cultivated genotypes was shallow. Prospects would be better if both types of root growth habits could be incorporated into a single genotype which will have an advantage of moisture absorption during frequent rain situations whereas the part of the deeper root system is utilized for drought situations. This may prove to be an ideotype root system for plants under both drought and normal conditions.

#### 2.1.6.4. Proposed ideotype for increased water use efficiency (WUE) under water stress in plants

Donald (1968) proposed the use of crop ideotype breeding as a way to increase crop yields. The ideotype breeding method offered the framework for applying concepts of light interception and plant competition in development of high-yielding cultivars. It emphasized selection for specific characteristics that affect photosynthesis in a plant and may contribute to higher yields, such as leaf size and position, plant height and larger tillering capacity.

**Highlights of proposed model**

– Longer palisade mesophyll cells
– Reduced air spaces in leaves
– Larger size and lower number of stomata
– Petiole with a higher number of conducting tissues
– Compact parenchyma cells
– Higher proportion of secondary phloem and conducting tissues in stem
– Deeper roots with wider and dense xylem poles
– Higher number of feeder roots and root hairs

Jaleel *et al.* (2009) reported that plants optimize the morphology, physiology and metabolism of their organs and cells in order to maximize productivity under the drought conditions. The reactions of the plants to water stress differs significantly at various organizational levels depending upon intensity and duration of stress as well as plant species and its stage of development. They also reported that the stress resistance in plant is divided into two categories, including stress tolerance and stress avoidance. Drought avoidance is the ability of plant to maintain high tissue water potential under drought conditions, while drought tolerance is a plant's stability to maintain its normal functions even at low tissue water potentials. Drought avoidance is usually achieved through morphological changes in the plant, such as reduced stomatal conductance, decreased leaf area, development of extensive root systems and increased root/shoot ratios. On the other hand, drought tolerance is achieved by cell and tissue specific physiological, biochemical, and molecular mechanisms, which include specific gene expression and accumulation of specific proteins. Anjum *et al*. (2011) stated that the dehydration process of drought-tolerant plants is characterized by fundamental changes in water relation, biochemical and physiological process, membrane structure, and ultrastructure of sub cellular organelles. It was also reported that some plants are able to cope with arid environments by mechanisms that mitigate drought stress, such as stomatal closure, partial senescence of tissues, reduction of leaf growth, development of water storage organs, and increased root length and density, in order to use water more efficiently. Water flux through the plant can be reduced or water uptake can be increased by several physiological adaptations.

# CHAPTER 3

# MATERIALS AND METHODS

The overall research work was consisted of four pot culture experiments in the field that were done within the period from 2012 to 2014. The experiments were done with specific materials and following several methods.

## 3.1. Experiment-I: The morpho-physiological responses of different transplanted *aman* rice varieties to different soil moisture levels.

The present experiment was undertaken with the following objectives-

» to assess the responses of different rice varieties to different soil moisture levels in respect of their morphological and physiological characters

» to identify the important morphological and physiological characters those help plant to withstand water stress.

### 3.1.1. Experimental location and duration

The experiment was conducted at the research farm and Plant Physiology Laboratory, Dept. of Agricultural Botany, Sher-e-Bangla Agricultural University, Dhaka-1207. The experimental field was in upland farm (plot no: 42) of Sher-e-Bangla Agricultural University. The research area was located under the Agro-ecological zone of Madhupur Tract (AEZ-28) which was in 23º77´N latitude and 90º22´E longitudes with an elevation of 9m above the sea level (BCA, 2004). The duration of the experiment was July to November (*aman* season), 2012. The detailed morphological characteristics of the experimental field are given in appendix 2.

### 3.1.2. Climatic condition and soil character

The experimental field was situated in the sub-tropical region characterized by heavy rainfall during the month from May to September and scantly rainfall in the rest of the year. The meteorological data on temperature, relative humidity and rainfall during the crop growth period are presented in appendix 3.

The soil used in this experiment is shallow red brown terrace soil cultivated for long time under Tejgaon series. The surface soil is silty clay and the sub-surface soil is silty clay loam. According to USDA soil classification, the experimental soil was under Ochrept sub-order of Inceptisol order. The soil analysis was done in the department of Soil Science, Sher-e-Bangla Agricultural University, Dhaka. The physical and chemical properties of soil are furnished in appendix 4.

### 3.1.3. Experimental design and treatments

Though the experiment was conducted in pot it was carried out following two factors Randomized Complete Block Design (RCBD) with four replications for better management and reduction of experimental error for analyzing some physiological traits in different days. Because analysis of these traits from each experimental unit was not possible within a day.

**Treatments**

A. Factor1(soil moisture level)
   i) 100% FC (field capacity) moisture = S0
   ii) 70% of the FC moisture = S1
   iii) 40% of the FC moisture = S2
B. Factor 2 (rice genotypes)
   i) BRRI dhan56
   ii) BRRI dhan57
   iii) Binadhan-7
   iv) BRRI dhan49

### 3.1.4. Plant materials (rice variety) collection

Seeds of the rice (*Oryza sativa* L) genotypes BRRI dhan56, BRRI dhan57, Binadhan-7 and BRRI dhan49 were collected from the gene bank of Bangladesh Rice Research Institute (BRRI).

### 3.1.5. Short description of the genotypes

**BRRI dhan49**

Rainfed lowland transplanted *aman* rice variety of BRRI.

**Special characters:**

1. Its vegetative size is like BR11 variety but shorter than BR11.
2. Matured plant height is 100-104 cm.
3. Lifecycle of this variety is 7-10 days earlier than BR11 and Indian SHORNA, that is 132-135 days.
4. The grains remain tightly arranged through the panicle.
5. Ripen paddy is straw colored.
6. Grain size is narrower than BR11 and BRRI dhan32.
7. Boiled rice is nonsticky and tasty.
8. Through proper intercultural operation yield may be 5 to 5.5 t $ha^{-1}$.

**BRRI dhan56**

Rainfed lowland drought tolerant transplanted *aman* rice variety of BRRI.

Special characters:

1. BRRI dhan56 is a drought tolerant, high yielding and short durated rice variety.
2. Its vegetative height is taller than BR11 variety.
3. Matured plant height is 115 cm.
4. Lifecycle of this variety ranges from 105 to 110 days.
5. 1000 filled grain weight is about 23.6 g.
6. Ripen paddy is reddish in color.
7. Grain size is long, heavy and white in color.
8. Boiled rice is non sticky and testy.
9. Yield: 4.5-5 t $ha^{-1}$

**BRRI dhan57**

Rainfed lowland drought avoidance transplanted *aman* rice variety of BRRI.

Special characters:

1. BRRI dhan57 is a moderately drought tolerant, high yielding and short durated rice variety.
2. Its vegetative size and shape is higher than BR11 variety.
3. Matured plant height ranges from 110 to115 cm.
4. Lifecycle of this variety ranges from 100 to105 days.
5. 1000 filled grain weight is about 19.2 g.
6. Ripen paddy is straw colored.
7. Grain size and shape is like traditional JIRASAIL and MINIKET.
8. Boiled rice is non sticky and testy.
9. Yield : 4-4.5 t $ha^{-1}$.

**Binadhan-7:**

Rainfed lowland transplanted *aman* rice variety of BINA.

Special characters:

1. High yielding and short durated rice variety.
2. Crop duration ranges from 110 to 120 days (from seed to seed).
3. More tolerant to diseases and insect-pest.
4. Grain yield ranges from 5 to 5.5 t $ha^{-1}$.
5. Rice long, fine and tasty.

### 3.1.6. Fertilizer application

The pots were fertilized with cow dung 40 g $pot^{-1}$, urea 1.72 g $pot^{-1}$, TSP 1.44 g $pot^{-1}$, MP 0.8 g $pot^{-1}$corresponding to 15 t $ha^{-1}$ cow-dung, 215 kg urea $ha^{-1}$, 180 kg TSP $ha^{-1}$and 100 kg MP $ha^{-1}$ as per recommendation of BRRI. All TSP, MP and 1/3 of the total Urea were applied as basal dose. The remaining 2/3 of the Urea was applied in two equal splits in each pot at 30 and 50 days after transplanting (DAT).

### 3.1.7. Calculation of field capacity

The pot soil was collected from the research field of Sher-e-Bangla Agricultural University. For the calculation of the field capacity a pot (with pore at the base) containing the soil was kept under water for 24 hrs in order to saturate the soil. Then the pot was taken out of water and transferred to normal condition and kept 48 hrs to allow the excess water to leach out of the soil and only capillary water remained in the soil. That moisture content of the soil was treated as 100% field capacity moisture. The weight of the water (needed to attain 100% field capacity) was calculated by subtracting the weight of oven dry soil from the total weight of wet soil at 100% field capacity. Then a little amount of soil was taken from the middle of the pot and the moisture content of the soil was calculated through gravimetric method. Then the moisture content of 70% and 40% field capacity of the soil were calculated respectively.

### 3.1.8. Pot (earthen pot) preparation and maintenance of field capacity

Sufficient number of earthen pots (of appropriate size) was taken as per treatment. Each pot was tagged with enamel paint and weighed separately. The soil was sun dried to reach its moisture content below 10%. Then the moisture content of the soil was calculated through gravimetric method and covered with polythene. Then each pot was filled up with 10 kg of soil with a poly bag to prevent excess water loss. As we predetermined the moisture content of 100%, 70% and 40% of the field capacity soil, then the treatment pots were standardized as 100%, 70% and 40% of FC moisture as per treatment, adding sufficient water. Then each pot was weighed by balance and recorded. These weights were maintained from seedling establishment (10 days after transplanting) to maturity. The fertilizer and plant fresh weights (which were added later on) were deducted from the standard weight. Plants fresh weight was calculated time to time from destructive samples. For the destructive samples there were extra treatment pots. The loss of water from each pot through evaporation and transpiration was compensated adding water every day with the help of balance. The pots were placed under transparent polythene shed in order to prevent rain water.

### 3.1.9. Lay out of the experiment with randomization

| Block-I | Block-II | Block-III | Block-IV |
|---|---|---|---|
| T6 | T1 | T5 | T2 |
| T12 | T5 | T7 | T8 |
| T7 | T8 | T8 | T6 |
| T8 | T6 | T4 | T10 |
| T4 | T12 | T12 | T5 |
| T2 | T7 | T11 | T1 |
| T10 | T4 | T10 | T4 |
| T3 | T10 | T2 | T12 |
| T1 | T11 | T3 | T7 |
| T9 | T2 | T6 | T9 |
| T5 | T9 | T1 | T3 |
| T11 | T3 | T9 | T11 |

### 3.1.10. Seed treatment, germination and sowing

The seeds were treated with Bavistin solution for 20 minutes. The solution was prepared by dissolving 3 g of Bavistin in 1 liter of water. When the treating was completed then the seeds were washed with clean water and then were kept for sprouting in a traditional practice using straw and cloth. After two days all the seeds were well sprouted. The seed beds were prepared in a plastic rack. Each rack was used for a single genotype. A plastic sheet was placed at the bottom of the soil. Fertilizer was applied as per recommendation in the seed beds. After pudling of the soil the sprouted seeds were sown in the separate seed beds as per 60-80 g seeds per 1 sq-meter. Then the seed beds were covered with mosquito net to prevent from bird damage and watered regularly. The seedlings were raised for 30 days in the seed beds.

### 3.1.11. Seed sowing date

Seeds of different genotypes were sown on 15 July 2012.

### 3.1.12. Transplanting

The 30 days old seedlings were transplanted to the pots. The upper soil of the pot was paddled in order to hold the seedling. Each pot contained one healthy seedling only. After seedling stablishment (10 days after transplanting) three soil moisture levels (100% FC, 70% FC and 40% FC) were maintained as per treatment until maturity adding required amount of water (described above). Different intercultural operations were done as and when necessary.

### 3.1.13. Harvesting

Plants were harvested from each experimental unit (earthen pot) by cutting the plants close to the ground. The roots were also collected from pot soil and dried. Harvested crop was allowed to dry in the threshing yard. After complete sun drying, the crop was threshed.

### 3.1.14. Packaging and drying

The plant parts were separated and packages with brown paper packet separately. At first, the packets were dried in the sun and then oven dried for 72 hrs at 70ºC temperature.

### 3.1.15. Data collection

Different data were collected during germination, seedling stage, at anthesis, during grain filling and at maturity on following parameters-

**iv)Plant height at maturity (cm)**

The plant height was measured at maturity from the soil level to the tip of the panicle. It was measured in cm unit.

**v) Tiller number at maturity**

The tiller number of the plant was counted at maturity.

### vi) Duration of different growth and developmental stages (days)

Days to flowering and days to maturity were recorded. The days to flowering was calculated from the period of germination to the date of 50% of the spikelet of the panicle were open for anthesis. The maturity of grain was determined when the grain weight was found maximum and the color of the grain turned yellowish.

### vii) Leaf area (LA), specific leaf area (SLA), specific leaf weight (SLW) and leaf weight ratio (LWR)

LA, SLA and SLW were calculated just after anthesis and LWR was calculated after harvest as Hossain *et al*. (2011). Plant samples were carefully uprooted and separated into leaf, stem and panicle. Leaf area was calculated from average of ten fully expanded leaf including flag leaf which were collected randomly from the whole plant. The area of each leaf blade was computed as follows-

$$\text{Leaf area} = k \times l \times w$$

Where,

k = adjustment factor

l = length of leaf blade

w = breadth of leaf blade

The value of k varied with the genotypes, growth stage and slope of the leaf. To determine the dry weight of leaves, the samples were at first air dried for 6 to 8 hours. Leaves were then packed in separate brown paper packet and were oven dried for 72 hours at 70ºC. Dry weight of leaves was recorded separately with an electronic balance (Type- ELB300, NO-D515710442, INPUT-DC9V/80mA). Average LA, SLA and SLW was taken finally. Dry weight of leaves, stem and panicle were altogether regarded as total above ground dry matter. Different growth and physiological parameters were calculated as follows-

Specific leaf weight (SLW)

$$\text{SLW} = \frac{\text{Dry weight of leaf}}{\text{Leaf area}}\ \text{g/cm}^2$$

Specific leaf area (SLA)

$$\mathrm{SLA} = \frac{\text{Leaf area}}{\text{Dry weight of leaf}}\ (\mathrm{cm^2/\,g})$$

Leaf weight ratio (LWR)

$$\mathrm{LWR} = \frac{\text{Leaf dry weight}}{\text{Above ground dry weight}}$$

**viii) Relative water content (RWC) of flag leaf**

The fully developed flag leave of each plant from four blocks (as per treatment) were carefully collected at anthesis stage. Their fresh weights were measured immediately. Thereafter, the leaves were immersed in distilled water for 24 hrs at room temperature in the dark. These leaves were weighed after removing excess water by gently wiping with paper towel to determine their turgid weight. The leaves were then dried in an oven for 72 hrs at 70 ºC to determine their dry weights. The fresh, turgid and dry weights of the leaves were used to calculate the relative water content of leaves according to Ghannoum *et al*. (2002) as follows-

$$\mathrm{RWC} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

**ix) Leaf rolling score**

Leaf rolling was assessed visually from each treatment. Several tillers were assessed and the pots were given a mean leaf rolling score, ranging from 0 to 5, with 0 being flat and 5 a tightly rolled leaf (O'Toole and Moya, 1978). These ratings were made during midday, i.e. about twice per week during the period of water deficit of all the treatments and the average values were taken.

**x) SPAD (Soil Plant Analysis Development) value**

The greenness of the flag leaf of main tiller of each pot was measured by SPAD meter (model-SPAD-502 Plus, Japan) starting from the day of anthesis to maturity at seven days intervals.

**xi) Leaf conductance / Stomatal conductance (mmol $m^{-2}s^{-1}$)**

The stomatal conductance of main tiller flag leaf of each pot was measured by leaf porometer (G9-Leaf Porometer, model: SC-1,USA) from the day of anthesis to maturity at seven days interval.

**xii) Photosynthetic gas exchange**

**a) Net assimilation rate of $CO_2$ from anthesis to maturity (μ mol $m^{-2}$ $s^{-1}$)**

The net assimilation rate was measured by 'LCpro+ photosynthesis gas exchange system'( model:LI-6400XT, USA) from flag leaf of main tiller of each plant from the day of anthesis to maturity at seven days interval. At the same time, the following data were also recorded from this equipment. The average value of each parameter was taken for analysis.

**b) Intercellular $CO_2$ concentration**

**c) Transpiration rate**

**xiii) Relative growth rate (g $g^{-1}day^{-1}$)**

RGR is a measure to quantify the speed of plant growth. It expresses the rate of increase in dry matter per unit amount of dry matter produced. RGR was calculated growth rate per unit plant weight according to Tanaka *et al*. (1996).

$$\text{RGR (g g}^{-1}\text{day}^{-1}) = \frac{W2 - W1}{W1(T2 - T1)}$$

Where,

W2 = Total dry weight (g) at the time T2

W1 = Total dry weight (g) at the time T1

T2 – T1 = Time interval between first and second sample collection

**xiv) Stem reserve translocation (%)**

To determine the pre-anthesis photosynthetic stem reserve translocation towards the final kernel weight the method described by Gallagher *et al*. (1975) has been used. This is based on the net loss in weight of above ground vegetative organs between anthesis and maturity with the difference in grain weight. It was calculated as follows-

$$\text{Stem reserve translocation (\%)} = \frac{S1 \; - S2}{G2 - G1} \times \; 100$$

Where,

$S_1$ = Stem dry weight at anthesis

$S_2$ = Stem dry weight at maturity

$G_1$ = Grain dry weight at anthesis

$G_2$ = Grain dry weight at maturity

The main stems or tillers were used to calculate stem reserve translocation. The main tillers were marked by color thread for easy identification during subsequent sampling.

**xv) Absolute grain growth rate**

During anthesis, the main panicles were tagged; some were sampled and packed in separate brown paper packet as per treatment. Then the packets were kept in an oven at 70 ºC for 72 hrs. The tagged panicles were collected after 15 days interval from anthesis to maturity. Then the panicles were packed and oven dried using the same procedure. After drying 20 grains were randomly collected from each panicles and the weight of one grain was calculated dividing by 20. The absolute grain growth rate (AGR) was calculated using the following formula according to Hasan (2009)-

$$\text{AGR (mg/grain/day)} = \frac{W2 \; - \; W1}{T2 \; - \; T1}$$

Where,

$W_1$ = Grain dry weight at initial time

$W_2$ = Grain dry weight at final time

$T_1$ = Initial time

$T_2$ = Final time

### xvi) Dry matter content of leaf blade, stem and root of whole plant (g)

The maturity of the plant was determined when the grain weight was found maximum and the color of the grain turned yellowish. Roots were uprooted at harvest and separated carefully. All the plant parts such as panicle, leaf blade, stem and roots of each hill were separated. The panicles were packed in separate brown paper packet according to treatments. To determine the dry weight of leaves, stems and roots the samples were at first air dried for 6 to 8 hours. Leaves, stems and roots were then packed in separate brown paper packet and were oven dried for 72 hours at 70ºC. Dry weight of leaves, stems and roots were measured separately with an electronic balance and recorded. Dry weight of leaves, stems, reproductive parts and roots were altogether regarded as total dry matter.

### xvii) Dry matter content of leaf blade and stem of main stem (g)

The panicle, leaf blade and stem of main stem were packed separately and were oven dried and weighted as the same procedure described above.

### xviii) Leaf number

The leaf number of the main stem of each plant was counted at maturity.

### xix) Germination percentage

The germination percentage was calculated after sprouting of the seeds.

### xx) Relative performance

The relative performance was calculated as Asana and Williams (1965) by the following formula-

$$\text{Relative performance} = \frac{\text{Variable measured under stress condition}}{\text{Variable measured under normal condition}}$$

### 3.1.16. Data analysis

The data were analyzed in two factor randomized complete block design and the means were separated by DMRT at 5% level of significance using the statistical computer package program MSTAT-C (Russell, 1986). Correlation analysis was also done.

## 3.2. Experiment-II: Response of different morphological and physiological characters of transplanted *aman* rice varieties to varying water stress conditions as imposed by different concentrations of polyethylene glycol (PEG).

The present experiment was under taken with the following objective-

» To find out the effect of moisture stress (induced by PEG) on morphological and physiological attributes of transplanted *aman* rice genotypes at early vegetative stage.

### 3.2.1. Experimental location and duration

Research field and Plant Physiology Lab. Dept. of Agricultural Botany, Sher-e-Bangla Agricultural University, Dhaka-1207 during the period from March to April 2013.

### 3.2.2. Design and treatments

The experiment was conducted in two factors Completely Randomized Design with 3 replications.

**Treatment**: (I) Factor A- Water stress conditions, induced by PEG (Polyethylene glycol)-8000

1. 0% PEG (control)
2. 5% PEG
3. 10% PEG
4. 15% PEG

5. 20% PEG
6. 25% PEG

(II) Factor B- Rice genotypes.

1. BRRI dhan56
2. BRRI dhan57
3. Binadhan-7
4. BRRI dhan49

**3.2.3. Plant materials collection**

The seeds of the rice (*Oryza sativa* L) varieties BRRI dhan56, BRRI dhan57, Binadhan-7 and BRRI dhan49 were collected from the gene bank of Bangladesh Rice Research Institute (BRRI).

**3.2.4. Water stress treatment**

Polyethylene glycol-8000 (PEG-8000) was used to impose water stress in nutrient solutions. Seeds were sterilized with 20% vitavex for 20 min. and rinsed with distilled water. Then the seeds were sprouted and placed on well prepared seed bed using the methodology has been described in experiment I. For the determination of the effect of PEG on rice seedlings, required number of 10 days old seedlings of uniform size were selected and transferred them to a hydroponic system in plastic pots (of appropriate size) containing Yoshida culture solution (collected from BRRI, detail in appendix 7). Required amount of PEG was added in each pot containing nutrient solution. The appropriate pH (5) was also adjusted by adding acid (HCL) or alkali (NaOH) solution every day. The plants were fixed to the lids with the pots as the roots suspended in the solution. The plants were grown in field condition with sufficient transparent plastic shed in order to protect from rain. After 7 days interval the old PEG solution in each pot was replaced by fresh solution. When the plant age was 50 days data were collected from different parameters.

### 3.2.5. Data collection

Different data were collected on the following parameters-

**i) Eye observation**

Different polyethylene glycol concentration in the nutrient solution imposed water stress condition for rice plant grown in that solution. Seven days after transplanting of the rice seedlings in the experimental pots the plants were observed.

**ii) Mortality percentage (M) of plant**

Mortality percentage was calculated with the following formula

$$M = \frac{\text{Number of dead plants}}{\text{Total number of plants}} \times 100$$

**iii) Shoot length and root length (cm)**

Shoot and root lengths were measured at the end of the experiment.

**iv) Leaf area ($cm^2$) and specific leaf weight ($g/cm^2$)**

The leaf area and specific leaf weight of upper two fully expanded leaves were calculated at the end of the experiment with the formula as same as Experiment I.

**v) Shoot dry matter and root dry matter (g)**

To determine the dry weight of shoot and roots, the samples were at first air dried for 6 to 8 hours. The shoots and roots were then packed in separate brown paper packet and were oven dried for 72 hours at 70ºC. Dry weight of shoots and roots were recorded separately with an electronic balance. Dry weight of shoots and roots were altogether regarded as total dry matter.

### vi) The relative water content (RWC) of leaf

Relative water content of leaf is one of the major determinants of leaf photosynthesis. The relative water content of upper two fully expanded leaves were also recorded from every treatment in the present experiment as same as experiment I, at the end of the experiment.

### vii) SPAD value and leaf conductance or stomatal conductance

The SPAD value and stomatal conductance were recorded from the upper fully expanded leaf of the main stem by SPAD meter and leaf porometer as described in Experiment I.

### 3.2.6. Data analysis

The data were analyzed in two factors Completely Randomized Design and the means were separated by DMRT at 5% level of significance using the statistical computer package program MSTAT-C (Russell, 1986). Correlation analysis was also done.

## 3.3. Experiment-III: Evaluating the yield and yield contributing characters response of transplanted *aman* rice varieties to different soil moisture levels.

The objectives of this study were as follows:

» to assess the effect of moisture stress at pre and post-flowering stages on yield components of rice.

» to assess the relative drought tolerance of different varieties of rice considering their yield characters.

The materials and methods of experiment III were as same as described in experiment I except the duration of the experiment. The duration of the experiment III was July to November 2013.

### 3.3.1. Data collection

In the experiment III, the data were collected on the following parameters-

**i) Plant height at maturity (cm)**

The plant height was measured at maturity from the soil level to the tip of the panicle. It was measured in cm unit.

**ii) Tiller number/plant**

The tiller number was counted at anthesis from each plant.

**iii) Effective tillers/plant**

The tiller that bears panicle was considered as effective tiller. The number of effective tiller was counted for each plant (or hill) at maturity.

**iv) Filled grain number of main stem**

The filled grain number of main tiller was counted from each plant after harvest.

**v) Unfilled grain number of main stem**

The unfilled grain number of main tiller was also counted from each plant after harvest. Total grain number per main stem was calculated by adding filled and unfilled grain per main tiller panicle.

**vi) Panicle length (cm)**

The panicle length was measured from the base of the panicle to the top during harvest.

**vii) Grain weight per plant (g)**

Grain weight of each plant was measured after harvest.

### viii) Grain size or individual grain weight (mg)

The grain weight was divided by the number of the grain to calculate the grain size.

### ix) Harvest index

Harvest index (HI) was calculated with the following formula-

$$HI = \frac{\text{Economic yield}}{\text{Biological yield}}$$

### x) Drought susceptibility index

The drought susceptibility index (DSI) was calculated for grain yield as heat susceptibility index described by Fisher and Maurer (1978).

$$DSI = (1- Y/Yp) / (1-X/Xp)$$

Where Y = Grain yield of genotype in a stress environment

Yp = Grain yield of genotype in a stress-free environment

X = Mean Y of all genotypes

Xp = Mean Yp of all genotypes

For the calculation of DSI, the S0 treatment was considered as normal treatment and S2 treatment was considered as water stress treatment

### xi) Relationship between grain filling duration and grain yield

### xii) Relationship between grain filling duration and harvest index

### xiii) Relationship between plant height and grain yield

### xiv) Relationship between plant height and harvest index

### xv) Relative performance

### 3.3.2. Data analysis

The data were analyzed in two factor randomized complete block design and the means were separated by DMRT at 5% level of significance using the statistical computer package program MSTAT-C (Russell, 1986). Correlation analysis was also done.

## 3.4. Experiment IV: Examining the biochemical and anatomical characters response of transplanted *aman* rice varieties to different soil moisture levels.

The objectives of this study were as follows:

» to assess the biochemical and anatomical response of different rice genotypes as influenced by different soil moisture stress

» identifying the biochemical parameters those play important roles in drought tolerance.

In the experiment IV, different materials and methods were as same as described in experiment I or III except the duration of the experiment. The duration of the experiment IV was July to November 2014.

### 3.4.1. Data collection

Different biochemical and anatomical data were collected on the following parameters-

**i) Membrane thermostability or relative injury of leaf tissue**

Cellular thermotolerance is termed as an indicator of crop plant stress tolerance. There is a strong relationship between membrane injury and yield under stress. A cell membrane system that remains functional during stress finally contributes to adaptation of plants to that unfavorable condition. This is very important during grain filling period. The membrane thermostability is also termed as electrolytic leakage.

**Measurement procedure:**

Hossain *et. al*. (1995) developed an improved method of determination of membrane thermostability for screening heat tolerance and sensitive varieties in Brassica. In case of rice, fully expanded leaf samples were collected from five randomly selected plants. Ten leaf discs (10 mm in diameter) were collected from a flag leaf using a leaf puncher. The leaf discs were washed three times with deionized water (collected from G14 Deionizer water set, model: TKA Micromed, Germany) to remove electrolytes adhering to leaf tissue as well as electrolytes released from cut cells on the periphery of the leaf discs. The beakers (50 ml) were also rinsed with deionized water. Then the leaf discs were placed inside the beakers. Thereafter 20 ml of deionized water was added in each beaker. The number of beakers were taken as per treatments.

The initial conductivity reading (I) was taken with an electric conductivity (EC) meter (model: CD-4303). The beakers were covered by aluminium foil and placed in a thermostatistically controlled water bath (model:DSB-1000E, Taiwan) incubator maintaining a constant temperature of $55^0$ C. The electrical conductivity reading (E) was taken at 30 minutes interval up to 4.5 h, subsequently the samples were autoclaved (model: DAC-45, Korea) at $121^0$ C for 15 minutes to kill the leaf tissues completely. After autoclaving the samples were again placed in water bath to adjust the elevated temperature of $55^0$ C. Final conductance (F) was measured after 30 minutes of incubation. The percentage of injury induced by the elevated temperature during the time course (30 minutes) was calculated as follows:

$$\text{Injury (\%)} = \frac{E - I}{F - I} \times 100$$

Where,

I = Initial conductance

E = Elevated temperature conductance

F = Final conductance after autoclaving

The relative injury (RI) was measured by the following formula-

$$RI = \frac{\text{Injury level after 4.5h treatment}}{\text{Injury level after autoclaving}}$$

### ii) Estimation of soluble sugar and starch

The soluble sugar and starch content of rice flag leaf was determined using the method described by Yoshida *et al.,* (1976). The plant sample was collected from all treatment and replications.

**Soluble sugar:** The dried composite plant samples from four replicates were finely ground in mortar and pastel. 100 mg of finely ground sample was placed in to a 15 ml centrifuge tube and with it 10 ml of 80% ethanol was added. The tubes were covered with aluminum foil and kept in a water bath at 80-85° C for 30 minutes. Then the samples were centrifuged and decanted in to a 50 ml beaker. This extraction was repeated three more times. The alcohol extract was evaporated on a water bath at 80-85° C until most of the alcohol was removed (e.g. the volume was reduced to about 3ml). The volume was made up to 25 ml with distill water.

5 ml of sugar extract was transferred to a 100 ml volumetric flask and made up to volume with distilled water. 5 ml of this diluted sugar extract was taken into a pyrex test tube and then these tubes and the tubes containing the glucose standards (0, .01, .025, .05, 0.1, 0.2 and 0.3 mg/5ml) were put into an ice bath. To each tube 10 ml of anthrone reagent (2 g of anthrone was dissolved in one liter of concentrated sulfuric acid and was stored in a refrigerator and a fresh solution was prepared ever 2 days) was added slowly, allowing the reagent to run down the side of the test tube. The solution ways stirred slowly with a glass rod. The tubes were then put into boiling water bath for exactly7.5 minutes and were cooled immediately in ice. Then the absorbance was measured at 630 nm and sugar content was estimated using standard curve.

**Starch:** The residue left in the centrifuge tube after collecting sugar extract was dried in an oven at 80° C and 2 ml of distilled water was added to each centrifuge tube. The tubes were put in a boiling water bath for 15 minutes and stirred occasionally. Then they were allowed to cool and 2 ml of 9.2N $HClO_4$ (Perchloric acid) was added to each tube while stirring constantly. Then the solution was stirred occasionally for 15 minutes. The suspension was then made up to about 10 ml and centrifuged. The supernatant was collected and 2 ml of 4.6N $HClO_4$ was added to the residue. This suspension was stirred for 15 minutes and was made up to 10 ml with distilled water then the suspension was centrifuged and the supernatants were combined. The combined supernatants were made up to 50 ml with distilled water. Then 5 ml sugar extract was transferred to a 50 ml volumetric flask and made up to volume with distilled water. Then 5 ml of this diluted starch extract was taken into a Pyrex test tube. Then these tubes and the tubes containing glucose standards (0.1, 0.25, 0.50, 0.75, 1 and 1.5 mg/5ml including 0.6 ml 0.46N $HClO_4$ solution for each tube) were put in to ice bath. To each tube 10ml of the anthrone reagent was added slowly allowing the reagent to run down the side of the test tube. The solution was stirred slowly with a glass rod. Then tubes were put in a boiling water bath for exactly 7.5 minutes and were cooled immediately in ice. Then the absorbance was measured at 630 nm and starch content was estimated.

### iii) Chlorophyll content

Chlorophyll content of flag leaf of the rice genotypes were determined on fresh weight basis using the method proposed by Witham *et al*., (1986). Hundred milligram of leaf sample was broken into small pieces and dipped into 80% acetone in a twenty five-milliliter vial. The vial was made up to the volume with 80% acetone. Then the sample was kept over forty eight hours in a dark place. Finally the absorbance of the filtrate was taken by spectrophotometer at 663 nm and 645 nm, respectively.

Amount of chlorophyll were calculated using the following equations / formula

Chlorophyll a (mg $g^{-1}$) = [12.7 ($OD_{663}$) - 2.69 ($OD_{645}$)] V/1000W

Chlorophyll b (mg $g^{-1}$) = [22.9 ($OD_{645}$) - 4.68 ($OD_{663}$)] V/1000W

Total chlorophyll (mg $g^{-1}$) = [20.2 ($OD_{645}$) + 8.02 ($OD_{663}$)] V/1000W

Where,

OD = Optical density regarding of the chlorophyll extract at the specific indicated wavelength (645 & 663 nm).

V = Final volume of the 80% acetone chlorophyll extract (ml).

W = Fresh weight in gram of the tissue extracted.

### iv) Proline content

Free proline content in the leaves was determined following the method of proposed by Bates *et al.* (1973). The protocol was based on the formation of red colored formazone by proline with ninhydrin in acidic medium, which was soluble in organic solvents like toluene.

**Instruments and glassware**

Test tubes, test tube stand, micro-pipettes (20-200 µl, 100-1000 µl and 5 ml), Whatman No. 1 filter papers, visible range spectrophotometer.

**Reagents**

1. Glacial acetic acid (Analytical grade)
2. Sulphosalycylic acid (3%): Three gram of sulphosalycylic acid was dissolved in 100 ml of distilled water.
3. Orthophosphoric acid (6 N): Required volume of orthophosphoric acid (38.1 ml) was taken and volume was made to 100 ml, using distilled water to get 6 N orthophosphoric acid.
4. Acid ninhydrin: Ninhydrin (1.25 g) was dissolved in a blend of 30 ml of glacial acetic acid and 20 ml of 6 N orthophosphoric acid.

**Procedure**

1. 0.5 g plant tissue was taken and homogenized in 5ml of 3% sulphosalycylic acid using pre-washed mortar and pastle.
2. Then the homogenate was filtered through Whatman No.1 filter paper and the collected filtrate was used for the estimation of proline content.
3. 2 ml of extract was taken in a test tube and add 2ml of glacial acetic acid and 2ml of ninhydrin reagent was added.
4. Then the reaction mixture was heated in a boiling water bath (by Stirrer hot water bath, Model: DSB-1000E, Taiwan) at 100ºC for 1 hour. Brick red colour was developed.

5. After cooling the reaction mixtures, 4ml of toluene was added and each tube shaken vigorously for 15-20 second in an electric shaker (Model: KS260 basic, Germany) then transferred to a separating funnel.
6. After thorough mixing, the chromospheres containing toluene was separated and its absorbance was read at 520 nm in spectrophotometer (G14 Spectrophotometer, Model: SB-10, Australia) against toluene blank.
7. The proline standard curve was prepared by taking 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 $\mu g\ ml^{-1}$ concentrations.
8. The proline content was determined from a standard curve and calculated on a fresh weight basis as follows-

µmoles proline / g of fresh plant material = {(µg proline / ml × ml toluene) / 115.5 µg / µmoles} / (g sample/5)

**v) Leaf anatomy under soil moisture stress**

The anatomical changes in of leaf, due to soil moisture stress in the present experiment, were observed under photomicroscope and the photographs were taken.

### 3.4.2. Data analysis

The data were analyzed in two factor randomized complete block design and the means were separated by DMRT at 5% level of significance using the statistical computer package program MSTAT-C (Russell, 1986). Correlation analysis was also done.

# CHAPTER 4

# RESULTS AND DISCUSSION

In this research work, the response of four transplanted *aman* rice varieties were studied under different soil moisture levels and in nutrient solution (where water stress treatments were induced by different concentrations of PEG) conducting four experiments. In the 1st experiment, the morphological and physiological responses were studied in pot experiment with soil. In the 2nd experiment, the morphological and physiological responses were further studied in pot experiment with nutrient solution. In the 3rd experiment, different yield contributing characters of those varieties under different soil moisture treatments (same as experiment1) were studied and total concentration had been paid on yield loss due to water stress. In the 4th experiment, the results of the previous three experiments were evaluated further along with some biochemical and anatomical parameters to find out the effect of different soil moisture levels. The overall research works were conducted within the year from 2012 to 2014.

## 4.1. Experiment-I: The morpho-physiological responses of different transplanted *aman* rice varieties to different soil moisture levels.

In this study, a number of morpho-physiological characters of four transplanted *aman* rice varieties were evaluated under three soil moisture treatments.

### 4.1.1. Plant height at maturity

The plant height was recorded during maturity stage from all the treatments which are presented in table 1. Considering all the varieties and soil moisture treatment, the tallest (114.05 cm) plant recorded was in BRRI dhan49 at S0 treatment which was statistically similar to S1 treatment of the same variety, with S0 and S1 treatments of BRRI dhan56. The shortest (83.21 cm) plant recorded was in BRRI dhan57 at S2 treatment which was statistically similar to S2 treatment of Binadhan-7. Considering all the rice varieties, it was clear that the control treatment (S0) produced the highest plant height, which was gradually decreased with decreasing the moisture level of the pot soil.

The shortest plant recorded was in S2 treatment in all the varieties. This might be due to lowest LA, SLA, chlorophyll content and RWC; as a result leaf rolling became higher and light harvest was lower. As a result stomatal conductance, transpiration rate, intercellular $CO_2$ concentration and RGR were also recorded lower which ultimately affected the total vegetative DM as well as the plant height. S2 treatment differs significantly with control treatment; indicated that S2 treatment had harmful effect on every variety. This might also due to inhibition of cell division or cell enlargement under water stress treatment (Hussain *et al*., 2008).

In BRRI dhan56 and in BRRI dhan49, S0 and S1 treatment did not differ significantly. This might be due to as the LA, NAR, RGR and soluble sugar content of those varieties in S1 treatment did not differ significantly with the control (S0) treatment. Variation in plant height among the varieties also indicated that different varieties had different water requirement. Under drought stress, plant roots were unable to supply required amount of water and the growth of the plant was retarded due to flaccid treatment. Bakul *et al.* (2009) stated that plant height was affected most by water stress at tillering and panicle initiation stages. They also reported that plant height at 40%FC treatment was found significantly lower than that of control (100%FC). Zubaer *et al*. (2007) reported that, at booting stage, the tallest plant was found at 100% FC treatment, which was followed by 70%FC treatment and the shortest plant was found at 40% FC treatment in different rice varieties. It was also reported that the water restrictions at the vegetative stage decreased both plant height and tiller number (Kima *et al*, 2014). It was revealed that the plant height was significantly affected by water stress at booting, flowering and grain filling stage (Sarvestani and Pirdashti, 2008) over the control. Islam *et al*. (1994b) found that moisture stress reduced plant height under 20% soil saturation at booting and flowering stages. Similar result has also been reported by Islam (1999). Prasad *et al*., (2012) found that the drought stress significantly reduced the plant height irrespective of rice varieties. They also reported that the decrease in height might be either due to inhibition of length.

**Table 1**. Effect of different soil moisture levels on plant height of various transplanted *aman* rice varieties

| Varieties | Soil moisture levels | Plant height at maturity (cm) | Relative to control (%) |
|---|---|---|---|
| BRRI dhan56 | S0 | 112.17 a | 100 |
| | S1 | 112.05 a | 99.89 |
| | S2 | 100.44 bc | 89.54 |
| BRRI dhan57 | S0 | 96.86 cd | 100 |
| | S1 | 91.14 def | 94.09 |
| | S2 | 83.21 g | 85.91 |
| Binadhan-7 | S0 | 103.01 b | 100 |
| | S1 | 90.09 ef | 87.46 |
| | S2 | 86.12 fg | 83.60 |
| BRRI dhan49 | S0 | 114.05 a | 100 |
| | S1 | 108.65 a | 95.27 |
| | S2 | 92.23 de | 80.87 |
| CV (%) | | 3.85 | |

S0=100%FC (field capacity) moisture, S1= 70% of the FC moisture, S2= 40% of the FC moisture. Values followed by same letter(s) did not differ significantly at 5% level of probability.

of cells or cell division by water deficits. Zain *et al.* (2014) also found that the plant height and number of tillers reduced with increased duration of water stress.

### 4.1.2. Effective and total tillers per plant

The number of effective tiller is the main yield determinant. In the present experiment, different varieties and soil moisture treatments did not differ significantly among them for the number of effective and total tiller per plant shown in table 2. The control (S0) treatment produced the highest effective tiller in all the varieties. On the other hand S2 treatment produced the lowest number of effective tiller in all the varieties (table 3.2). The S0 and S1 treatments produced similar number of effective tiller in all the varieties except in BRRI dhan57. The LA, RGR, sugar and starch content of S1 treatments were also statistically similar to the respective control treatment in those varieties (except BRRI dhan57).

Considering all the varieties and soil moisture treatments, the number of tiller recorded was the highest (12) in S0 treatment of Binadhan-7 and the number of tiller recorded was the lowest (10) in S2 treatment of BRRI dhan57. But the varieties and soil moisture treatments did not differ significantly among them considering the total tiller number per plant. In most of the varieties S1 treatment produced the similar number of effective as well as total tillers compared to the control treatment. The S2 treatment of BRRI dhan57 and Binadhan-7 produced the lower number of tiller than the control. This might be due to decrease in RWC, chlorophyll and leaf gas exchange under S2 treatment in the present study.

Reduced tiller production under limited soil moisture content might be due to the fact that the stressed plants were unable to produce enough assimilates for inhibited photosynthesis (Chaves *et al.,* 2002; Medrano *et al.,* 2002). It might also be happened for less amount of water uptake to prepare sufficient food and inhibition of cell division of meristematic tissue under soil moisture stress (Prasad *et al*., 2012). The results also agree with Murty (1987), Castilo *et al.* (1987), Cruz *et al.* (1986), IRRI (1974) and Islam *et al.* (1994a). Due to the reduction in turgor pressure under stress, cell growth is severely impaired (Taiz and Zeiger, 2006). Drought affects both elongation as well as expansion growth (Shao *et al*., 2008), and inhibits cell enlargement more than cell division (Jaleel *et al*., 2009). However, the negative effect of drought observed on the number of tillers corroborated the results of Efisue (2006) and Ndjiondjop *et al.* (2010), who explained the phenomenon as a result of poor plant development. Zubaer *et al.* (2007) reported that soil moisture level and varieties interacted significantly on number of tillers per hill. They also observed that at all growing stages (booting, flowering and maturity), the highest number tillers per hill were obtained from 100%FC treatment and the lowest number of tillers per hill was obtained from 40%FC treatment. Number of tillers / hill also varied due to genotypic difference. Sellammal *et al.* (2014) also found that the tiller number per plant gradually reduced under the moderate stress and severe water stress. Drought reduced the number of tillers which was also reported by many authors such as Mostajeran and Rahimi-Eichi, 2009; Ashfaq *et al.*, 2012; Bunnag and Pongthai, 2013. Akram *et al*., (2013) found that the moisture stress at all growth stages in different rice cultivars had non-significant effect on number of tillers per hill.

**Table 2**. Effect of different soil moisture treatments on the number of effective and total tillers of transplanted *aman* rice varieties

| Varieties | Soil moisture Levels | Number of effective tillers plant$^{-1}$ | Number of total tillers plant$^{-1}$ |
|---|---|---|---|
| BRRI dhan56 | S0 | 11 $^{NS}$ | 11 $^{NS}$ |
| | S1 | 11 $^{NS}$ | 11 $^{NS}$ |
| | S2 | 9 $^{NS}$ | 11 $^{NS}$ |
| BRRI dhan57 | S0 | 11 $^{NS}$ | 11 $^{NS}$ |
| | S1 | 10 $^{NS}$ | 11 $^{NS}$ |
| | S2 | 9 $^{NS}$ | 10 $^{NS}$ |
| Binadhan-7 | S0 | 11 $^{NS}$ | 12 $^{NS}$ |
| | S1 | 11 $^{NS}$ | 11 $^{NS}$ |
| | S2 | 10 $^{NS}$ | 11 $^{NS}$ |
| BRRI dhan49 | S0 | 11 $^{NS}$ | 11 $^{NS}$ |
| | S1 | 11 $^{NS}$ | 11 $^{NS}$ |
| | S2 | 10 $^{NS}$ | 11 $^{NS}$ |
| CV (%) | | 17.83 | 11.53 |

S0=100%FC (field capacity) moisture, S1= 70% of the FC moisture, S2= 40% of the FC moisture, NS= Not significant.

### 4.1.3. Days to anthesis, grain filling duration and the duration (days) from germination to maturity

Days to anthesis, the duration of grain filling period and the duration of germination to maturity of different rice varieties under various soil moisture treatments are presented in the table 3. The days to anthesis was found much earlier in BRRI dhan57 than any other varieties under control and water stress treatments. Anthesis was delayed in BRRI dhan49 variety compare to other varieties under control and water stress treatments. Considering all the varieties and soil moisture treatments, the days to anthesis was found the highest (99 days) at S2 treatment in BRRI dhan49, which was statistically similar to S0 and S1 treatments of the same variety and the lowest (85 days) days to anthesis recorded was in BRRI dhan57 at control and S2 treatment.

It was reported that the drought stress applied at the beginning of the reproductive stage usually results in a delay in flowering (Saini and Westgate, 2000). This is mainly due to slowed elongation of the panicle and supporting tissues (Lafitte *et al.*, 2004). This trait can be an effective drought avoidance mechanism if the period of water deficit is short, as panicle elongation resumes after relief from a brief period of stress. However, if flowering is delayed by more than a few days, severe yield losses usually occur. In the present experiment little (single day) delayed in flowering compare to control occurred in most of the varieties under S2 treatment and this response might no effect on final grain yield. It has been reported that the greater the delay in flowering, the greater the yield and harvest index reduction due to drought (Pantuwan *et al*., 2002). Improving drought resistance may therefore involve selection for plants exhibiting little or no flowering delay due to drought (Pantuwan *et al.,* 2002; Bernier *et al.,* 2007). It was also reported that the delay in flowering under drought is a consequence of a reduction in plant dry matter production and of a delay in panicle exsertion (Murty and Ramakrishnayya, 1982). Novero *et al.* (1985) reported that the delay in flowering depends on the intensity, time, and period of drought. Wopereis *et al.* (1996) observed longer flowering delay when drought occurred during early tillering than when it occurred in mid-tillering stage. Pantuwan *et al.* (2002) made similar observations and concluded that under prolonged drought, flowering time is an important determinant of rice grain yield.

In the present experiment, considering all the varieties and soil moisture treatments, the grain filling duration was found the highest (39 days) in control treatment of BRRI dhan57, which was statistically similar to S2 treatment of the same variety and the lowest (26 days) grain filling duration recorded was in S1 treatment of Binadhan-7 which was statistically similar to S2 treatment of BRRI dhan49. In all the varieties, the grain filling duration was lower under water limiting condition compare to control (S0). It was clear that the variety in which the anthesis become earlier get longer grain filling period and the variety in which the anthesis was delayed get shorter grain filling period.

The decreased grain filling duration under S2 treatment in all the varieties might be due to the reduction in chlorophyll content, lower RWC and higher leaf rolling under S2 treatment which ultimately affected the total light harvest and consequently the stomatal conductance as well as the soluble sugar and starch content. It was also recorded that, in BRRI dhan49 and in BRRI dhan56, the grain filling duration at S1 treatment was statistically similar to control treatment. This might be due to, in this treatment, the LA, NAR, RGR, chlorophyll content, sugar-starch content was statistically similar to control treatment in those varieties. It was reported that drought during the grain-filling process induced early senescence and shortened the grain-filling period, but increased remobilization of assimilates from the straw to the grains (Plaut *et al.*, 2004).

Considering all the varieties and soil moisture treatment, the duration of germination to maturity was found the highest (128 days) in control (S0) treatment of BRRI dhan49 which was significantly higher than any other treatments and the lowest (118 days) life duration recorded was in S2 treatment of BRRI dhan56 and in S1 treatment of Binadhan-7. In all the varieties, S2 treatment showed the lowest and S0 or control treatment showed the highest life duration except in Binadhan-7 in which the life durations was equal both in S0 (control) and S2 treatment (123 days). In all the varieties, the S1 treatment did not show any remarkable effect on life duration compare to control except in Binadhan-7.

These results indicated that the effect of soil moisture stress on the vegetative and reproductive were different for different varieties. Due to moisture stress the decreased life cycle in BRRI dhan56 was mainly due to decrease in reproductive duration rather than vegetative duration.

Decrease in life duration under limited soil moisture condition is one of the escape strategies of rice plant which was also recorded in the present experiment. Lower reproductive duration might give lower seed size as well as lower grain yield. It was reported that when the stress is terminal and predictable, drought escape through the use of shorter duration varieties is often the preferable method of improving yield potential. Drought avoidance and tolerance mechanisms are required in situations where the timing of drought is mostly unpredictable (Pantuwan *et al.,* 2002). Lauteri *et al*. (2014) also found that life span of rice varieties decreased with the increasing drought stress. Rice plant that can escape or evade drought through the adjustment of the life cycle is also an important trait for Drought resistance as in BRRI dhan56 and BRRI dhan49 in the present experiment.

**Table 3**. Effect of different soil moisture levels on the duration of various developmental stages of transplanted *aman* rice varieties

| Varieties | Soil moisture Level | Duration from germination to anthesis (days) | Grain filling duration (days) | Duration from germination to maturity (days) |
|---|---|---|---|---|
| BRRI dhan56 | S0 | 88 de | 36 bc | 124 c |
| | S1 | 88 de | 34 cd | 122 d |
| | S2 | 89 d | 29 e | 118 e |
| BRRI dhan57 | S0 | 85 f | 39 a | 124 c |
| | S1 | 88 de | 37 b | 125 bc |
| | S2 | 85 f | 38 ab | 123 cd |
| Binadhan-7 | S0 | 90 cd | 33 d | 123 cd |
| | S1 | 92 b | 26 f | 118 e |
| | S2 | 91 bc | 32 d | 123 cd |
| BRRI dhan49 | S0 | 98 a | 30 e | 128 a |
| | S1 | 98 a | 29 e | 127 ab |
| | S2 | 99 a | 27 f | 126 b |
| CV (%) | | 2.13 | 4.93 | 0.65 |

S0=100%FC (field capacity) moisture, S1= 70% of the FC moisture, S2= 40% of the FC moisture. Values followed by same letter(s) did not differ significantly at 5% level of probability.
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### 4.1.4. Leaf area

Leaf is the main light harvesting organ. Biswal and Kohli (2013) observed a positive correlation between leaf traits and yield under drought. In the present experiment, leaf area was also affected due to water stress treatment. Water stress decreased the leaf area in all the varieties. But there were no significant difference among the treatments in every variety. In all the varieties, S0 or control treatment produced the highest leaf area (Figure 1). Considering all the varieties and soil moisture treatment, the leaf area was found the highest (48.06 $cm^2$) in S0 treatment of BRRI dhan57 which was followed by S0 treatment of BRRI dhan49 variety; but was statistically similar to other soil moisture treatments of those varieties and with all the soil moisture treatments of BRRI dhan56. The lowest (25.18 $cm^2$) leaf area recorded was in S1 treatment of Binadhan-7 which was statistically similar to other soil moisture treatments of the same variety. In BRRI dhan57 the leaf area at S0 treatment was much higher than the other soil moisture treatments. But in Binadhan-7 and BRRIdhan49 the leaf area of S0 and S2 treatments was similar.

The LA was little affected under water stress treatment in BRRI dhan49 and BRRI dhan56 and this might be due little effect on RGR, sugar-starch, chlorophyll content, NAR and increased dry matter investment to leaf under water limiting condition in those varieties. It was reported that reduced soil moisture levels produced lower leaf area and this might be due to inhibition of cell division of meristematic tissue under water starved treatment (Zubaer *et al.,* 2007). The results of the present experiment are also in agreement with Aggarwall and Kodundal (1988) and Hossain (2001). Gloria *et al.* (2002) reported that the water deficit in rice caused a larger reduction in leaf area than shoot dry matter, demonstrating the greater sensitivity of leaf enlargement to water stress than dry matter accumulation. It was also revealed that many aspects of plant growth are affected by drought stress, these include leaf expansion, production and promotes senescence and abscission (Hayatu and Mukhtar, 2010). Kumar *et al.* (2014) found that drought stress at reproductive stage caused reduction in leaf area (34.87%).

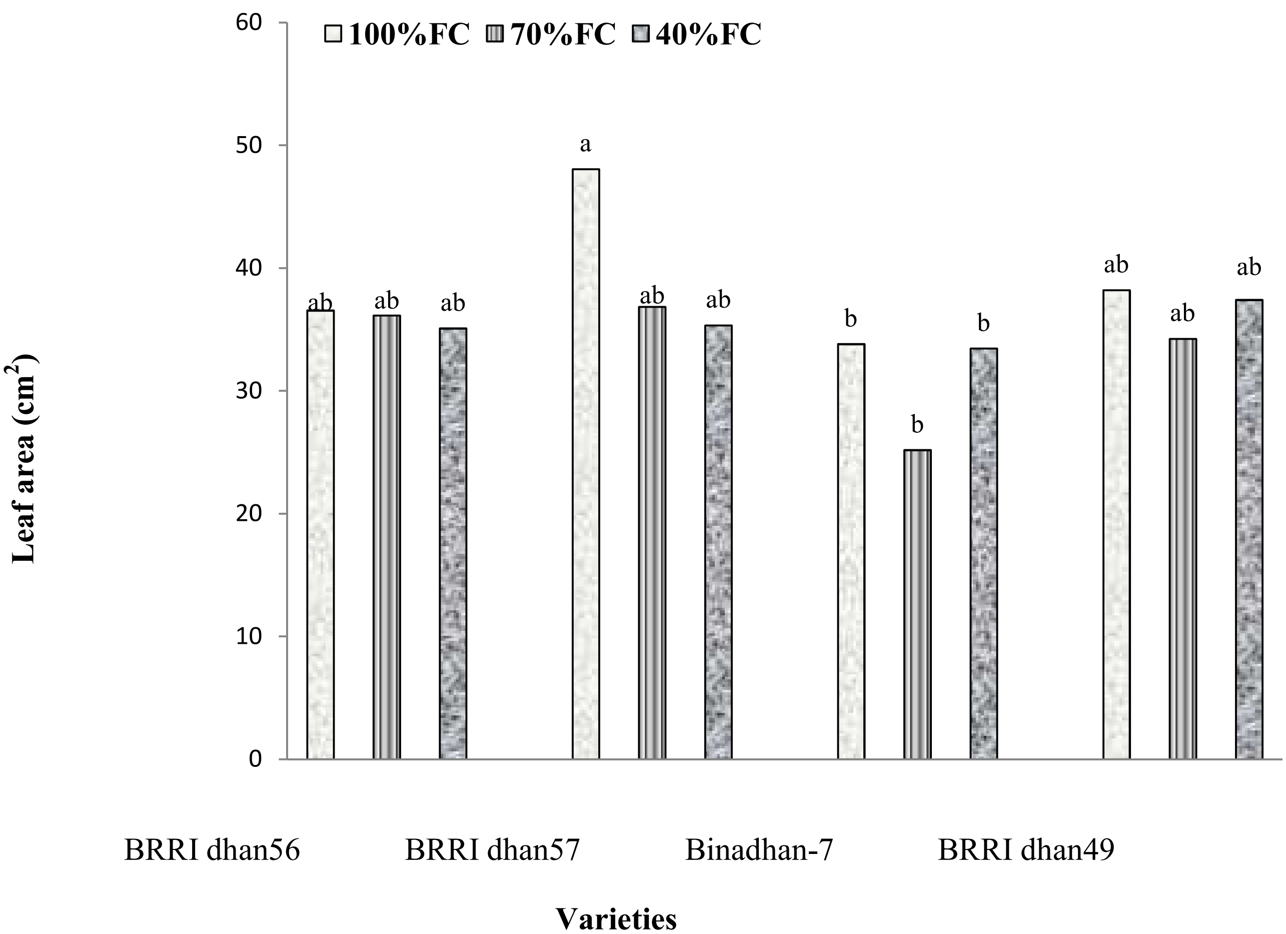


**Figure 1**. Leaf area of different rice varieties as influenced by various soil moisture treatments. S0=100%FC (field capacity) moisture, S1= 70% of the FC moisture, S2= 40% of the FC moisture. Values followed by same letter(s) did not differ significantly at 5% level of probability.

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### 4.1.5. Specific leaf area (SLA)

Specific leaf area (SLA) is defined as the ratio of leaf area to dry mass. In the present experiment, the SLA was determined during grain filling period from each plant at the same time. The specific leaf area (SLA) recorded was the highest at control (S0) treatment in all the varieties (Figure 2). The S2 treatment produced the lowest SLA in all the variety also. Considering all the varieties and soil moisture treatment the SLA was found the highest (276.88 $cm^2/g$) in S0 treatment of BRRI dhan49 which was significantly higher than any other treatments and lowest (176.29 $cm^2/g$) SLA recorded was in S1 treatment of BRRI dhan56 which was statistically similar to the S2 treatment of the same variety and with S2 treatment of Binadhan-7.

In BRRI dhan56, the highest SLA recorded was in $S_0$ treatment which was significantly higher than any other treatments of the same variety and the lower SLA recorded was in S1 treatment, which was statistically similar to $S_2$ treatment. In BRRI dhan57, the highest SLA recorded was in control treatment which was statistically similar to other treatments of the same variety. In Binadhan-7, the highest SLA recorded was in control treatment also which was statistically similar to other treatments of the same variety. In BRRI dhan49, the highest SLA recorded was in control treatment, which was significantly higher than any other treatments of the same variety and the lowest SLA recorded was in S2 treatment which was statistically similar to $S_1$ treatment. The overall results indicated that the SLA was decreased under drought treatment. The reduction in SLA under severe water stress is an adaptive mechanism to water stress helps in reducing water loss from the evaporative surfaces ((Hayatu and Mukhtar, 2010) and the reduction in transpiration under water stress treatment was also recorded in the present experiment. The DM investment to leaf was also recorded higher under water stress compare to control. Farooq *et al*. (2010) stated that broader leaves result in better performance of *indica* rice under drought stress.

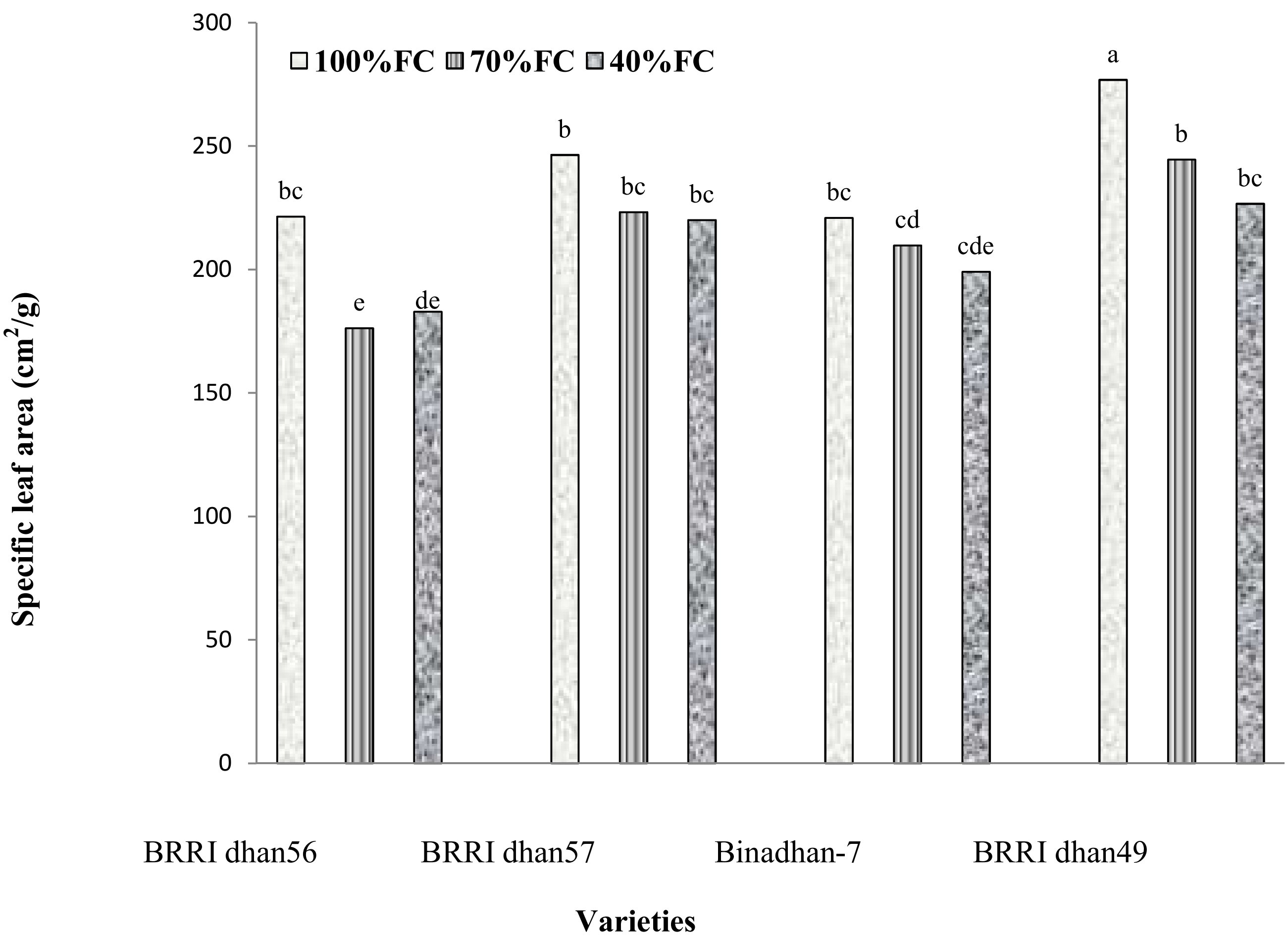


**Figure 2**. Specific leaf area of different rice varieties as influenced by various soil moisture treatments. S0=100%FC (field capacity) moisture, S1= 70% of the FC moisture, S2= 40% of the FC moisture. Values followed by same letter(s) did not differ significantly at 5% level of probability.

### 4.1.6. Specific leaf weight (SLW)

Specific leaf weight (SLW) is defined as the mass of leaf dry matter per unit of leaf area. SLW also expressed the thickness of the leaf. The plant with higher SLW (thick leaf) possesses more mesophyll cells for photosynthesis. Specific leaf weight (SLW) gradually increased with decreasing soil moisture content from S0 to S2 in BRRI dhan57, Binadhan-7 and in BRRI dhan49 (Figure 3). But there were no significant difference among the treatments in those varieties. The highest SLW in BRRI dhan56 recorded was at S1 treatment which was followed by S2 treatment in the same variety and they were statistically similar. Considering all the varieties and soil moisture treatments, the SLW recorded was the lowest (3.61 $mg/cm^2$) in control treatment of BRRI dhan49 which was statistically similar to other treatments of the same variety, with control (S0) treatment of BRRI dhan56 and Binadhan-7, with S0 and S1 treatment of BRRI dhan57. Considering all the varieties and soil moisture treatment the SLW recorded was the highest (5.67 $mg/cm^2$) in S1 treatment of BRRI dhan56 which was statistically similar to S2 treatment of the same variety, with S2 treatment of BRRI dhan57 and with all the treatments of Binadhan-7. It is clear from the figure that the leaf becomes thicker under lower soil moisture condition and it was also recorded in another table that the DM investment to leaf was also increased under S2 treatment. Boontang and Tantisuwichwong (2013) reported that SLW was increased under drought treatment that helps to minimize transpiration loss under limited soil moisture condition and the similar result was also recorded in the present experiment. SLW and RWC are physiological parameters related to drought tolerance (Nigam and Aruna, 2008; Nautiyal *et al*., 1995).

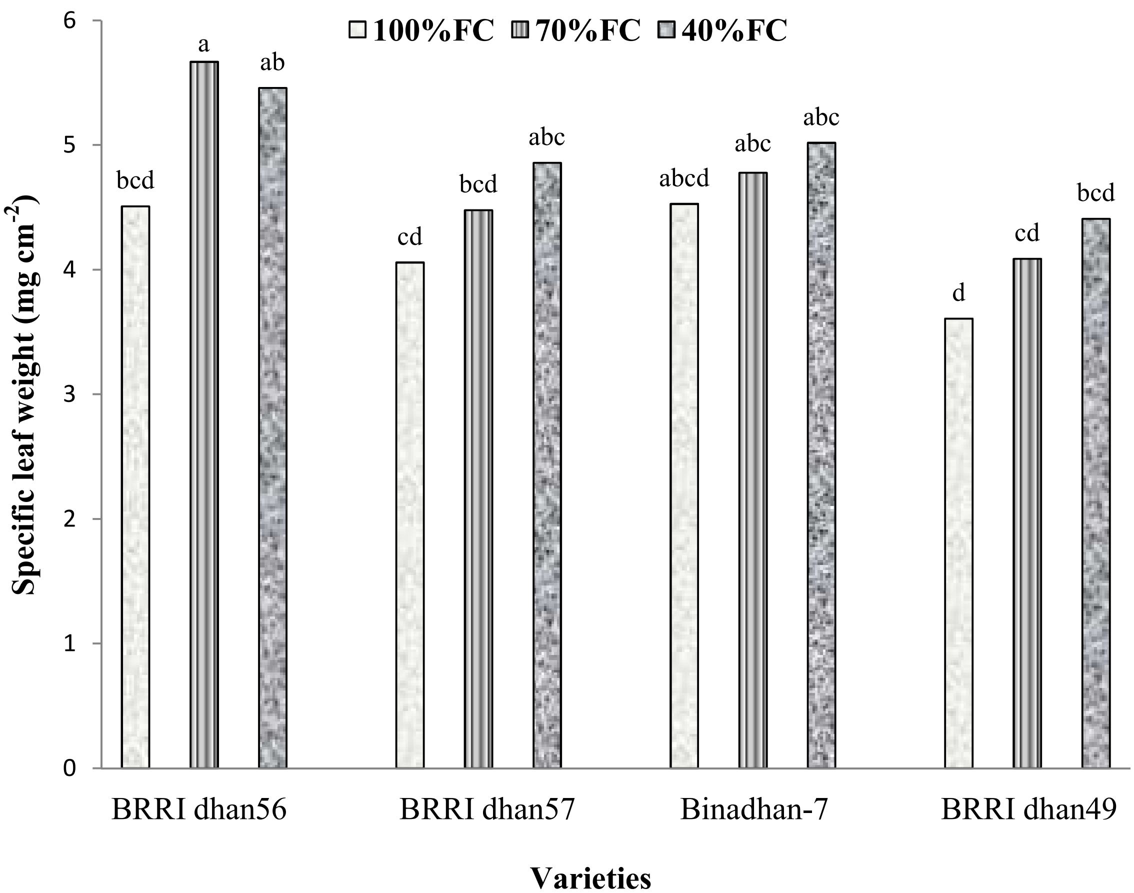


**Figure 3**. Specific leaf weight of different rice varieties as influenced by various soil moisture levels. S0=100%FC (field capacity) moisture, S1= 70% of the FC moisture, S2= 40% of the FC moisture. Values followed by same letter(s) did not differ significantly at 5% level of probability.

### 4.1.7. Leaf weight ratio (LWR)

The LWR indicates the dry matter investment towards leaf. In the present experiment, the LWR was calculated after harvest. The results indicated that there was a significant difference among the soil moisture treatments for LWR in all the varieties and dry matter investment recorded increased in leaf under low soil moisture treatment compared to control in all the varieties. It was also recorded that the dry matter investment to leaf recorded was higher at S2 treatment compared to S0 treatment in all the varieties (Figure 4). Considering all the varieties and soil moisture treatments, the highest (16.54) leaf investment was observed in S2 treatment of Binadhan-7 which was significantly higher than any other treatment and the lowest (10.12) leaf investment recorded was in S0 or control treatment of BRRI dhan57 which was statistically similar to S0 treatment of BRRI dhan56. The leaf weight ratio (LWR) gradually increased in BRRI dhan56 from control to S2 treatment. In BRRI dhan56, the highest (14.21) LWR recorded was in S2 treatment which was significantly higher than any other treatments of the same variety. In BRRI dhan57, the highest (14.60) LWR recorded was at S1 treatment which was significantly higher than any other treatments of the same variety. In Binadhan-7, the highest (16.54) LWR recorded was at S2 treatment which was significantly higher than any other treatments of the same variety. In BRRI dhan49, the highest (13.55) LWR recorded was in S1 treatment, which was significantly higher than any other treatments of the same variety. In the present experiment, the leaf became thicker due to water stress treatment and the transpiration rate was decreased. The increased LWR under limited soil moisture condition indicated that leaf dry mass was less affected and total biomass was more affected in this situation. Sarani *et al*. (2014) stated that soil moisture stress directly affect the biomass of shoots.

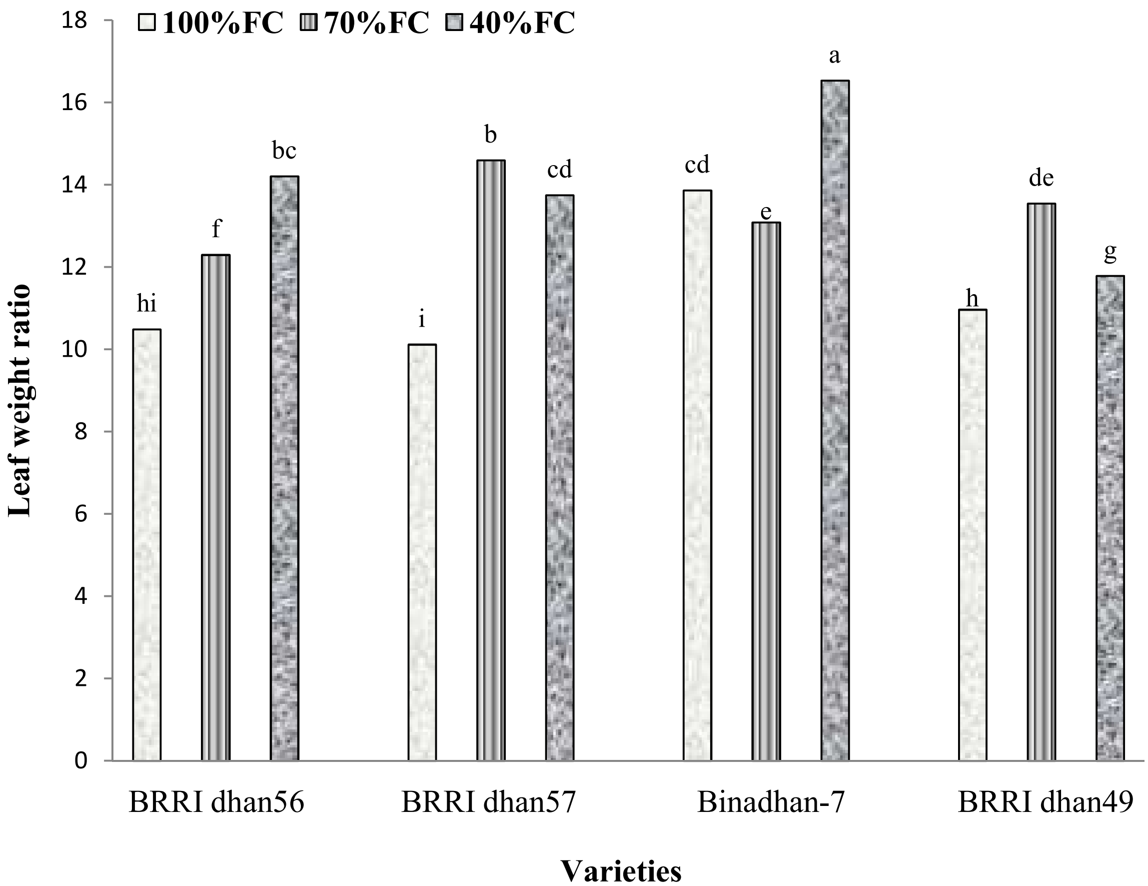


**Figure 4**. Leaf weight ratio of different rice varieties as influenced by various soil moisture levels. S0=100%FC (field capacity) moisture, S1= 70% of the FC moisture, S2= 40% of the FC moisture. Values followed by same letter(s) did not differ significantly at 5% level of probability.

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#### 4.1.8. Relative water content (RWC) of flag leaf during anthesis

The relative water content of leaf depends on the moisture content of the soil and the water absorbing capacity of the root. In mid 80s, the RWC was introduced as a best criterion for plant water status which, afterwards was used instead of plant water potential as RWC referring to its relation with cell volume, accurately can indicate the balance between absorbed water by plant and consumed through transpiration (Arjenaki *et al*., 2012). In the present experiment, the relative water content of flag leaf recorded was during anthesis. There was a significant difference among the soil moisture treatments for relative water content in every variety. The relative water content of leaf gradually decreased with decreasing soil moisture from control to S2 treatment in all the rice varieties (Figure 5). But the difference of RWC between S0 and S2 was lower in BRRI dhan49 and higher in BRRI dhan56. Considering all the varieties and soil moisture treatments, the highest (96.43%) RWC recorded was in S0 treatment of BRRI dhan56 and Binadhan-7, which was significantly higher than other treatments. The lowest (74.55%) RWC recorded was in S2 treatment of BRRI dhan56, which was significantly lower than any other treatments.

Under S2 treatment, the soil moisture level was much lower which ultimately affect the water content of the whole plant as well as the RWC of the leaf in all the varieties. But in BRRI dhan49, the RWC of the leaf was much higher compared to other varieties under S2 treatment; this might be due to higher proline accumulation in the leaf of this variety under S2 treatment compared to other varieties. The dry matter investment to root was also higher in BRRI dhan49 under S2 treatment compared to other varieties and these results indicated that BRRI dhan49 is more drought tolerant than others. The capacity to maintain higher RWC under drought stress treatment has been suggested as a possible water scarcity tolerance mechanism in rice (Gupta and Guhey, 2011). So, the varieties that retain more water in leaf under water stress treatment are might be considered as more water stress tolerant, as in BRRI dhan49. It is also suggested that the high relative water content could help the tolerant variety to perform physio-biochemical processes more efficiently under water stress treatments than susceptible variety (Moussa and Aziz, 2008). Zulkarnain *et al.* (2009) stated that the relative water contents of different rice varieties were similar under the well-watered treatment on different measurement occasions and it declined progressively in stressed plots with the development of severe water deficit and this might be due to that the corresponding root was unable to keep peace with the transpiration demand of the shoot when the soil water level was limited. The differences among the rice varieties in terms of the rate of decline in the leaf RWC could also be associated with the variations in other physiological responses to water stress, such as reduction in stomatal conductance. Kumar *et al.* (2014) found a significant difference in RWC among different rice varieties between drought stress and irrigated treatment.

#### 4.1.9. Leaf rolling score

Leaf rolling score is an eye estimation process of leaf rolling under control and limited soil moisture treatment. In the present experiment, no leaf rolling was yet observed at S0 or control treatment (Figure 6) in all the varieties. The highest leaf rolling score recorded was at S2 treatment in all the varieties. Under S1 treatment, the score recorded was the highest (3.0) in Binadhan-7 which was significantly higher than any other varieties and the lowest (1.34) score was observed in BRRI dhan56 which was statistically similar to other varieties. Under S2 treatment, there existed a significant different among the varieties for leaf rolling score and the score recorded was the highest (3.67) in Binadhan-7 also, which was followed by BRRI dhan56 and the lowest (1.67) score was observed in BRRI dhan49. Leaf rolling under water stress condition helps plant to minimize transpiration loss and protect the plants from drying.

The highest leaf rolling at S2 treatment in all the varieties might be due to the lowest RWC of leaf under this treatment. But in BRRI dhan49, the leaf rolling was relatively lower in S2 treatment compared to others and this might be due to higher proline accumulation in this variety under S2 treatment. Higher proline content increased the RWC of the leaf and the leaf rolling was lower in this variety. But in Binadhan-7, the highest leaf rolling under S2 treatment might be due to lower proline accumulation as well as lower RWC under this treatment. Therefore, leaf rolling is commonly used as an

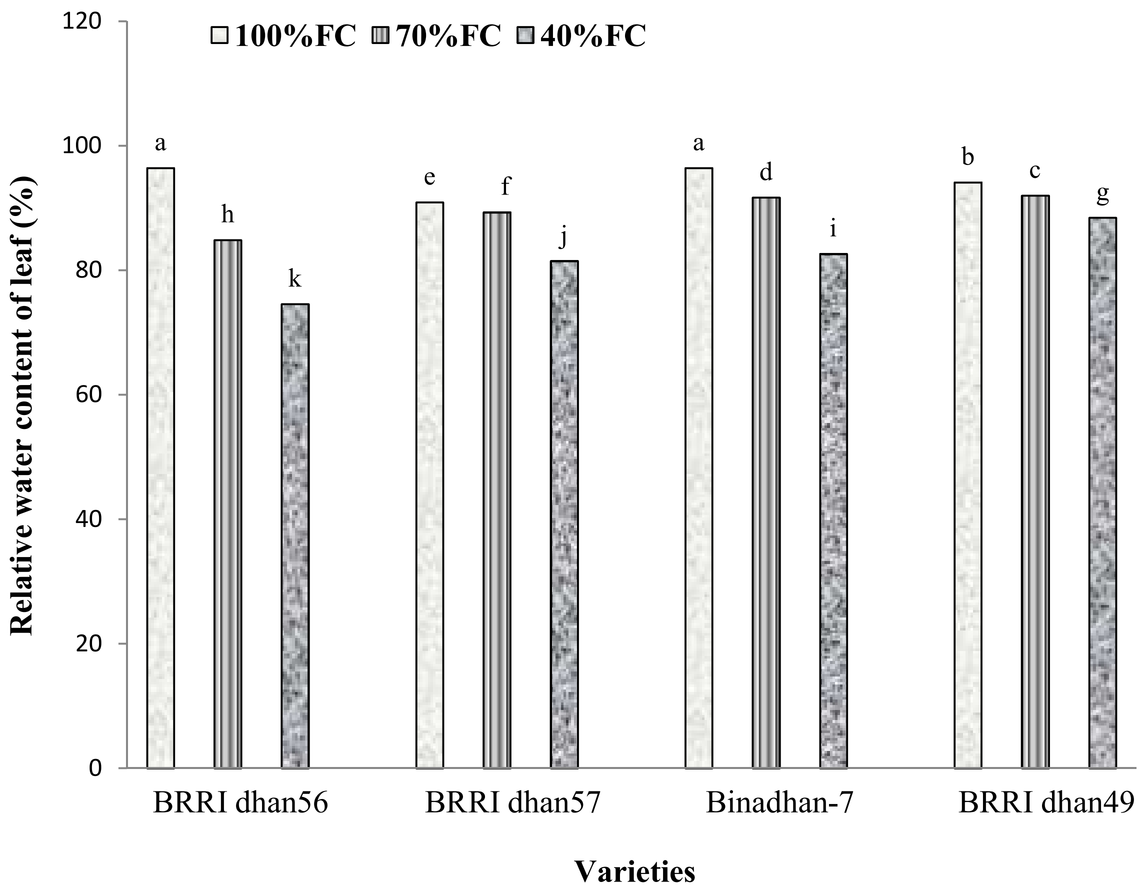


**Figure 5**. Relative water content of leaf of different rice varieties under various soil moisture treatments. S0=100%FC (field capacity) moisture, S1= 70% of the FC moisture, S2= 40% of the FC moisture. Values followed by same letter(s) did not differ significantly at 5% level of probability.

important criterion during screening of varieties for drought tolerance (Cutler *et al*., 1980; Sloane *et al*., 1990; Rosario *et al*., 1992; Lilley and Fukai, 1994). Accordingly, in the present experiment, Binadhan-7 was found to be sensitive and BRRI dhan49 was found to be tolerant to drought. Leaf rolling and leaflet closure during periods of soil moisture depletion have also been observed in other varieties of rice (Lilley and Fukai, 1994). These leaf movements, such as the adjustment of leaf angle or modification of leaf orientation to reduce the interception of solar radiation and, thus, decrease leaf temperature and water loss by transpiration, are regarded as one of the drought avoidance mechanisms evolved in plants (Pugnaire *et al*., 1999; Carr, 2001). There is positive correlation between these flag leaf traits and yield under drought (Biswal and Kohli, 2013). The leaf rolling score of different rice varieties under drought stress was also stated by Zulkarnain *et al*. (2009). Blum (1988) reported the use of delayed leaf rolling under water stress as important selection criteria for dehydration avoidance. Leaf rolling was considered to be a response to leaf water potential and has been found to correlate with leaf water potential in rice. Delayed leaf rolling was considered as a desirable character in rice (Maji, 1994) as in BRRI dhan49 in the present experiment. According to Blum (1988), a plant that maintains high leaf water potential would show less leaf rolling. Mackill (1991) reported that delayed leaf rolling was positively related to drought resistance and recovery from drought. It was also reported that the leaf rolling is one of the acclimation responses of rice and is used as a criterion for scoring drought tolerance (Pandey and Shukla, 2015). Kadioglu and Terzi (2007) stated that the leaf rolling is hydronasty that leads to reduced light interception, transpiration and leaf dehydration. It may help in maintaining internal plant water status (Turner *et al.,* 1986; Abd Allah, 2009; Gana, 2011; Ha, 2014). If cell turgor is maintained under drought stress, it will result in delayed leaf rolling. However, it was also reported that increased leaf rolling under severe stress has the advantage of preventing water loss and radiation damage and variation in leaf rolling among varieties has a genetic basis (Subashri *et al*., 2009; Salunkhe *et al*., 2011).Thus, leaf rolling is an adaptive response to water deficit in rice, and leaf angle is a character usually associated with plasticity in leaf rolling when internal water deficit occurs (Chutia and Borah, 2012).

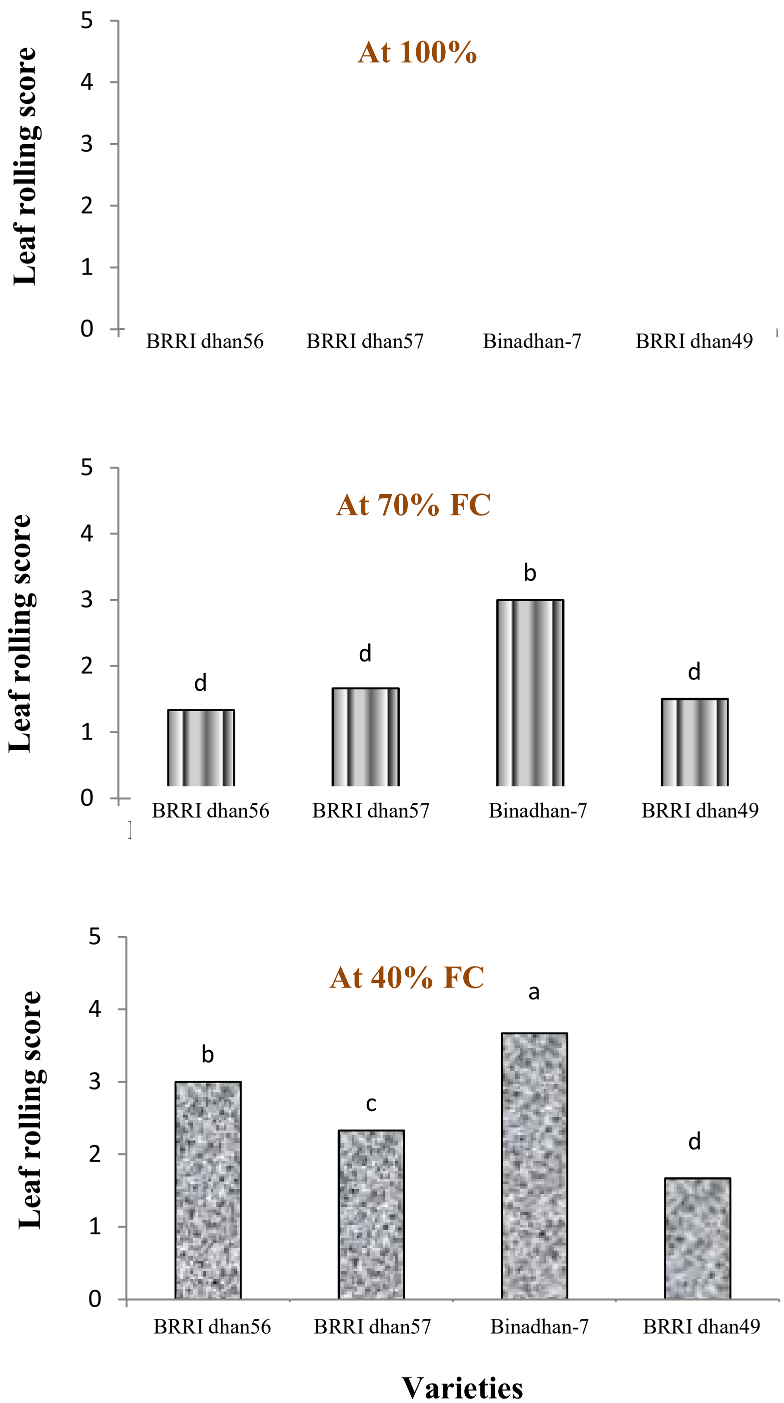


**Figure 6**. Leaf rolling score of different rice varieties as influenced by various soil moisture levels. S0=100%FC (field capacity) moisture, S1= 70% of the FC moisture, S2= 40% of the FC moisture. Values followed by same letter(s) did not differ significantly at 5% level of probability.

### 4.1.10. Relationship between leaf rolling and grain yield

In the present experiment, it was recorded that the grain yield was also influenced by leaf rolling. The leaf rolling helped plants to minimize transpiration loss under water limiting treatment. As a result, the relative water content of leaf might be less affected under water stress treatment. On the other hand, higher leaf rolling might decreased light harvest as well as different physiological processes in leaf, which ultimately affect grain yield. The relationship between leaf rolling and grain yield of different rice varieties under various soil moisture treatments was recorded in the present experiment. It was observed that there was a highly negative linear relation between leaf rolling and grain yield (Figure 7). Grain yield was found the lowest when the leaf rolling was the highest. The grain yield recorded was the highest when the leaf rolling was the lowest. The grain yield was gradually decreased with the increasing leaf rolling score. Kumar *et al.* (2014) found that grain yield was significantly and negatively correlated with leaf rolling, leaf drying and spikelet sterility under drought stress treatment.

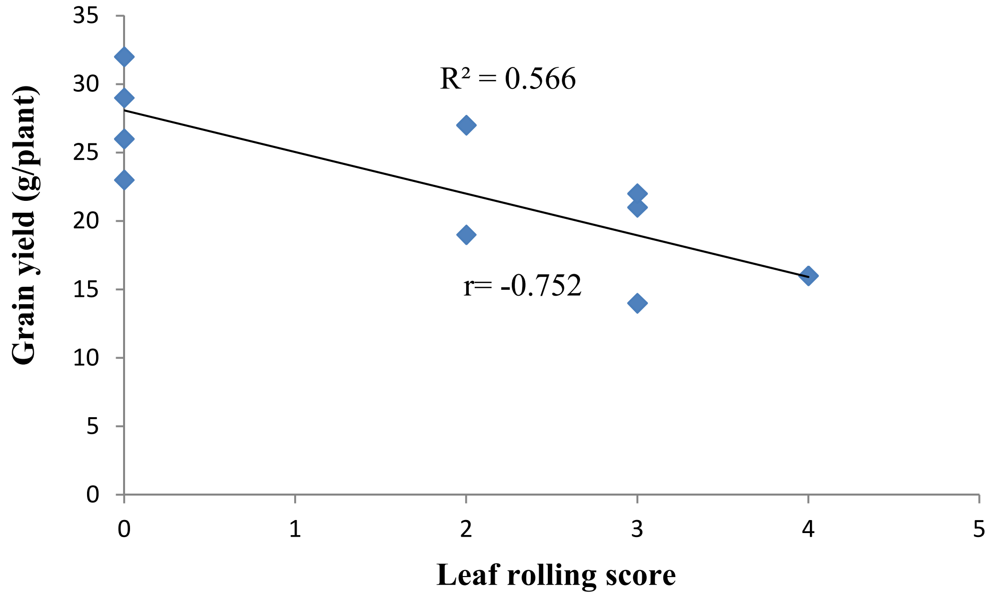


**Figure 7**. Relationship between leaf rolling score and grain yield of different rice varieties under various soil moisture treatments.

### 4.1.11. SPAD reading from anthesis to maturity

The SPAD (Soil Plant Analysis Development) value represents the greenness of the leaf. SPAD is a tool for measuring leaf chlorophyll content by which plant N level can be indirectly estimated. The instrument measures transmission of red light at 650 nm, at which chlorophyll absorbs light, and transmission of infrared light at 940 nm, at which no absorption occurs. On the basis of these two transmission values the instrument calculates a SPAD value that is quite well correlated with chlorophyll content (Markwell *et al*., 1995). The SPAD value has been well recognized as a mean of determining the onset of senescence process in a leaf (Rajcan *et al*., 1999). The sharp decrease in SPAD value indicates the onset of leaf senescence. The SPAD value recorded was from the flag leaf of all tillers and the average value was recorded during the grain filling period after 7 days interval from anthesis to maturity. In the present study, sharp decrease in SPAD value, which occurs at a SPAD reading around 20, was used as an indication of the onset of flag leaf senescence. During anthesis period the SPAD value was found around 40 in all the varieties. In some rice varieties the SPAD value slightly increased after anthesis then gradually decreased and in other varieties the SPAD value gradually decreased from anthesis to maturity (Figure 8). Considering all the varieties, the difference in SPAD value among the three water levels was not clear but the variety BRRI dhan49 maintained a strong steady trend at all soil moisture treatment compare to other varieties. At maturity stage, the SPAD values were recorded around 20 in all the soil moisture treatments of every variety.

The lowest SPAD value at S2 treatment might be due to lower chlorophyll content under S2 treatment. The SPAD values were much better in BRRI dhan49 under water stress treatment compare to other and this might be due to lower reduction of chlorophyll under water stress treatment in this variety. On the other hand, the chlorophyll content of this variety recorded was the highest at S1 treatment among the treatments.  Rajcan *et al.* (1999) reported that the leaf senescence of maize occurred at a SPAD reading between 25 and 30. In an experiment, Hasan (2009) recognized the SPAD values ranging from 25 to 30 as an indicator of flag leaf senescence in wheat. Kumar *et al.* (2014) found significant variations among different rice varieties for drought tolerance parameters such as

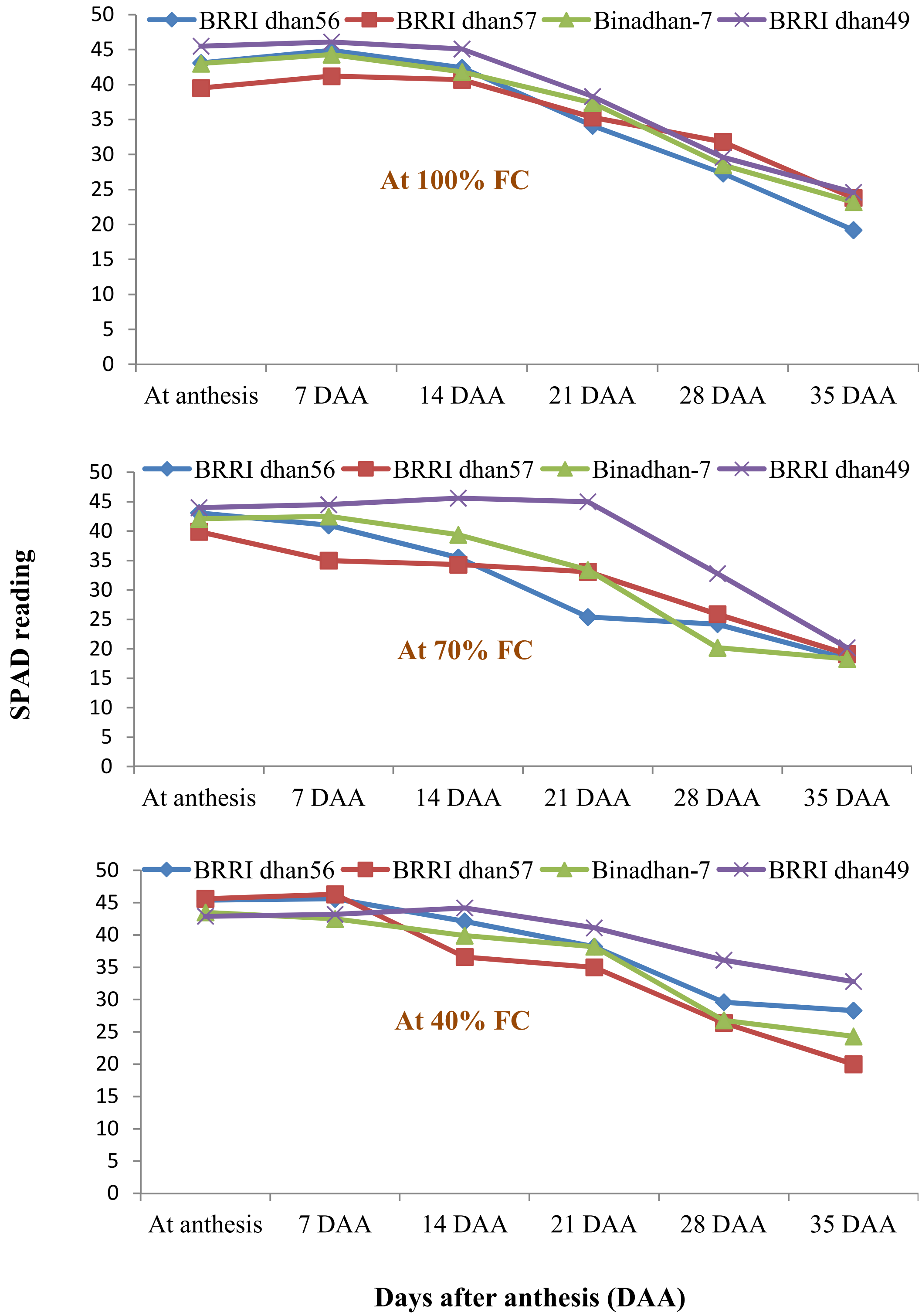


**Figure 8**. SPAD reading during grain filling period of different rice varieties under various soil moisture levels.

greenness of leaf. Ndjiondjop *et al.* (2010) stated that plant height and grain yield were reduced under drought and under drought, plant development is reduced as a consequence of reduced in leaf color.

### 4.1.12. Stomatal conductance from anthesis to maturity

The stomatal conductance was recorded from the flag leaf of all tillers and the average value was recorded during the grain filling period after 7 days interval from anthesis to maturity. The stomatal conductance was recorded up to maturity when the SPAD value recorded was around 20. The results have been shown in figure 9. The stomatal conductance recorded was the highest during anthesis and it was gradually decreased from anthesis to maturity in all the varieties. The decreasing rate of stomata conductance towards maturity was very sharp at S2 treatment compare to S0 and S1 treatments in all the varieties. At S0 treatment, the stomatal conductance of all the varieties recorded around 1200 $\mu$ mol $m^{-2}s^{-1}$. At S1 treatment, the stomatal conductance of all the varieties recorded ranging from 1000 to 1200 $\mu$ mol $m^{-2}s^{-1}$. At S2 treatment, the stomatal conductance of Binadhan-7 recorded was much lower (around 800 $\mu$ mol $m^{-2}s^{-1}$) and recorded much higher in BRRI dhan49 (around 1000 $\mu$ mol $m^{-2}s^{-1}$). Under water stress treatment (under S1 and S2 treatment) the variety BRRI dhan49 showed much better stomatal conductance during grain filling period compare to other varieties.

The stomatal conductance was recorded the lowest at S2 treatment in all the varieties. This might be due to lower RWC in this treatment. But in BRRI dhan49, the stomatal conductance was much better under S2 treatment compared to other varieties in the same soil moisture treatment. This might be due higher RWC in BRRI dhan49 under S2 treatment, as the proline content was higher under this treatment. It was reported that the stomatal conductance is dependent on some factors that are also altered by drought; among which are external factors such as soil water availability, vapor pressure deficit, and so on, and internal factors such as abscisic acid (ABA), xylem conductivity, leaf water status, and so on. Under drought a chemical signal is produced by ABA in plant roots, which is synthesized in the roots in response to soil drying. This signal is transported from the root to leaf through the xylem by the transpiration stream; this is

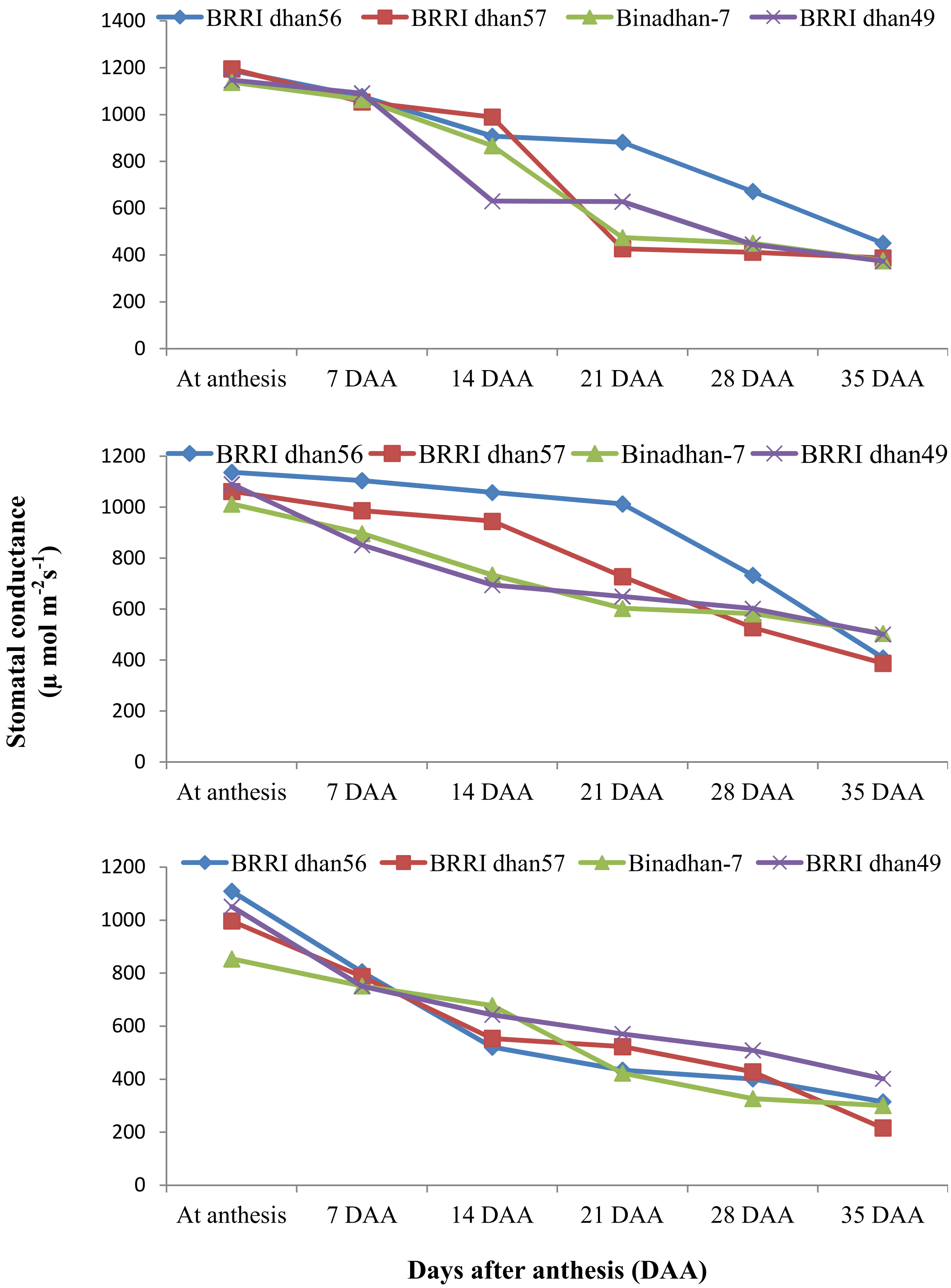


**Figure 9**. Stomatal conductance during grain filling period of different rice varieties under various soil moisture levels.

thought to induce stomatal closure (Wilkinson and Davies, 2008), some other factors like precursors of ABA or cytokinins, mineral composition, and pH of the xylem have a role in stomatal movement (Hartung *et al*., 1998). Lawlor (2002) stated that during the onset of drought, stomatal conductivity declines before photosynthesis, and the inhibition of photosynthesis during mild stress is mainly due to the reduction of $CO_2$ diffusion.

**4.1.13. The stomatal conductance and transpiration rate recorded with gas analyzer**

The importance of flag leaf in grain filling is well recognized. The stomatal conductance and transpiration rate were recorded from flag leaf during anthesis period (with gas analyzer) which have been shown in the table 4. The highest stomatal conductance was found at control (S0) treatment in all the varieties except in Binadhan-7 in which the S1 treatment showed the highest stomatal conductance. The lowest stomatal conductance recorded was at S2 treatment in all the rice varieties (Table 4). Considering all the varieties and soil moisture treatments, the highest (1.6 m mol $m^{-2}$ $s^{-1}$) stomatal conductance recorded was in S0 treatment of Binadhan-7 which was significantly higher than any other treatments and the lowest (0.8 m mol $m^{-2}$ $s^{-1}$) stomatal conductance recorded was in S2 treatment of Binadhan-7 which was significantly lower than any other treatments.

The stomatal conductance recorded was the lowest at S2 treatment in all the varieties. This might be due to lower RWC in this treatment. But in BRRI dhan49 and BRRI dhan57, the stomatal conductance was much better under S2 treatment compared to other varieties. This might be due to comparatively higher RWC, which was initiated by higher proline accumulation under water stress. Ahmad *et al*. (2014) stated that ABA (which is a stress hormone) accumulation under drought stress, regulates stomatal movement and thus is an important component of drought tolerance strategy for reduced water loss, by closing stomata. When the soil moisture becomes lower the stomatal conductance also become lower. Zulkarnain *et al.* (2009) found that the stomatal conductance was decreased in different rice varieties, as the intensity of water deficit stress increased with the time of soil drying. Such a reduction in stomatal conductance appeared to be the primary response and a common phenomenon during water stress, which is believed to be one of the most important desiccation-avoidance mechanisms evolved in plants (Pugnaire *et al*., 1999; Carr, 2001). The stomatal conductance is dependent on some factors that are also altered by drought; among which are external factors such as soil water availability, vapor pressure deficit, and so on, and internal factors such as abscisic acid (ABA), xylem conductivity, leaf water status, and so on. Under drought a chemical signal is produced by ABA in plant roots, which is synthesized in the roots in response to soil drying. This signal is transported from the root to leaf through the xylem by the transpiration stream; this is thought to induce stomatal closure (Wilkinson and Davies, 2008). Some other factors like precursors of ABA or cytokinins, mineral composition, and pH of the xylem have a role in stomatal movement (Hartung *et al*., 1998).

The transpiration rate was also become lower at S2 treatment in all the varieties. Higher transpiration rate was observed at S0 treatment in all the rice varieties (Table 4). Considering all the varieties and soil moisture treatments, the transpiration rate recorded was the highest (4.15 m mol $m^{-2}$ $s^{-1}$) in S0 (control) treatment of BRRIdhan56 which was statistically similar to S0 treatments of Binadhan-7 and BRRI dhan49 and the transpiration rate recorded was the lowest (2.32 m mol $m^{-2}$ $s^{-1}$) at S2 treatment of BRRI dhan57. This might be due to lower RWC in this treatment which ultimately affect the stomatal conductance as well as the transpiration rate. The lower transpiration rate under S2 treatment might also due to lower availability of soil water. Lipiec *et al*. (2013) also found that the transpiration rate was substantially lower under water deficit than under well-watered treatments. Islam (2010) found stomatal conductance, transpiration rate decreased with water stress imposed on rice varieties which was agreed with those of Pieters and Nunez (2008) who observed decreased photosynthesis, transpiration rate and stomatal conductance with water deficit in rice.

**Table 4**. Effect of different soil moisture levels on stomatal conductance and transpiration rate of transplanted *aman* rice varieties

| Varieties | Soil moisture Levels | Stomatal conductance (m mol $m^{-2}$ $s^{-1}$) | Transpiration rate (m mol $m^{-2}$ $s^{-1}$) |
|---|---|---|---|
| BRRI dhan56 | S0 | 1.5 b | 4.15 a |
| | S1 | 1.2 c | 3.47 bc |
| | S2 | 1.1 d | 3.44 bc |
| BRRI dhan57 | S0 | 1.2 c | 3.61 b |
| | S1 | 1.2 c | 3.31 cd |
| | S2 | 1.1 d | 2.32 f |
| Binadhan-7 | S0 | 1.6 a | 4.02 a |
| | S1 | 1.5 b | 3.64 b |
| | S2 | 0.8 e | 3.10 de |
| BRRI dhan49 | S0 | 1.5 b | 4.09 a |
| | S1 | 1.2 c | 3.00 e |
| | S2 | 1.2 c | 3.01 e |
| CV (%) | | 9.97 | 4.51 |

S0=100%FC (field capacity) moisture, S1= 70% of the FC moisture, S2= 40% of the FC moisture. Values followed by same letter(s) did not differ significantly at 5% level of probability.

### 4.1.14. Intercellular $CO_2$ concentration and net assimilation rate (NAR) recorded with gas analyzer

The intercellular $CO_2$ concentration and net assimilation rate was also measured during anthesis period with the same instrument of transpiration rate measurement. Considering all the varieties and soil moisture treatments, the intercellular $CO_2$ concentration recorded was the highest (295 $\mu$ mol mol$^{-1}$) in Binadhan-7 at control (S0) treatment (Table 5). The lowest (242 $\mu$ mol mol$^{-1}$) intercellular $CO_2$ concentration recorded was also in Binadhan-7 at S2 treatment.

The intercellular $CO_2$ concentration was decreased with the increasing water stress in all the varieties. This might be due to lower RWC under water stress treatment, which ultimately affected the stomatal conductance as well as the intercellular $CO_2$ concentration. It was reported that the decline in the photosynthetic rate under drought stress is frequently attributed to lower intercellular $CO_2$, inhibition of photosynthetic enzymes (*eg* Rubisco) and synthesis of ATP (Zlatev and Lidon, 2012) and lower intercellular $CO_2$ might be due to lower stomatal conductance under limited soil moisture treatment. Zain *et al*. (2014) stated that water stress has significant effect on leaf gas exchange in rice variety.

The net assimilation rate is the rate of increase in dry weight per unit of leaf area. So, NAR is a good measurement of photosynthetic efficiency. Considering all the varieties and soil moisture treatments, the net assimilation rate was also recorded the highest (5.74 $\mu$ mol m$^{-2}$ s$^{-1}$) in Binadhan-7 at S0 treatment. The lowest (3.57 $\mu$ mol m$^{-2}$ s$^{-1}$) net assimilation rate was also recorded in Binadhan-7 at S2 treatment (Table 5). So it was clear that the net assimilation rate was decreased under water stressed treatment but the S1 treatment did not show any remarkable effect on net assimilation rate compare to control in all the varieties.

In the present experiment, as the RWC at S2 treatment recorded was lower, the stomatal conductance was also lower and consequently the gas exchange became lower which might affect the NAR. But the NAR at S1 and S0 treatment did not differ markedly; this might be due to no significant different between S1 and S0 treatment in RWC, stomatal conductance, relative injury and chlorophyll content which ultimately contributed in the highest NAR at S1 treatment in BRRI dhan49, BRRI dhan56 and BRRI dhan57. Many authors reported that the photosynthesis is one of the main metabolic process determining crop production and is affected by drought stress (Ji *et al*., 2012; Lauteri *et al*., 2014; Yang *et al*., 2014). They aso reported that the major components limiting photosynthesis are the $CO_2$ diffusional limitation due to early stomatal closure, reduced activity of photosynthetic enzymes, the biochemical components related to triose-phosphate formation and decreased photochemical efficiency of PSII. Change in any of these components alters the final photosynthesis rate. Severe drought treatments limit photosynthesis due to a decline in Rubisco activity, which is an enzyme of the Calvin cycle (Bota *et al*., 2004; Zhou *et al.*, 2007). As the photosynthesis as well as net assimilation rate was decreased under water stress (S2) treatment compare to control treatment (S0), the dry matter content might also lower under water stress treatment which ultimately affected the grain yield. Islam (2010) found photosynthetic rate decreased with water stress imposed on rice varieties.

**Table 5**. Effect of different soil moisture treatments on intercellular $CO_2$ concentration and net assimilation rate of transplanted *aman* rice varieties

| Varieties | Soil moisture levels | Intercellular $CO_2$ concentration ($\mu$ mol mol$^{-1}$) | Net assimilation rate ($\mu$ mol m$^{-2}$ s$^{-1}$) |
|---|---|---|---|
| BRRI dhan56 | S0 | 281 b | 4.59 d |
| | S1 | 264 d | 4.58 d |
| | S2 | 260 de | 4.43 e |
| BRRI dhan57 | S0 | 283 b | 5.04 b |
| | S1 | 260 de | 4.94 b |
| | S2 | 248 f | 4.80 c |
| Binadhan-7 | S0 | 295 a | 5.74 a |
| | S1 | 271 c | 5.04 b |
| | S2 | 242 g | 3.57 f |
| BRRI dhan49 | S0 | 269 c | 4.97 b |
| | S1 | 256 e | 4.93 b |
| | S2 | 249 f | 4.50 de |
| CV (%) | | 1.10 | 1.5 |

S0=100%FC (field capacity) moisture, S1= 70% of the FC moisture, S2= 40% of the FC moisture. Values followed by same letter(s) did not differ significantly at 5% level of probability.

#### 4.1.15. Relative growth rate (RGR)

The relative growth rate simply indicates the change in dry weight per unit dry weight over a period of time. In the present experiment, control treatment (S0) showed the highest relative growth rate in all the varieties (Figure 10). After control treatment the RGR gradually decreased with decreasing soil moisture content in all the varieties. In BRRI dhan56 and BRRI dhan49, the S1 treatment showed more or less similar RGR compared to control treatment. Considering all the varieties and soil moisture treatments, BRRI dhan56 showed the highest (9.75 g $g^{-1}day^{-1}$) RGR at control (S0) treatment which was statistically similar to S1 treatment of the same variety, with S0 and S1 treatments of BRRI dhan57 and with all the treatments of Binadhan7 and BRRI dhan49. BRRI dhan57 showed the lowest (5.09 g $g^{-1}day^{-1}$) RGR at S2 treatment which was statistically similar to the other treatments of the same variety and with S2 treatment of BRRI dhan56, Binadhan-7 and BRRI dhan49. In all the varieties, the RGR of S1 treatment did not show any remarkable compare to control.

In the present experiment, drought stress resulted in various physiological changes in plants that included the reduction in RWC, net assimilation rate, transpiration rate, stomatal conductance, chlorophyll degradation and lower soluble sugar and starch, resulting in decreased RGR which was also reported by Tuna *et al*. (2010). But the RGR at S1 and S0 treatments were statistically similar in BRRI dhan49 and in BRRI dhan56; this might be due to no significant different between S1 and S0 treatment in LA, RWC, stomatal conductance, relative injury and chlorophyll content, sugar-starch content and NAR. As the cell division and cell enlargement become lower under water stress treatment (Prasad *et al*., 2012), relative growth rate might decreased under this limited soil moisture treatment. It was also reported that the water deficit in the rhizosphere leads to an increased rate of root respiration, leading to an imbalance in the utilization of carbon resources, reduced production of adenosine triphosphate and enhanced generation of reactive oxygen species (Farooq *et al.,* 2009) and RGR become lower. However, it was also stated that mild water stress decreased biomass production without a significant effect on photosynthesis (Verelst *et al*., 2012). This demonstrates that plants reduce their

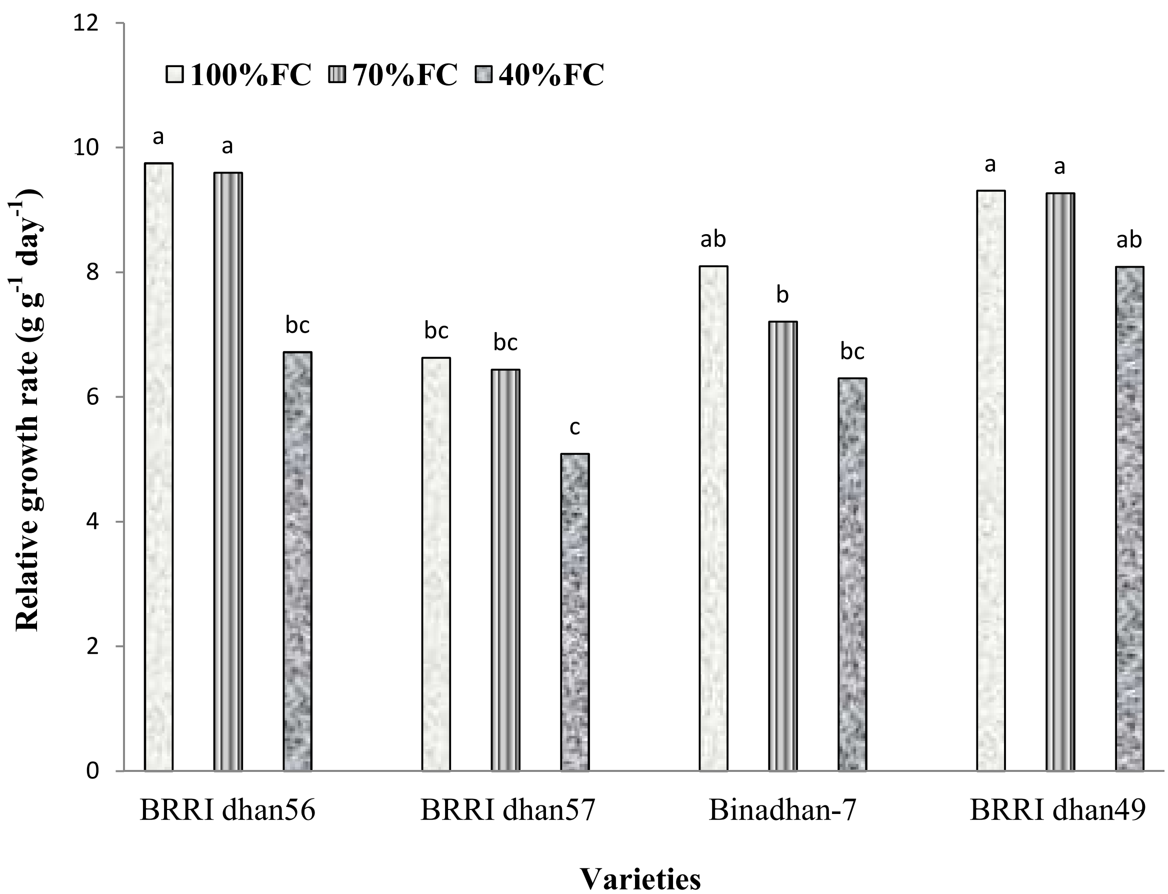


**Figure 10**. Relative growth rate of different rice varieties under various soil moisture levels. S0=100%FC (field capacity) moisture, S1= 70% of the FC moisture, S2= 40% of the FC moisture. Values followed by same letter(s) did not differ significantly at 5% level of probability.

growth as an adaptation response to stress rather than as a secondary consequence of resource limitations (Rollins *et al*., 2013).

**4.1.16. Stem reserve translocation**

The stem reserve translocation plays an important role in grain yield under water stress treatment. Among the varieties and soil moisture treatments, the highest (36.70%) stem reserve translocation recorded was in S2 treatment of BRRI dhan57 which was statistically similar to other treatments of the same variety and significantly higher than any treatment of the other variety and the lowest (24.10%) stem reserve translocation recorded was in BRRI dhan49 at S0 treatment which was statistically similar to S1 treatment of the same variety and with control treatment of BRRI dhan56 (Figure 11). In all the varieties, the lowest stem reserve translocation recorded was at control (S0) treatment and was gradually increased with decreasing soil moisture treatment.

In the present experiment, under water stress treatment the LA, SLA, plant height, effective tiller decreased. The RWC was also lower under water stress treatment that caused increased leaf rolling, decreased chlorophyll content which ultimately affected the harvesting of light. As a result, the stomatal conductance, gas exchange, RGR, AGR, and the grain filling duration became lower under water stress treatment. In this situation as the contribution of current photosynthesis towards grain become lower, the grain filling become more dependent on stem reserve. Therefore, the SRT was increased with the increasing water stress. But the difference between control stem reserve and water stress stem reserve found significant only in BRRI dhan49 and BRRI dhan56. It was clear from the present experiment that soil moisture stress increased the pre-anthesis stem reserve translocation from vegetative part to the developing grain. Extensive studies demonstrated that water deficit treatments resulted in early senescence and more remobilization of pre-anthesis stored assimilates to grains in cereals (Yang *et al.,* 2001; Yang *et al*., 2003). Yang *et al.* (2001) stated that drought induced earlier mobilization of non-structural reserve carbohydrates (largely, fructans) from stem and leaf sheaths, which provided a greater proportion of kernel dry weight at maturity and it can account for 70-92% of grain dry matter, under treatments of drought. Usually, drought during the grain-

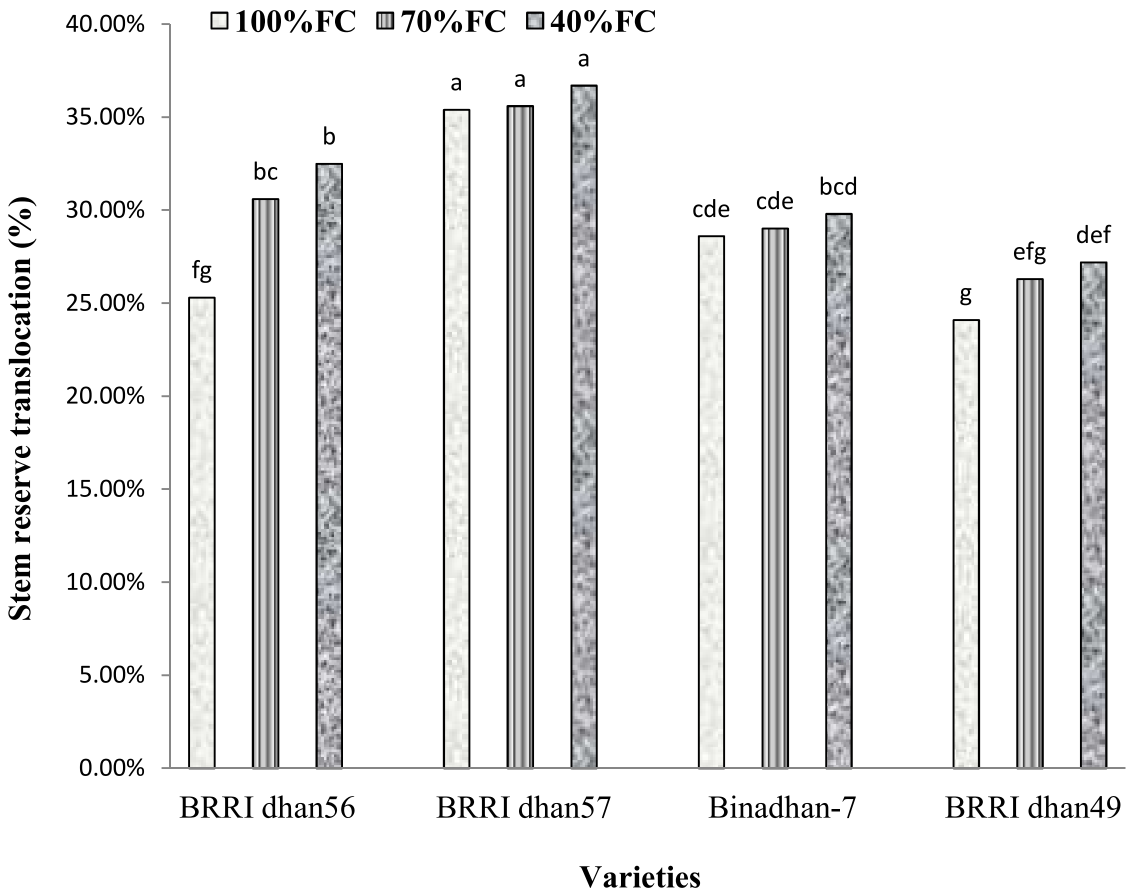


**Figure 11**. Stem reserve translocation towards grain of different rice varieties under various soil moisture levels. S0=100%FC (field capacity) moisture, S1= 70% of the FC moisture, S2= 40% of the FC moisture. Values followed by same letter(s) did not differ significantly at 5% level of probability.

filling process induces early senescence and shortens the grain-filling period, but increases remobilization of assimilates from the straw to the grains (Plaut *et al.*, 2004) plays an important role in grain development. Blum (2005) stated that as photosynthesis becomes inhibited by drought, the grain filling process becomes increasingly reliant on stem reserve utilization.

### 4.1.17. Absolute grain growth rate (AGR)

The absolute grain growth rate (AGR) indicates the growth rate of a single grain. The AGR was estimated during grain filling period from each treatment of every variety. The absolute grain growth rate was gradually decreased with the increasing water stress treatment in all the varieties (Figure 12). In the present experiment, the absolute grain growth rate differed significantly among the treatments by the interaction effect of the rice varieties and soil moisture contents. Considering all the varieties and soil moisture treatments, the highest (1.45 mg $grain^{-1}day^{-1}$) absolute grain growth rate recorded was in control (S0) treatment of BRRI dhan56 which was significantly higher than any other treatments and the lowest (0.37 mg $grain^{-1}day^{-1}$) absolute grain growth rate recorded was in S2 treatment of Binadhan-7 at which was significantly lower than any other treatments. Under water stress treatment the absolute grain growth rate was much higher in BRRI dhan56 and BRRI dhan49 compare to other two varieties.

In the present experiment, drought stress resulted in various morpho-physiological changes in plants, included the reduction in LA, SLA, RWC, chlorophyll content; which ultimately affect the harvesting of light (PAR) and stomatal conductance. Finally it affected the gas exchange and sugar-starch content. As a result the AGR became lower under water stress treatment. It was also recorded that, in the present experiment, the AGR in BRRI dhan49 and BRRI dhan56 was relatively higher than other varieties under water stress treatment. This might be due to higher proline accumulation in those varieties under water stress treatment, which contributed in higher RWC, lower leaf rolling and relatively higher chlorophyll content. As a result, the light harvesting was comparatively higher in those varieties. Consequently, the stomatal conductance, gas exchange, soluble sugar and starch content were also higher in those varieties under water limiting condition. The dry matter investment to root was also recorded higher in those varieties under water stress treatment. As a result, the AGR was less affected in BRRI dhan49 and BRRI dhan56 compare to other varieties under water stress treatment. Due to increase in water stress, the rate of photosynthesis was seriously hampered due to lack of water (Yang *et al*., 2014) and consequently the grain growth rate recorded was much lower under water stress treatments. A positive relationship between photosynthetic rate and grain growth rate under water stress (at S2 treatment) was observed by Islam (2010), which was fully supported by Huqu *et al*. (2002) who observed a completely synchronous relationship of photosynthate in leaves and the demand of grain filling in super high yielding hybrid rice. Drought stress at vegetative growth especially booting stage (Pantuwan *et al*., 2002), flowering and terminal periods can interrupt floret initiation, causing spikelet sterility and slow grain filling, resulting in lower grain weight and ultimately poor paddy yield (Kamoshita *et al*., 2004; Botwright Acuña *et al*., 2008). They also suggest that higher photosynthetic rate in leaves at fertilization and its complete synchronization with grain filling and to get higher yield of rice under water stress treatment.

**4.1.18. The leaf number, leaf dry weight and stem dry weight of main stem**

The main stem has the major contribution to grain yield in rice (*Martinez-Eixarch et al., 2015).* In the present experiment, different data on main stem was also recorded. The leaf number, leaf dry weight and stem dry weight of main stem was recorded after harvest. There was recorded no remarkable effect of different soil moisture treatments on main stem leaf number. Considering all the varieties and soil moisture treatments, the leaf number found was the highest (6) in BRRI dhan49 at S2 treatment which was statistically similar to S0 and S1 treatments of the same variety and with S0 treatment of BRRI dhan56 (Table 6). The lowest (4.25) leaf number was recorded in BRRI dhan57 at S0 treatment which was statistically similar to other treatments of the same varieties, with S1 and S2 treatments of BRRI dhan56 and with all the treatments of Binadhan-7. In the present experiment, the increased dry matter investment to leaf under water stress treatment might contribute in higher leaf number under water stress treatment.

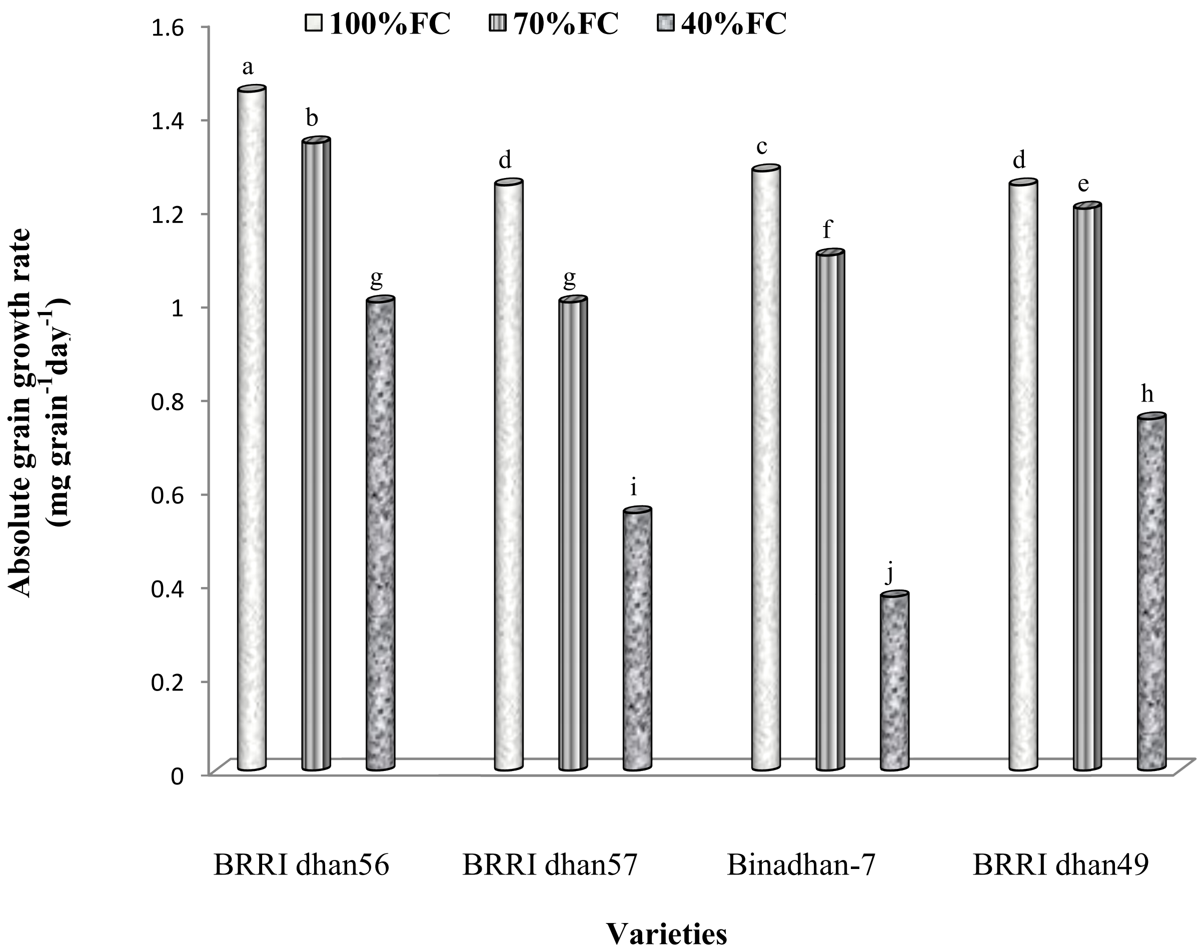


**Figure 12**. Absolute grain growth rate during active grain filling stage of different rice varieties under various soil moisture levels. S0=100%FC (field capacity) moisture, S1= 70% of the FC moisture, S2= 40% of the FC moisture. Values followed by same letter(s) did not differ significantly at 5% level of probability.

In the present experiment, the different soil moisture treatments had no significant effect on leaf dry matter content of main stem. Among the varieties and soil moisture treatments, the leaf dry weight was found the highest (0.795 g) in BRRI dhan49 at S0 treatment which was statistically similar to other treatments of the same variety and with S0 treatment of BRRI dhan56. The leaf dry weight recorded was the lowest (0.510 g) in BRRI dhan57 at S0 treatment which was statistically similar to other treatments of the same variety, with S2 treatment of BRRI dhan56 and with all the soil moisture treatments of Binadhan-7. At S2 treatment, though the leaf number was slightly higher compared to control in BRRI dhan57 and BRRI dhan49, but the leaf dry matter recorded was lower in BRRI dhan49 at S2 treatment compared to control (S0) treatment. In the present experiment, the increased dry matter investment to leaf under water stress treatment might contribute in lower reduction of leaf dry matter under water stress treatment. Sarani *et al*. (2014) stated that soil moisture stress directly affect the biomass of shoots.

The main stem dry matter recorded was lower under water stress treatment compared to control treatment in all the varieties. But different soil moisture treatments did not differ significantly among them in BRRI dhan49. Considering all the varieties and soil moisture treatments, the stem dry weight of main stem recorded was the highest (1.76 g) in BRRI dhan49 at S0 treatment which was significantly higher than S2 treatment of BRRI dhan57 and S1 treatment of Binadhan-7. The lowest (1.238 g) stem dry weight of main stem recorded was in Binadhan-7 at S1 treatment which was statistically similar to other treatments of the same variety and with all the treatments of BRRI dhan56 and BRRI dhan57.

Under water stress treatment as the different morpho-physiological parameters were affected (described before), the main tiller stem dry matter content was also affected. But in BRRI dhan49, the main stem dry matter was less affected under water stress treatment (reasons described before). As the photosynthesis become lower under water stress the dry matter content leaf and stem become lower. Sarani *et al*. (2014) stated that soil moisture stress directly affect the biomass of shoots.

**Table 6**. Effect of different soil moisture levels on leaf number, leaf dry weight and stem dry weight of the main stems of transplanted *aman* rice varieties

| Varieties | Soil moisture Levels | Leaf number of the main stem | Leaf dry weight of the main stem (g) | Stem dry weight of the main stem (g) |
|---|---|---|---|---|
| BRRI dhan56 | S0 | 5.50 abc | 0.813 a | 1.688 abc |
| | S1 | 4.75 cd | 0.738 abc | 1.455 abc |
| | S2 | 5.00 bcd | 0.608 cd | 1.417 abc |
| BRRI dhan57 | S0 | 4.25 d | 0.510 d | 1.485 abc |
| | S1 | 4.75 cd | 0.608 cd | 1.417 abc |
| | S2 | 4.75 cd | 0.645 bcd | 1.338 bc |
| Binadhan7 | S0 | 5.00 bcd | 0.605 cd | 1.455 abc |
| | S1 | 4.75 cd | 0.618 cd | 1.238 c |
| | S2 | 5.00 bcd | 0.632 bcd | 1.443 abc |
| BRRI dhan49 | S0 | 5.75 ab | 0.823 a | 1.878 a |
| | S1 | 5.75 ab | 0.795 ab | 1.800 ab |
| | S2 | 6.00 a | 0.795 ab | 1.760 ab |
| CV (%) | | 11.32 | 15.55 | 18.27 |

S0=100%FC (field capacity) moisture, S1= 70% of the FC moisture, S2= 40% of the FC moisture. Values followed by same letter(s) did not differ significantly at 5% level of probability.

### 4.1.19. Leaf, stem and root dry weight per plant

The Leaf, stem and root dry weight per plant recorded was after harvest. There was no remarkable effect of FC treatments on leaf dry matter in all the varieties. But, it was also recorded that S1 treatment produced the highest leaf dry matter compare to control in all the varieties. Considering all the varieties and soil moisture treatment, the leaf dry weight recorded was the highest (6.81 g) in BRRI dhan49 at S1 treatment which was statistically similar to Binadhan-7 at S0. The lowest (3.93 g) leaf dry weight recorded was in BRRI dhan57 at S2 treatment, which was statistically similar to S0 treatment of the same variety.

In all the varieties, S2 treatment produced the lower leaf dry weight than control (S0) treatment (Table 7). This might be due to reduction in different morpho-physiological parameters, LA, SLA, RWC, chlorophyll content, light harvesting, stomatal conductance, gas exchange, soluble sugar and starch content. All these affected the total dry matter content as well as the leaf dry matter content of the plant. The reduction of leaf dry matter might be due to rapid decline in cell division and leaf elongation under drought (Pandey and Shukla, 2015). Rucker *et al*. (1995) stated that water deficit reduces the leaf number per plant.

All the soil moisture treatments of BRRI dhan56 produced statistically similar stem dry matter and S1 treatment produced the highest stem dry matter among the treatments in Binadhan-7 and BRRI dhan49. Considering all the varieties and soil moisture treatments, stem dry weight was also recorded the highest (13.81 g) in BRRI dhan49 at S1 treatment which was statistically similar to Binadhan-7 at control. The lowest (7.91 g) stem dry matter was also recorded in BRRI dhan57 at S2 (same as leaf dry weight) which was statistically similar to S0 treatment of the same variety and with S2 treatment of BRRI dhan49. Considering all the varieties the stem dry matter was found lower in S2 treatment compare to S0 treatment.

In the present experiment, it was also recorded that different physiological processes like stomatal conductance, NAR, RGR were decreased and SRT was increased due to soil moisture stress (mainly at S2 treatment) which must affected the stem dry matter content of plant. The rate of photosynthesis was seriously hampered due to lack of water (Yang *et al*., 2014) and rapid decline in cell division and leaf elongation under drought (Pandey and Shukla, 2015) might be the major causes of stem dry matter reduction in this situation.

The root dry matter of different soil moisture treatments did not differ significantly among them in all the varieties except in BRRI dhan49. Considering all the varieties and soil moisture treatment, the root dry matter was recorded the highest (3.43 g) in Binadhan-7 at S2 treatment which was statistically similar to the other soil moisture treatments of the same variety and the lowest (1.63 g) root dry matter was recorded in BRRI dhan49 at S0 or control treatment (Table 7) which was statistically similar to S1 treatment of the same variety and with all the soil moisture treatments of BRRI dhan56 and BRRI dhan57. Considering the three soil moisture treatments, all the varieties except BRRI dhan56 produced the highest root dry matter at S2 treatment. The relative root dry matter was much higher in BRRI dhan49 compare to other varieties.

Increased dry matter investment to root under limited soil moisture condition is very important for water uptake. In the present experiment, the root dry matter was increased with the increasing water stress in all the varieties except in BRRI dhan56. In BRRI dhan49 the relative root dry matter was much higher under water stress treatment and this might help this variety to perform better under limited soil moisture condition. Total root length is strongly related to drought tolerance in rice under drought treatment (Ingram *et al*., 1994). Greater root length density during drought stress might cause better water uptake, as indicated by their higher grain yield (Abd Allah *et al.,* 2010) as in BRRI dhan49. But the relationships between root growth and grain yield under drought are complex. Positive associations between root length and grain yield have been documented in rice (Mambani and Lal, 1983a; Lilley and Fukai, 1994). Hussain *et al.*, (2008) stated that drought caused impaired mitosis; cell elongation and expansion resulted in reduced growth and yield traits. A common adverse effect of drought is the reduction in biomass production (Farooq *et al*., 2009a, 2010). Many studies indicate significant decrease in fresh and dry weights of shoots (Centritto *et al*., 2009; Mostajeran and Rahimi-Eichi, 2009) and roots (Ji *et al*., 2012) under drought. Reduced fresh shoot and root weights as well as their lengths ultimately reduce the photosynthetic rate of physiology and biochemical processes of rice (Usman *et al*., 2013).

**Table 7**. Effect of different soil moisture levels on leaf, stem and root dry weight of plant of transplanted *aman* rice varieties

| Varieties | Soil moisture levels | Leaf dry weight per plant (g) | Stem dry weight per plant (g) | Root dry weight per plant (g) | |
|---|---|---|---|---|---|
| | | | | Actual | Relative (%) |
| BRRI dhan56 | S0 | 5.36 bc | 11.71 abc | 2.90 abc | 100 |
| | S1 | 5.85 ab | 11.70 abc | 2.49 c | 86 |
| | S2 | 4.56 cd | 10.60 bc | 2.37 c | 82 |
| BRRI dhan57 | S0 | 3.96 d | 9.48 cd | 2.63 c | 100 |
| | S1 | 5.31bc | 11.02 bc | 2.74 bc | 104 |
| | S2 | 3.93 d | 7.91 d | 2.76 bc | 105 |
| Binadhan-7 | S0 | 6.74 a | 13.31 a | 3.36 ab | 100 |
| | S1 | 5.29 bc | 10.70 bc | 3.37 ab | 100 |
| | S2 | 6.12 ab | 12.52 ab | 3.43 a | 102 |
| BRRI dhan49 | S0 | 5.25 bc | 10.54 bc | 1.63 d | 100 |
| | S1 | 6.81 a | 13.81 a | 2.61 c | 160 |
| | S2 | 4.62 cd | 9.60 cd | 2.52 c | 155 |
| CV (%) | | 13.28 | 12.44 | 14.58 | |

S0=100%FC (field capacity) moisture, S1= 70% of the FC moisture, S2= 40% of the FC moisture. Values followed by same letter(s) did not differ significantly at 5% level of probability.

#### 4.1.20. Root-shoot (without panicle) ratio and total dry matter (TDM) of plant

Water stress had a significant effect on root and shoots dry matter as well as the total dry matter production in rice plant. There were genotypic variations also. The root-shoot ratio and TDM of the plants was measured after harvest. In the present experiment, the root shoot ratio differed significantly by the interaction effect of varieties and soil moisture treatments. Considering all the varieties and soil moisture treatments, the root:shoot ratio recorded was the highest (0.232) in BRRI dhan57 at S2 treatment which was significantly higher than any other treatments and the lowest (0.100) ratio recorded was in BRRI dhan49 at S0 treatment which was significantly lower than any other treatments. The root:shoot ratio recorded was higher at S2 treatment compare to S0 treatment in all the varieties except in BRRI dhan56 (Table 8). This might be due to higher dry matter investment to root rather than the investment to shoot under water stress treatment. Increased root:shoot ratio has a positive role under water stress treatment, because increased root investment help plant to absorb more water from the soil under lower soil moisture condition.

Total dry matter content is the major determinants of growth and development. Among the varieties and soil moisture treatments, the total dry matter content was found the highest (54.01 g) in BRRI dhan56 at S0 treatment which was statistically similar to S0 treatment of Binadhan-7 and with S0 treatment of BRRI dhan49 (Table 8). In BRRI dhan57, the highest TDM content was also found in S0 treatment. In all the varieties, the highest TDM was recorded at S0 or control treatment and the lowest TDM was produced at S2 treatment. Considering all the varieties and soil moisture treatments, the TDM recorded was the lowest (31.35 g) in BRRIdhan57 at S2 treatment which was statistically similar to S2 treatment of BRRI dhan56. In BRRI dhan56, the percent reduction of total dry matter of plant was 7.35% of the control at S1 treatment and the reduction percentage was 36.18% in case of S2 treatment in the same variety. In BRRI dhan57, the reduction percentage at S1 treatment recorded was 2.97% of the control and in the same variety the reduction percentage was 24.93% of the control at S2 treatment. In Binadhan-7, the reduction of TDM of plant was 15.76% of the control at S1 treatment and the reduction percentage was 22.19% of the control at S2 treatment. In BRRI dhan49, the reduction of TDM of plant was much lower (only 0.08% of the control) at S1 treatment and at S2 treatment the reduction of TDM recorded was 21.09% of the control.

So, in the present experiment, the total dry matter production was decreased with decreasing soil moisture levels. This might be due to the reduction of various morphological and physiological processes, that included reduction in LA, SLA, RWC, stomatal conductance, gas exchange, sugar-starch synthesis and NAR etc, resulting in decreased water use efficiency and TDM content of plant. These results confirmed with Tuna *et al*. (2010) and Hossain (2001). Sokoto and Muhammad (2014) stated that water stress at tillering resulted in lower biomass than water stress at flowering and grain filling or no stress control which were statistically similar. Rice grown in drought stress treatment produced significantly less total biomass than irrigated rice (Kumar *et al*., 2014)

**Table 8**. Effect of different soil moisture levels on root-shoot (without panicle) ratio and total dry matter (TDM) production of transplanted *aman* rice varieties

| Varieties | Soil moisture levels | Root-Shoot (without panicle) ratio | Total dry matter per plant (g) | Reduction Percent of TDM per plant (%) |
|---|---|---|---|---|
| BRRI dhan56 | S0 | 0.170 f | 54.01 a | |
| | S1 | 0.145 i | 50.04 b | 7.35 |
| | S2 | 0.157 h | 34.47 d | 36.18 |
| BRRI dhan57 | S0 | 0.198 c | 41.76 c | |
| | S1 | 0.168 g | 40.52 c | 2.97 |
| | S2 | 0.232 a | 31.35 d | 24.93 |
| Binadhan-7 | S0 | 0.168 g | 51.96 ab | |
| | S1 | 0.210 b | 43.77 c | 15.76 |
| | S2 | 0.185 d | 40.43 c | 22.19 |
| BRRI dhan49 | S0 | 0.100 k | 52.91ab | |
| | S1 | 0.125 j | 52.87 ab | 0.08 |
| | S2 | 0.178 e | 41.71 c | 21.17 |
| CV (%) | | 7.26 | 5.28 | |

S0=100%FC (field capacity) moisture, S1= 70% of the FC moisture, S2= 40% of the FC moisture. Values followed by same letter(s) did not differ significantly at 5% level of probability.

### 4.1.21. Percent dry matter distribution to leaf, stem, panicle and root per plant

In the present experiment, the dry matter distribution to leaf, stem, panicle and root per plant was recorded after harvest. Considering all the varieties and soil moisture treatment, the dry matter distribution to leaf was recorded the highest (15.02%) in Binadhan-7 at S2 treatment which was significantly higher than any other treatments and the lowest (9.46%) distribution was recorded in BRRI dhan57 at S0 treatment which was statistically similar to S0 treatment of BRRI dhan56, with S0 and S2 treatments of BRRI dhan49. The dry matter distribution to leaf was increased under stressed treatment compare to control treatment in all the varieties (Table 9). In the present experiment, the leaf became thicker due to water stress treatment and as a result the transpiration rate was decreased. The increased leaf investment under limited soil moisture treatment indicated that leaf dry mass was less affected and total biomass was more affected in this situation. Rucker *et al*. (1995) stated that water deficit reduces the leaf number and weight per plant.

Considering all the varieties and soil moisture treatment, the dry matter distribution to stem was found the highest (30.88) in Binadhan-7 at S2 treatment which was statistically similar to S2 treatment of BRRI dhan56 and the lowest (21.23%) stem dry matter distribution was recorded in BRRI dhan49 at S0 treatment which was statistically similar to S2 treatment of the same variety, with S0 and S1 treatments of BRRI dhan56 and with S0 treatment of BRRI dhan57. In all the varieties, the leaf and stem investment were found higher at S2 treatment compare to S0 treatment. So, the results indicated that the leaf and stem investment increased under limited soil moisture condition compared to control in all the varieties. In the present experiment, the increased dry matter investment to stem under water stress treatment indicated that the vegetative dry matter was less affected and the reproductive dry matter was more affected under water stress treatment. Sarani *et al.* (2014) stated that soil moisture stress directly affect the biomass of shoots.

Among the varieties and soil moisture treatments, the dry matter distribution to panicle was found the highest (64.93%) in BRRIdhan49 at S0 FC treatment which was statistically similar to S2 treatment of the same variety, with S0 and S1 treatments of BRRI dhan56 and with S0 treatment of BRRI dhan57. The panicle investment recorded was the lowest (45.64%) in Binadhan-7 at S2 treatment which was statistically similar to S2 treatment of BRRI dhan56. In all the varieties, the panicle investment was found lower at S2 compare to S0 treatment (Table 9). In the present experiment, the decreased dry matter investment to panicle under water stress treatment indicated that the reproductive dry matter was more affected and the vegetative dry matter was less affected under water stress treatment. Maisura *et al*. (2014) found that drought stress reduced grain yield irrespective of rice varieties.

Considering all the varieties and soil moisture treatments, the dry matter investment to root was found the highest (8.73%) in BRRI dhan57 at S2 treatment (Table 9) which was statistically similar to S2 treatment of Binadhan-7 and the lowest (3.26%) root investment recorded was in BRRI dhan49 at S0 treatment which was significantly lower than any other treatments. In all the varieties the root investment was found higher at S2 compare to S0 treatment. In BRRI dhan49, the root investment was found almost double at S2 treatment than that in S0 treatment. Increased root investment under limited soil moisture condition help plant to absorb more water from the soil. Greater root dry matter during drought stress might cause better water uptake (Abd Allah *et al.,* 2010). In BRRI dhan49, the increased dry matter investment to root under water stress treatment might help this variety to perform better under limited soil moisture condition. Zhao *et al*. (2006) also found a common adverse effect of water stress on crop plants; was the reduction in fresh and dry biomass production.

**Table 9**. Effect of different soil moisture levels on dry matter partitioning to leaf, stem, panicle and root in plant of transplanted *aman* rice varieties

| Varieties | Soil moisture levels | Dry matter partitioning to leaf (%) | Dry matter partitioning to stem (%) | Dry matter partitioning to panicle (%) | Dry matter partitioning to root (%) |
|---|---|---|---|---|---|
| BRRI dhan56 | S0 | 9.92 ef | 21.68 ef | 63.04 a | 5.36 ef |
| | S1 | 11.66 bcde | 23.33 cdef | 60.06 ab | 4.96 f |
| | S2 | 13.16 b | 30.67 a | 49.32 de | 6.85 cd |
| BRRI dhan57 | S0 | 9.46 f | 22.58 def | 61.68 a | 6.27 de |
| | S1 | 13.11 b | 27.03 b | 53.16 cd | 6.71d |
| | S2 | 12.41 bc | 25.15 bcd | 53.71 cd | 8.73 a |
| Binadhan-7 | S0 | 12.98 b | 25.61 bcd | 54.96 c | 6.47 d |
| | S1 | 12.09 bcd | 24.43 bcde | 55.80 bc | 7.69 bc |
| | S2 | 15.02 a | 30.88 a | 45.64 e | 8.46 ab |
| BRRI dhan49 | S0 | 10.58 def | 21.23 f | 64.93 a | 3.26 g |
| | S1 | 12.86 b | 26.07 bc | 56.14 bc | 4.93 f |
| | S2 | 11.01 cdef | 22.89 def | 60.10 ab | 6.01 de |
| CV (%) | | 9.42 | 7.66 | 5.66 | 10.12 |

S0=100%FC (field capacity) moisture, S1= 70% of the FC moisture, S2= 40% of the FC moisture. Values followed by same letter(s) did not differ significantly at 5% level of probability.

### 4.2. Experiment-II: Response of different morphological and physiological characters of transplanted *aman* rice varieties to varying water stress conditions as imposed by different concentrations of polyethylene glycol (PEG).

In the present experiment, different morphological and physiological characters of four transplanted *aman* rice varieties, grown in nutrient solutions treated with different concentrations of polyethylene glycol (PEG), were studied to find out the effect of moisture stress (induced by PEG) on morphological and physiological attributes of those rice varieties.

Among the treatment, all plants were died at 15, 20 and 25% PEG concentration. At 10% PEG concentration plants of Binadhan-7 did not survive.

#### 4.2.1 Eye observation

Different polyethylene glycol (PEG) concentration in the nutrient solution imposed water stress for rice plant grown in that solution. Seven days after transplanting of the rice seedlings in the experimental pots, it was observed that all plant of every variety did not survive in some PEG concentration. Some rice seedlings were more sensitive to PEG (Appendix 8). It was found that no plants of all the varieties were survived in 25%, 20% and 15% PEG concentration (Appendix 9 and 10). All plants of Binadhan-7 did not survive in 10% PEG concentration also.

So, in the second experiment, the variety Binadhan-7 also showed higher sensitivity to PEG induced drought stress compared to other varieties. Though the plants of different varieties were survived under lower concentration of PEG, their growth was much lower compared to control plant which was easily identified by their phenotypic appearance. The living plants grown in PEG solution also looked very weak. The phenotypic appearance of plants in different treatment pots were also shown in photographs (shown in appendix 8-10).

#### 4.2.2. Seedling mortality percentage

Seedling mortality percentage was calculated at the end of the experiment after removing the plant from PEG solutions. All plants of every variety found dead in 15%, 20% and in 25% PEG solutions. In BRRI dhan56, all plants were alive in 0% PEG concentration. 66.66% plants were alive in 5% and 10% PEG concentration in the same variety (Table 10). But, there were significant difference in seedling mortality percentage between control (no PEG) and other two (5% and 10%) PEG treatments. All plants of BRRI dhan57 were survive in 0% PEG concentration and 66.66% plants were survived in 5% PEG solution and 33.33% plants were survived in 10% PEG concentration in the same variety. But there existed significant difference in seedling mortality percentage among the treatment of BRRI dhan57. In Binadhan-7, all plants were survive in 0% PEG concentration. But only 33.33% plants were survive in 5% PEG treatment which was significantly lower than control treatment and no plants were survive in 10% PEG concentration in the same varieties. In BRRI dhan49, all plants were survived in 0% PEG concentration. Consequently, 66.66% plants were survived in 5% and in 10% PEG treatment in the same varieties. There was significant difference in seedling mortality percentage among the treatments in BRRI dhan49. So, it was clear that Binadhan-7 was more sensitive and BRRI dhan56 and BRRI dhan49 were less sensitive in FEG-induced drought treatment. More or less same results were also recorded in experiment I. Polyethylene glycol (PEG) of high molecular weights have been long used to simulate drought stress in plants as non-penetrating osmotic agents lowering the water potential in a way similar to soil drying (Larher *et al*., 1993). Though, there was enough water in the pot, the rice root was unable to absorb those water and ultimately dead in some treatments.

**Table 10**. Seedling mortality percentage of different rice varieties to various PEG concentrations

| Varieties | PEG concentration | Seedling mortality% |
|---|---|---|
| BRRI dhan56 | 0% | 0 d (2.87)* |
| | 5% | 33.33 c (35.26) |
| | 10% | 33.33 c (35.26) |
| | 15% | 100 a (87.13) |
| | 20% | 100 a (87.13) |
| | 25% | 100 a (87.13) |
| BRRI dhan57 | 0% | 0 d (2.87) |
| | 5% | 33.33 c (35.26) |
| | 10% | 66.66 b (54.73) |
| | 15% | 100 a (87.13) |
| | 20% | 100 a (87.13) |
| | 25% | 100 a (87.13) |
| Binadhan-7 | 0% | 0 d (2.87) |
| | 5% | 66.66 b (54.73) |
| | 10% | 100 a (87.13) |
| | 15% | 100 a (87.13) |
| | 20% | 100 a (87.13) |
| | 25% | 100 a (87.13) |
| BRRIdhan49 | 0% | 0 d (2.87) |
| | 5% | 33.33 c (35.26) |
| | 10% | 33.33 c (35.26) |
| | 15% | 100 a (87.13) |
| | 20% | 100 a (87.13) |
| | 25% | 100 a (87.13) |
| CV (%) | | 12.69 |

Values followed by same letter(s) did not differ significantly at 5% level of probability.
*Values within the parenthesis indicate the arcsine transformation value.

### 4.2.3. Shoot length

The shoot length of 50-days old seedling was recorded after taking from treatment pot. The shoot length was found the highest at 0% PEG treatment in all the varieties and the shoot length decreased with the increasing PEG concentration (Figure 13). The plants of Binadhan-7 were not survive at 10% PEG concentration. In BRRI dhan56, the highest (50.83 cm) shoot length recorded was at 0% PEG treatment, which was significantly higher than any other treatments. The lowest (37.20 cm) shoot recorded was at 10% PEG treatment which was statistically similar to 5% PEG treatment. In BRRI dhan57, the longest (50.43 cm) shoot was also recorded in 0% PEG solution, which was significantly higher than any other treatments in the same variety and the lowest (44.50 cm) shoot length recorded was in 10% PEG solution, which was statistically similar to 5% PEG solution. In the variety Binadhan-7, the highest (49.17 cm) shoot length recorded was in 0% PEG treatment also which was significantly higher than 5% PEG treatment in the same variety. In BRRI dhan49, the highest (49.18 cm) shoot length recorded was at 0% PEG treatment, which was significantly higher than any other treatments of the same variety and the lowest (29 cm) shoot length, in this variety, recorded was in 10% PEG treatment, which was significantly lower than any other treatments of the same variety.

The shortest plant recorded was at 10% PEG treatment in BRRI dhan49, BRRI dhan56 and in BRRI dhan57 and in 5% PEG treatment in Binadhan-7. This might be due to lowest LA, SLA, SPAD reading and RWC; as a result light harvest might be lower and stomatal conductance was also recorded lower which ultimately affected the shoot length. It was reported that as the cell division and cell enlargement become lower under water stress treatment (Hussain *et al*., 2008), the length of the shoot also become lower in that situation. The results of the PEG experiment, regarding shoot length, were similar to plant height of experiment I. it was also reported that reduced fresh shoot and root weights as well as their lengths ultimately reduce the photosynthetic rate of physiology and biochemical processes of rice (Usman *et al*., 2013). Govindaraj *et al*. (2010) found that the shoot length was decreased with an increasing in external water stress.

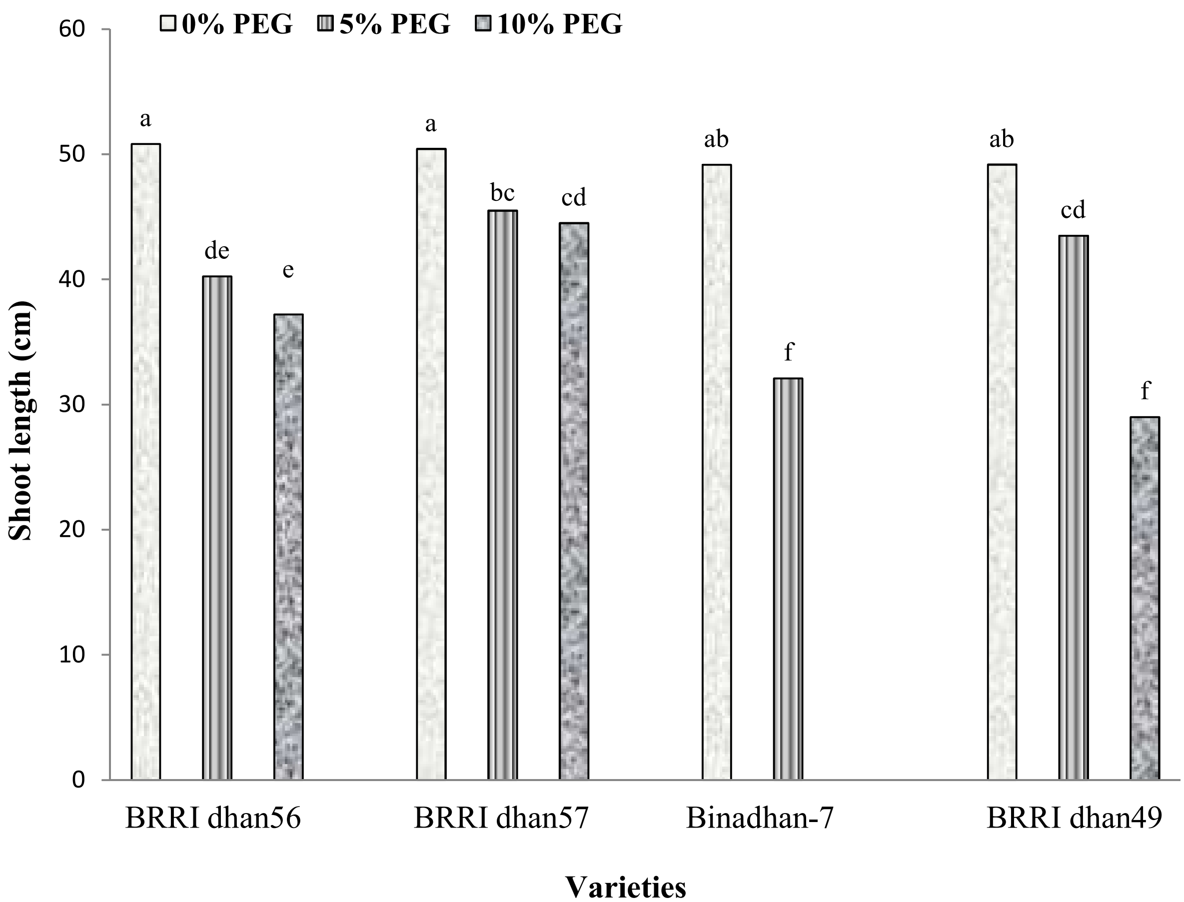


**Figure 13**. Shoot length of different rice varieties under nutrient solution treated with different concentrations of Polyethylene glycol-8000. Values followed by same letter(s) did not differ significantly at 5% level of probability.

### 4.2.4. Root length

In the present experiment, the root length of 50-days old seedling was also recorded at the end of the experiment (Figure 14). The highest root length was also recorded at 0% PEG solution in all variety (same as shoot length). After control treatment, the root length gradually decreased with the increasing PEG concentration in all the varieties (Figure 14). Considering all the varieties and PEG treatment, the lowest (13.04 cm) root length recorded was in BRRI dhan56 at 10% PEG concentration which was statistically similar to 10% PEG treatment of BRRI dhan57 and BRRI dhan49. The highest (24.73 cm) root length recorded was in BRRI dhan57 at 0% PEG concentration which was statistically similar to 5% PEG treatment of the same variety, with control treatment of BRRI dhan56, Binadhan-7 and with control and 5% PEG treatments of BRRI dhan49.

In the present experiment, different morphological and physiological processes (described in shoot length) were hampered which ultimately affect the root length. It was reported that as the cell division and cell enlargement is lower due to imposition of water stress (Hussain *et al.*, 2008), the root length was also become lower under this treatment. A variety with high root length and root weight is expected to be drought tolerant (Roy *et al*., 2009) as BRRI dhan49 in the present experiment. Govindaraj *et al.* (2010) stated that the root length significantly declined with increased external water potential and consequently, all treatments caused a decrease in root elongation in all varieties compared to their controls. Reduced fresh shoot and root weights as well as their lengths ultimately reduce the photosynthetic rate of physiology and biochemical processes of rice (Usman *et al*., 2013)

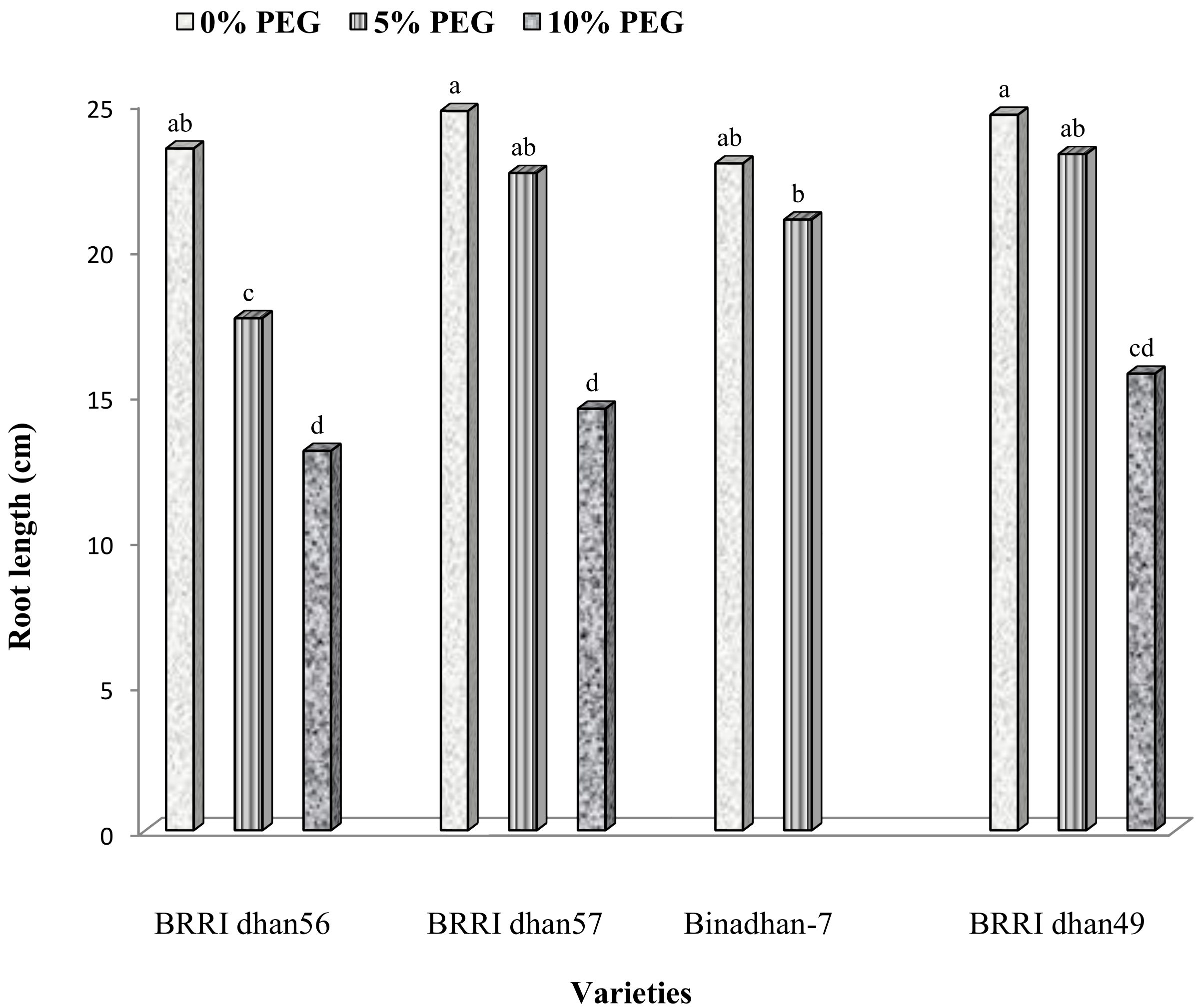


**Figure 14**. Root length of different rice varieties under nutrient solutions treated with different concentrations of Polyethylene glycol-8000. Values followed by same letter(s) did not differ significantly at 5% level of probability.

### 4.2.5. Leaf area and specific leaf weight

In the present experiment, the leaf area was also affected due to PEG treatment. Water stress decreased the leaf area in all the varieties. Leaf area was calculated from the upper two fully expanded leaves at the end of the experiment. Considering all the varieties, the leaf area recorded was the highest at 0% PEG treatment and lowest at 10% PEG treatment and at 5% PEG treatment in Binadhan-7 (Table 11). Considering all the varieties and PEG treatments, the highest (46.52 $cm^2$) leaf area recorded was in BRRI dhan56 at 0% PEG treatment which was statistically similar to the control treatment of BRRI dhan57 and BRRI dhan49 and the lowest (18.78 $cm^2$) leaf area recorded was in Binadhan-7 at 5% PEG concentration, which was statistically similar to 10% PEG treatment of BRRI dhan49. There existed a significant difference among the treatments in BRRI dhan57 and in Binadhan-7. The 5% and 10% PEG treatments in BRRI dhan56 did not differ significantly. The 0% PEG and 5% PEG treatments of BRRI dhan49 also did not differ significantly. The results, related to leaf area of the present experiment were similar to that of experiment I. The lower RWC of leaf might affect the stomatal conductance as well as the gas exchange and finally dry matter production. It was clear that PEG induced drought treatment decreased leaf area and Binadhan-7 was very much susceptible to PEG solution. Chutia and Borah (2012) found that the leaf growth significantly decreased from control plant due to PEG treatment.

The specific leaf weight indicates the thickness of leaf, which was calculated from the upper two fully expanded leaves at the end of the present experiment. Considering all the varieties, the specific leaf weight recorded was the lowest at 0% PEG concentration and the highest at 10% PEG concentration and 5% PEG concentration in Binadhan-7 (Table 11). The SLW was recorded increased with the increasing PEG concentration as in experiment I, where SLW was also increased with the increasing water stress treatment in all the varieties. Among the varieties and PEG treatments, the highest (3.78 g/cm) SLW recorded was in Binadhan-7 at 5% PEG concentration which was statistically similar to the control treatment of all the varieties and with 5% PEG treatment of BRRI dhan49 and the lowest (2.54 g/cm) Specific leaf weight recorded was in BRRI dhan49 at 0% PEG concentration which was statistically similar to control treatment of all the

**Table 11**. Leaf area and specific leaf weight of different rice varieties under various PEG concentrations

| Varieties | PEG concentrations | Leaf area ($cm^2$) | SLW ($g\ cm^{-2}$) |
|---|---|---|---|
| BRRI dhan56 | 0% | 46.52 a | 2.57 d |
| | 5% | 35.91 cd | 2.65 cd |
| | 10% | 31.11 de | 3.65 ab |
| BRRI dhan57 | 0% | 43.84 ab | 2.69 cd |
| | 5% | 36.54cd | 3.01 bcd |
| | 10% | 26.00 e | 3.42ab |
| Binadhan-7 | 0% | 39.65 bc | 2.66 cd |
| | 5% | 18.78 f | 3.78 a |
| | 10% | Dead | Dead |
| BRRI dhan49 | 0% | 43.86 ab | 2.54 d |
| | 5% | 39.30 bc | 3.31 abc |
| | 10% | 19.67 f | 3.42 ab |
| CV (%) | | 10.57 | 13.48 |

PEG= Polyethylene glycol. Values followed by same letter(s) did not differ significantly at 5% level of probability.

varieties and with 5% PEG treatments of BRRI dhan56 and BRRI dhan57. Leaf became thicker (the SLW was increased) due to PEG treatment sas well as water stress treatment. Boontang and Tantisuwichwong (2013) reported that SLW was increased under drought treatment.

### 4.2.6. Dry matter production

The shoot dry matter at 0% PEG treatment was significantly higher than other PEG treatments of all the varieties have been shown in table 12. Among the varieties and PEG treatments, the highest (1.38 g) shoot dry matter recorded was in Binadhan-7 at 0% PEG treatments, the lowest (0.21 g) shoot dry matter recorded was at 5% PEG treatments in Binadhan-7. In BRRI dhan57 and in Binadhan-7, the root dry matter was significantly higher at 0% PEG compared to other PEG levels of the same varieties. Among the varieties and PEG treatments, the highest (0.417 g) root dry matter recorded was in control (0% PEG) treatment of BRRI dhan49 which was statistically similar to the 0% PEG treatment of Binadhan-7. The lowest (0.09 g) root dry matter recorded was in BRRI dhan56 at 10% PEG treatments and in Binadhan-7 at 5% PEG treatment. The highest (1.79 g) total dry matter content recorded was in BRRI dhan49 at 0% PEG treatments which was statistically similar to 0% PEG treatment of Binadhan-7 and the lowest (0.30 g) TDM recorded was in Binadhan-7 at 5% PEG treatment which was statistically similar to 5% and 10% PEG treatments of BRRI dhan56 and with 10% PEG treatment of BRRI dhan57 and BRRI dhan49. The percent reduction in TDM content in PEG experiment was similar to experiment I with soil moisture treatment. This might be due to the reduction in LA, RWC and chlorophyll content (SPAD) of leaf which ultimately affected the stomatal conductance as well as the gas exchange and finally dry matter production. In the present experiment, the reduction of TDM in BRRI dhan49 was coparatively lower than other varieties as same as the experiment I. Hamayun *et al.* (2010) found that plant dry weight significantly reduced with 16% PEG, applied at pre-flowering growth stage. Many studies also indicated that drought stress significantly decrease the dry weights of shoots (Centritto *et al.,* 2009; Mostajeran and Rahimi-Eichi, 2009).

**Table 12**. Shoot dry matter, root dry matter and total dry matter of different rice varieties under various PEG concentrations

| Varieties | PEG concentrations | Shoot dry matter (g) | Root dry matter (g) | Total dry matter (g) | % reduction of total dry matter |
|---|---|---|---|---|---|
| BRRI dhan56 | 0% | 0.947 bc | 0.263 bcd | 1.210 bc | |
| | 5% | 0.393 de | 0.167 def | 0.483 ef | 60.08 |
| | 10% | 0.220 ef | 0.090 fg | 0.310 ef | 74.38 |
| BRRI dhan57 | 0% | 1.243 ab | 0.337 ab | 1.580 ab | |
| | 5% | 0.503 de | 0.220 cde | 0.723 de | 54.24 |
| | 10% | 0.260 ef | 0.110 efg | 0.370 ef | 76.58 |
| Binadhan-7 | 0% | 1.380 a | 0.407 a | 1.787 a | |
| | 5% | 0.210 ef | 0.090 fg | 0.300 ef | 83.21 |
| | 10% | 0 | 0 | 0 | 0 |
| BRRI dhan49 | 0% | 1.373 a | 0.417 a | 1.790 a | |
| | 5% | 0.703 cd | 0.317 abc | 1.020 cd | 43.02 |
| | 10% | 0.310 ef | 0.180 def | 0.490 ef | 72.63 |
| CV (%) | | 30.53 | 30.83 | 31.24 | |

FC= field capacity. Values followed by same letter(s) did not differ significantly at 5% level of probability.

### 4.2.7. Dry matter distribution

In the present experiment, the dry matter distribution of plant to shoot and root was calculated after oven drying of shoot and root from each treatment. In the variety BRRI dhan56, the dry matter distribution to shoot was found the highest (75%) at 0% PEG treatment, which was statistically similar to the 5% PEG treatment in the same variety. The percent dry matter distribution to shoot decreased with the increasing PEG concentration from 0% to 10% in this variety (Figure 15). On the other hand, in the same variety, dry matter distribution to root was found the lowest (25%) at 0% PEG treatment and the percent dry matter distribution to root increased with the increasing PEG concentration from 0% to 10% (Figure 15), but there was no significant difference among the treatments. Increased dry matter distribution to root (compare to control) under water stress condition was also observed in experiment I which might help plants to absorb more water under water stress.

In the variety BRRIdhan57, the dry matter distribution to shoot was recorded the highest (76%) at 0% PEG or control treatment which was significantly higher than any other treatments of this variety and was decreased with the increasing PEG concentration from 0% to 10% (Figure 16). There was no significant difference between 5% and 10% PEG treatments in this variety for dry matter distribution to shoot. In the same variety, the dry matter distribution to root was found the highest (37%) at10% PEG concentration and the lowest (24%) at 0% PEG concentration. But there was significant difference among the treatments in this variety considering the dry matter distribution to root. Similar result was also observed in experiment I for this variety.

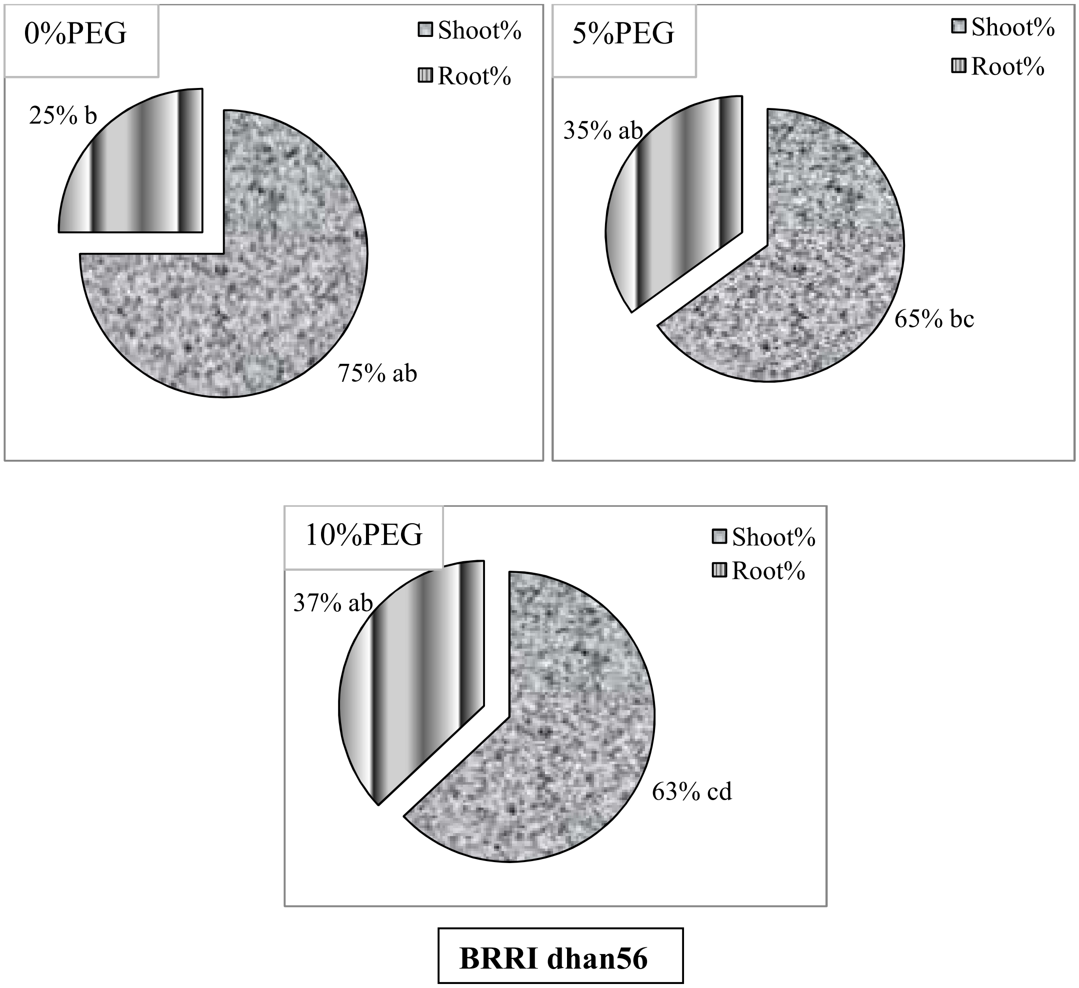


**Figure 15**. Dry matter distribution to shoot and root of BRRI dhan56 under nutrient solutions treated with different concentrations of Polyethylene glycol-8000. Values followed by same letter(s) did not differ significantly at 5% level of probability.

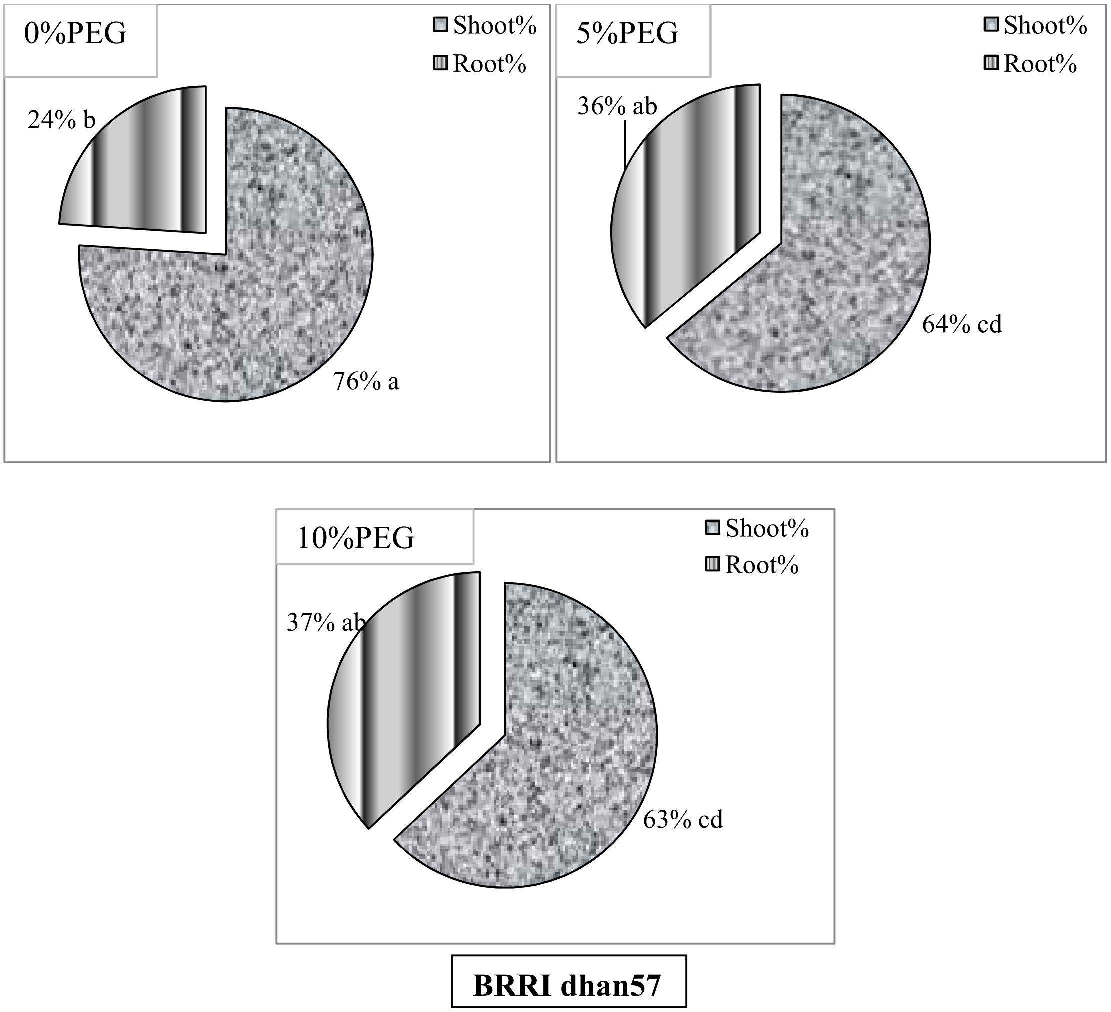


**Figure 16**. Dry matter distribution to shoot and root of BRRI dhan57 under nutrient solutions treated with different concentrations of Polyethylene glycol-8000. Values followed by same letter(s) did not differ significantly at 5% level of probability.

In Binadhan-7, the percent dry matter distribution to shoot decreased and the percent dry matter distribution to root increased with the increasing PEG concentration from 0% to 5% (Figure 17). Considering shoot dry matter distribution, there was significant difference between the treatments. But in case of dry matter distribution to root, there were no significant difference between the treatments in this variety. Similar results were also observed in experiment I for Binadhan-7.

In the variety BRRI dhan49, the similar trend of dry matter distribution to shoot and to root were recorded as in BRRI dhan56, BRRI dhan57, Binadhan-7 due to PEG treatments. Dry matter distribution to shoot recorded was the highest (74%) at 0% PEG treatment and decreased with the increasing PEG concentration from 0 to 10% (Figure 18) in this variety. At the same time dry matter distribution to root recorded was the lowest (26%) at 0% PEG concentration and it was increased with the increasing PEG concentration from 0 to 10%. But in this variety, the dry matter distribution of shoot at control treatment was statistically similar to that in 5% PEG treatment and was significantly higher than 10% PEG treatment. But in the same variety, the dry matter distribution to root among the treatments did not differ significantly among them. Similar results were also observed in experiment I for this variety.

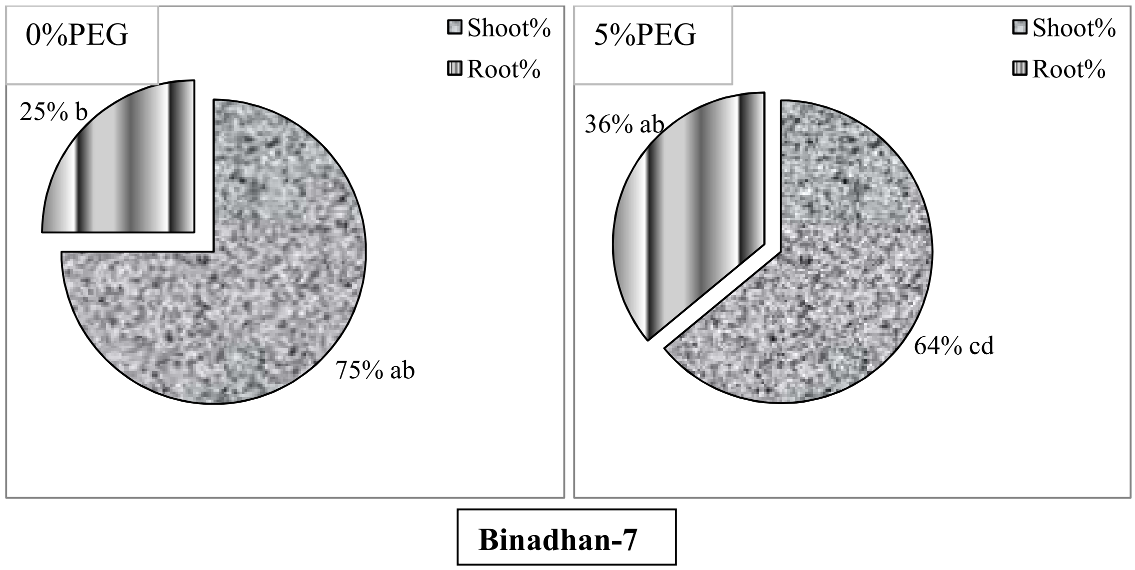


**Figure 17**. Dry matter distribution to shoot and root of Binadhan-7 under nutrient solutions treated with different concentrations of Polyethylene glycol-8000. Values followed by same letter(s) did not differ significantly at 5% level of probability.

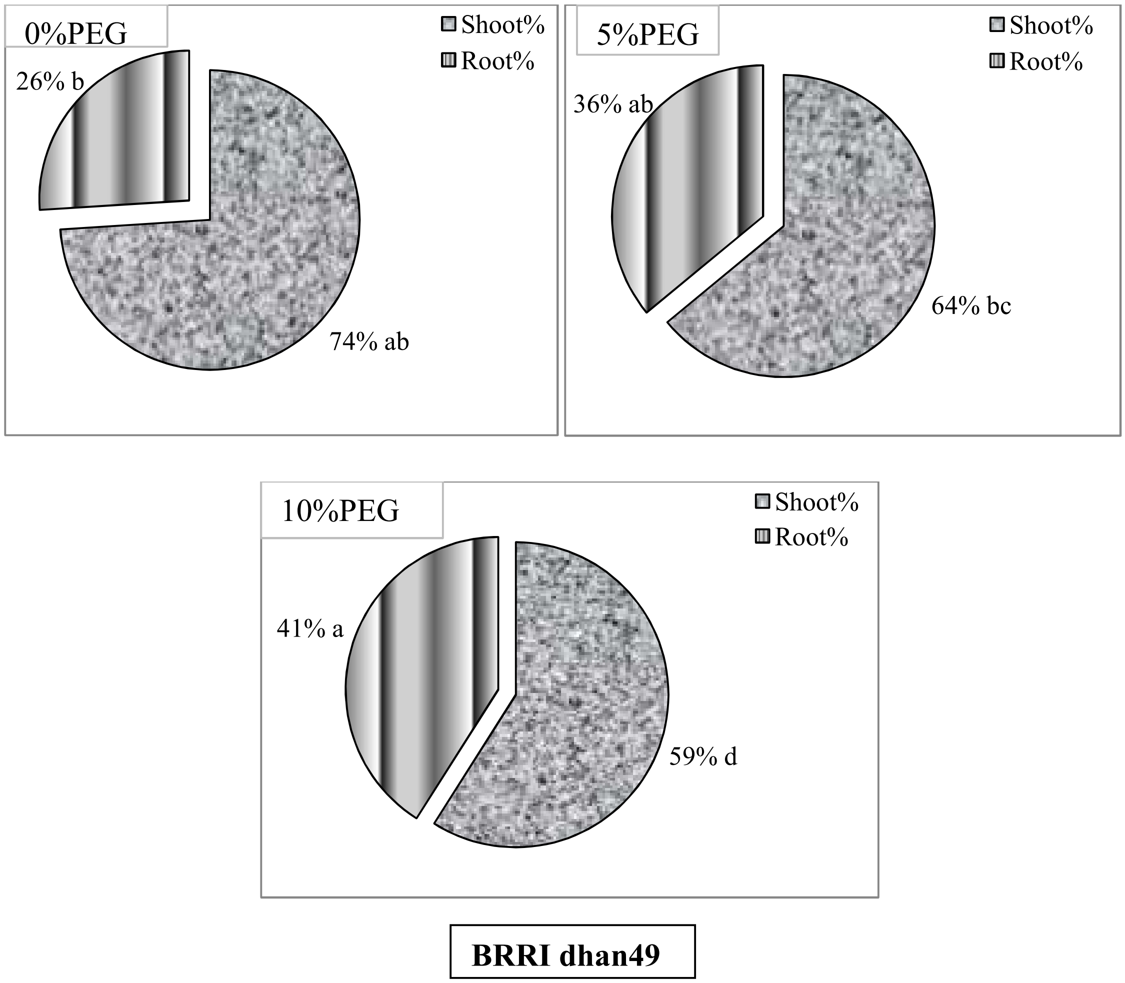


**Figure 18**. Dry matter distribution to shoot and root of BRRI dhan49 under nutrient solutions treated with different concentrations of Polyethylene glycol-8000. Values followed by same letter(s) did not differ significantly at 5% level of probability.

### 4.2.8. Relative water content (RWC) of leaf

The relative water content of leaf is one of the major determinants of leaf photosynthesis. The relative water content of leaves was also recorded from every treatment in the present experiment. Considering all the varieties and PEG treatments, the highest (97.62%) RWC was recorded at control treatment of BRRI dhan56 which was statistically similar to control treatment of BRRI dhan57 and BRRI dhan49. The lowest (75%) RWC was recorded at 10% PEG treatment of BRRI dhan57 which was significantly lower than any other treatments. The relative water content of leaf recorded was the highest at 0% PEG treatment which was gradually decreased with the increasing PEG concentration in every variety (Figure 19). The lowest relative water content recorded was at 10% PEG concentration in all the varieties. This might be due to lower availability of water under higher PEG concentration. Similar result was also observed in previous experiment (Experiment I) that water stress decreased the RWC of leaf. Lian *et al.* (2006) found that treatment with 20% PEG rapidly and significantly decreased the water content and caused about 3% water loss in leaves of upland rice.

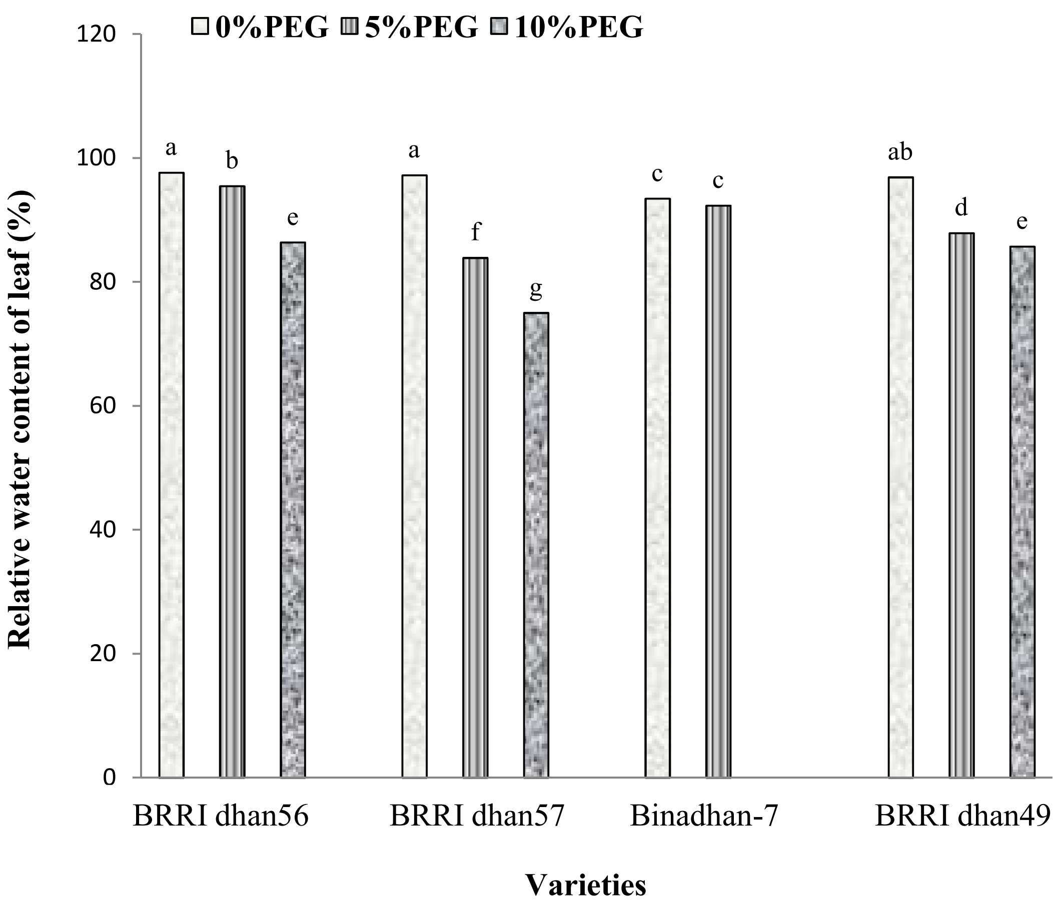


**Figure 19**. Relative water content of different rice varieties under nutrient solution treated with different concentrations of Polyethylene glycol-8000. Values followed by same letter(s) did not differ significantly at 5% level of probability.

**4.2.9. SPAD value**

The SPAD (Soil Plant Analysis Development) value represents the greenness of the leaf. The SPAD value has been well recognized as a mean of determining the onset of senescence process in a leaf (Rajcan *et al*., 1999). The sharp decrease in SPAD value indicates the onset of leaf senescence. In the present experiment, the SPAD value was recorded from the upper two fully expanded leaves of the main tiller and the average value was recorded which have been shown in figure 20. The SPAD value recorded was the highest at 0% PEG concentration which was gradually decreased with the increasing PEG concentration from 0 to 10% in all the varieties (Figure 20). This might be due to decrease in chlorophyll content due to water stress treatment. Considering all the varieties and PEG treatments, the highest (39.6) SPAD value was recorded in BRRI dhan49 at 0% PEG concentration which was statistically similar to 5% PEG treatment of the same variety and the lowest (30.9) SPAD value was recorded in BRRI dhan57 at 10% PEG concentration which was statistically similar to 5% and 10% PEG treatments of BRRI dhan56 and with 5% PEG treatment of Binadhan-7. The chlorophyll content of BRRI dhan49 might be affected lower compare to other varieties and the result was similar to experiment I. It was reported that under the water stress, the population varied widely for all the physiological traits studied *viz*., canopy-air temperature difference, leaf drying score, leaf rolling score, leaf senescence, relative water content, osmotic potential and osmotic adjustment (Sellammal *et al.*, 2014). It was also reported that the chlorophyll degradation is one of the consequences of drought stress that may result from sustained photoinhibition and photo-bleaching and even though other plant processes, such as cell division and cell expansion are the earliest to respond to water deficit stress, a decline in SPAD index is a sensitive and readily measurable trait that could be used to screen for stress tolerance (O'Neill *et al*., 2006). The chlorophyll content of leaf significantly decreased from control plant due to PEG treatment also observed by Chutia and Borah ( 2012).

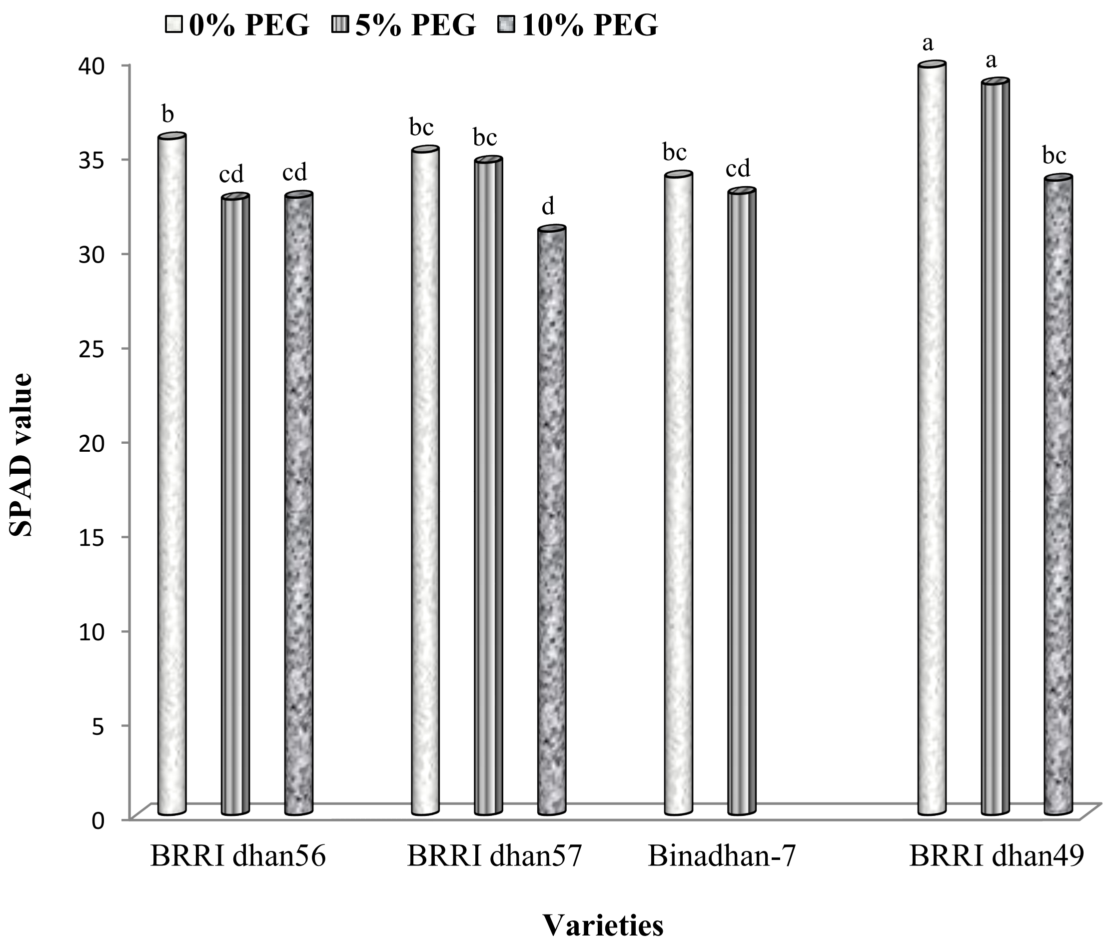


**Figure 20**. Average of SPAD value of leaf of different rice varieties under nutrient solutions treated with different concentrations of Polyethylene glycol-8000. Values followed by same letter(s) did not differ significantly at 5% level of probability.

### 4.2.10. Stomatal conductance

In the present experiment, the stomatal conductance was also recorded from the upper fully expanded two leaf of main tiller and the average value was recorded for analysis. The stomatal conductance was also recorded the highest at 0% PEG concentration which was gradually decreased with the increasing PEG concentration from 0 to 10% in all the varieties (Figure 21). Considering all the varieties and PEG treatments, the highest (509.3 µmol $m^{-2}s^{-1}$) stomatal conductance was recorded in BRRI dhan49 at 0% PEG concentration which was significantly higher than any other treatments of the same variety and other varieties and the lowest (309 µmol $m^{-2}s^{-1}$) stomatal conductance was recorded in BRRI dhan57 at 10% PEG concentration which was significantly lower than any other treatment of the same variety and all other treatments of other varieties. Similar results were also recorded in experiment I.

In the present experiment, the stomatal conductance recorded was the lower under water stress treatment which might be due to lower RWC under this condition. But in BRRI dhan49, the higher accumulation of proline (estimated in experiment IV) contributed in relatively higher RWC as well as stomatal conductance under water stress condition. It was reported that abscisic acid (ABA) accumulation in stressed plants was found to be protective against drought damage, causing stomata closure that reduces water loss via transpiration (Morillon and Chrispeels, 2001; Wan and Zwiazek, 2001).

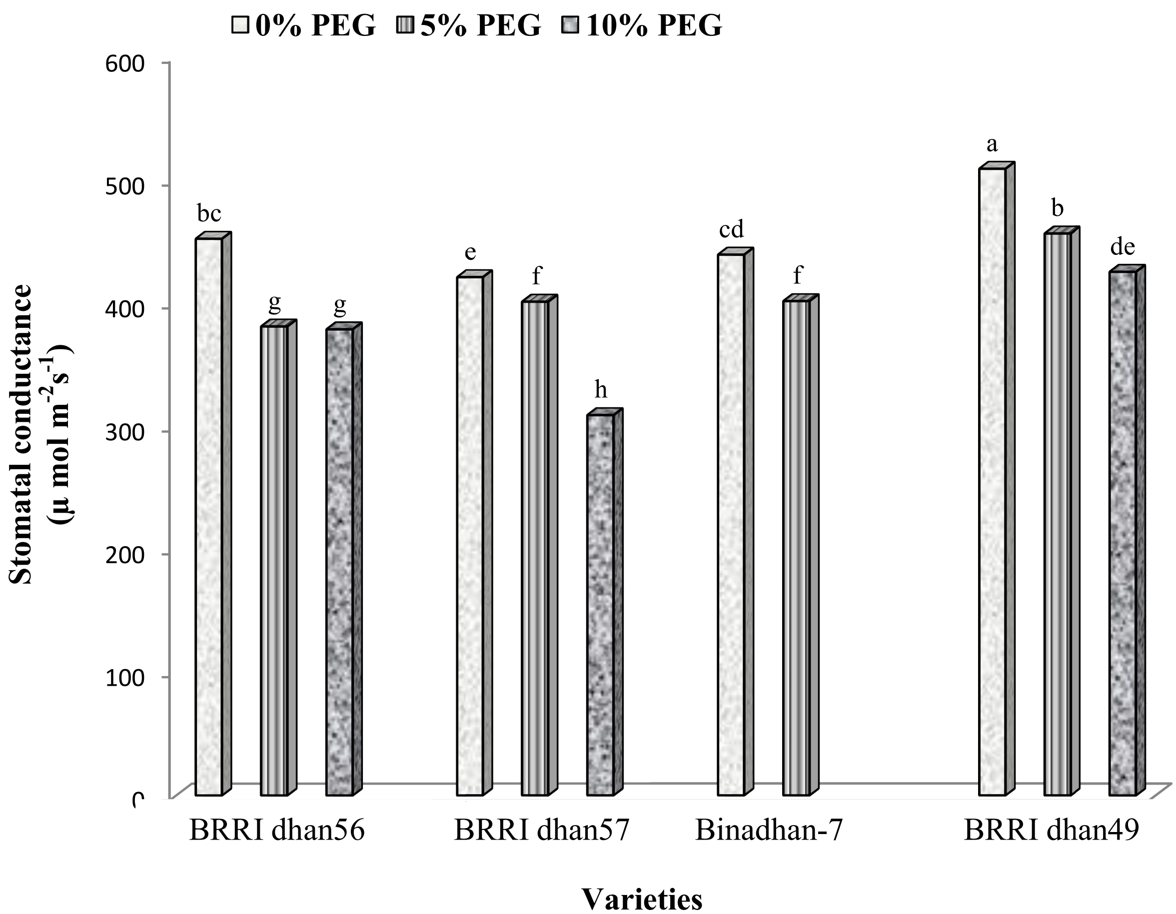


**Figure 21**. Average of stomatal conductance of leaf of different rice varieties under nutrient solutions treated with different concentrations of Polyethylene glycol-8000. Values followed by same letter(s) did not differ significantly at 5% level of probability.

### 4.2.11. Relation between stomatal conductance and SPAD value

Both SPAD value and stomatal conductance recorded was the higher at control treatment and decreased with the increasing PEG treatments from 0% to 10% PEG in all the varieties. In the present experiment, the relationship between SPAD value and stomatal conductance was also observed. It was a strong positive correlation (r = 0.895**) between stomatal conductance and SPAD value (Figure 22) in PEG treated plants. When the SPAD value was higher, the stomatal conductance was also higher and when the SPAD value remained lower the stomatal conductance was also lower. The SPAD value indicates the greenness of the leaf. When the leaf remains green the harvesting of light becomes higher (Taiz and Zeiger, 2006) and the stomatal conductance as well as photosynthesis becomes higher.

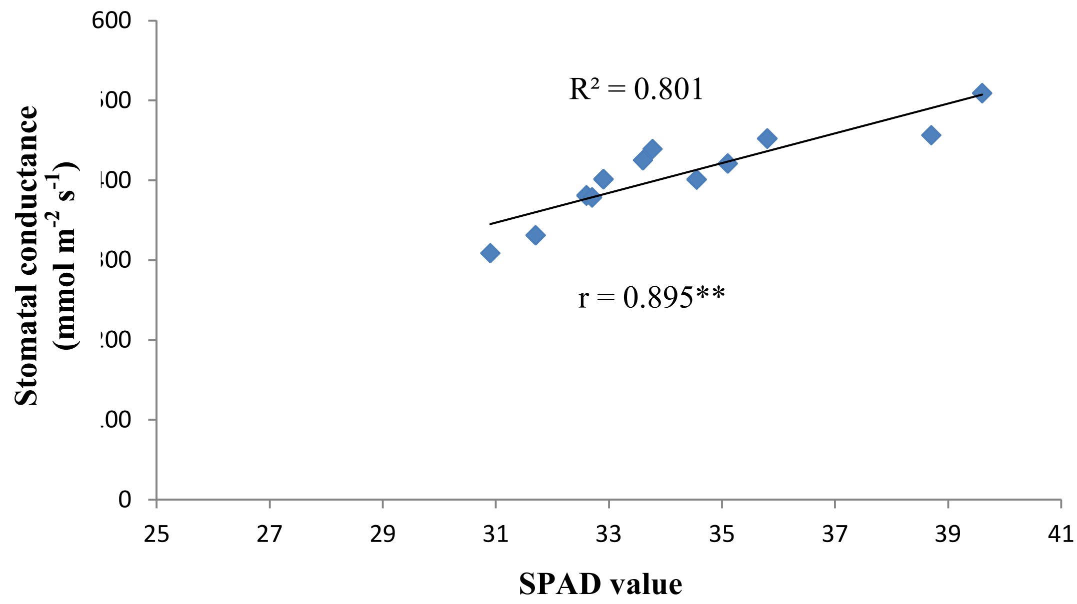


**Figure 22**. Relationship between SPAD value and stomatal conductance of leaf of different rice varieties under various nutrient solutions treated with different concentrations of Polyethylene glycol-8000.

### 4.3. Experiment-III: Evaluating the yield and yield contributing characters response of transplanted *aman* rice varieties to different soil moisture levels.

In the present experiment different yield contributing characters of four transplanted *aman* rice varieties, grown in different soil moisture levels were studied to find out the effect of water stress on yield attributes of those rice varieties.

#### 4.3.1. Spikelet number of main stem

The data of main stem represents the characters of the whole plant. The main stem has the major contribution to grain yield in rice (*Martinez-Eixarch et al., 2015).* For this reason, in the present experiment, different yield data of main stem were also recorded. Considering all the varieties and soil moisture treatments, the filled spikelet number of main stem was found the highest (206.75) in BRRI dhan49 at S0 (control) treatment which was significantly higher than any other treatment. The lowest (101.50) number recorded was in BRRI dhan56 at S2 treatment which was statistically similar to S2 treatment of Binadhan-7 (Table 13). In all the varieties, the S0 or the control treatment produced the highest number of filled spikelet of main stem which was gradually decreased from S0 to S2 treatment. Consequently, the lowest number of filled spikelet was recorded in S2 treatment in all the varieties. At S1 treatment, the variety BRRI dhan49 performed better compared to other varieties. The results showed that the number of filled grains per main stem decreased under lower soil moisture level but the degree of reduction was different for different varieties.

In the present experiment, decreased number of filled grains per main stem under lower soil moisture levels might be due decrease in LA, SLA, RWC, chlorophyll content under water stress treatment which affected the stomatal conductance, gas exchange and RGR. As a result, the total vegetative dry matter became lower which ultimately affected the filled grain number/main stem. These results agree with Hossain (2001), O' Toole and Moya (1981). Rahman and Yoshida (1985) observed that water stress reduced the number of grains/panicle. The RRDI (1999) reported that water stress at or before panicle initiation reduces the spikelet number.

On the other hand, considering all the varieties and soil moisture treatments, the unfilled spikelet number of main stem was found the highest (62.00) in BRRI dhan57 at S2 treatment which was statistically similar to S2 treatment of BRRI dhan56 and with S1 treatment of Binadhan-7. The lowest (25.50) number of unfilled spikelet was recorded in BRRI dhan56 at S0 treatment which was statistically similar to S1 treatment of BRRI dhan56, with S0 and S1 treatments of BRRI dhan57, with S0 and S2 treatments of Binadhan7 and with all the treatments of BRRI dhan49 (Table 13). Among the treatments in BRRI dhan56 and BRRI dhan57, the highest number of unfilled spikelets was recorded at S2 treatment but in Binadhan-7 and BRRI dhan49 the lowest number of unfilled spikelet was recorded at S2 treatment. This might be due to decrease in different morphological and physiological processes (described above) under water stress treatment. It was also documented that increased unfilled grains per main stem under lower soil moisture level might be due to inactive pollen grain for dryness, incomplete development of pollen tube; insufficient assimilates production and its distribution to grains (Hossain, 2001). The results agreed with Yamboo and Ingram (1988), Begum (1990) and Islam *et al.* (1994a). Sokoto and Muhammad (2014) stated that water stress increase the empty grains.

The filled spikelet number of the main stem mainly contributed to the total spikelet number of main stem in every variety. The total spikelet number of main stem found was the highest (245) in BRRI dhan49 at S0 treatment which was statistically similar to S1 treatment of the same variety and the lowest (141) in Binadhan-7 at S2 treatment which was statistically similar to S2 treatment of BRRI dhan56 (Table 13). In all the varieties, the total spikelet number of main stem was found the highest at S0 treatment and the lowest was also recorded at S2 treatment with an exception in BRRI dhan57, where S1 treatment produced the lowest number of total spikelet of main stem. This might be due to decrease in different morphological and physiological processes (described above) under water stress condition. Rahman and Yoshida (1985) stated that water stress reduced the number of grains/panicle.

In all the varieties, S0 (control) treatment showed the higher filled spikelet percentage than S2 treatment. Considering all the varieties and soil moisture treatments, the filled spikelet percentage of main stem recorded was the highest (87.121) in BRRI dhan56 at S0 treatment and the lowest (64.74) at S2 treatment in the same variety (Table 13). It was also recorded that S2 treatment of all varieties produced the lowest filled spikelet percentage of main stem with an exception in Binadhan-7 where S1 treatment produced the lowest filled spikelet percentage of main stem.

Under water stress condition, BRRI dhan49 produced the relatively higher filled spikelet percentage than the other varieties. This might be due to the plant height, LA, RGR, dry matter distribution to root, SPAD, RWC and stomatal conductance were statistically similar at S1 and S0 treatments of this variety. The filled spikelet percentage of S2 treatment was better in BRRI dhan49 compared to others and this might be due to higher proline accumulation under this treatment helped this variety to maintain better RWC as well as other physiological functions. It was reported that the drought stress during the vegetative growth, flowering, and terminal stages of rice cultivation can cause spikelet sterility and unfilled grains (Kamoshita *et al.*, 2004). Kumar *et al.* (2014) found that drought stress at reproductive stage caused reduction in number of spikelet (15.9%) and spikelet fertility (17.13%),

### 4.3.2. The panicle length, filled, unfilled and total spikelets weight of main stem

The panicle length, filled, unfilled and total spikelets weight were also recorded from main stem. Considering all the varieties and soil moisture treatments, the main stem panicle length recorded was the highest (26.675 cm) in BRRI dhan57 at S0 treatment which was statistically similar to other treatments of same variety, with all the treatments of BRRI dhan56 and Binadhan-7 and with S0 and S1 treatments of BRRI dhan49 and the lowest (24.40 cm) panicle length recorded was in BRRI dhan49 at S2 treatment which was significantly lower than S0 treatment of BRRI dhan56 and BRRI dhan57 (Table 14). Among the treatments, the panicle length recorded was the highest at S0 treatment in all the varieties.

**Table 13**. Effect of different soil moisture levels on filled spikelet number, unfilled spikelet number, total spikelet number and filled spikelet % of main stem of transplanted *aman* rice varieties

| Varieties | Soil moisture Levels | Filled spikelet number per main stem | Unfilled spikelet number per main stem | Total spikelet number per main stem | Filled spikelet % per main stem |
|---|---|---|---|---|---|
| BRRI dhan56 | S0 | 172.5 cd | 25.50 d | 198.00 c | 87.121 a |
| | S1 | 144.75 e | 31.25 cd | 176.00 cde | 82.395 ab |
| | S2 | 101.50 g | 56.00 ab | 157.50 ef | 64.74 e |
| BRRI dhan57 | S0 | 162.00 d | 31.50 cd | 193.50 c | 83.77 ab |
| | S1 | 138.25 e | 32.25 cd | 170.50 de | 80.935 ab |
| | S2 | 118.00 f | 62.00 a | 180.00 cd | 66.858 de |
| Binadhan-7 | S0 | 134.25 e | 40.75 bcd | 175.00 cde | 76.722 bc |
| | S1 | 120.50 f | 46.75 abc | 167.25 de | 72.075 cd |
| | S2 | 103.25 g | 37.75 cd | 141.00 f | 73.15 cd |
| BRRI dhan49 | S0 | 206.75 a | 38.25 bcd | 245.00 a | 84.427 ab |
| | S1 | 194.50 b | 37.50 cd | 232.00 ab | 83.773 ab |
| | S2 | 182.25 c | 37.50 cd | 219.75 b | 82.945 ab |
| CV (%) | | 5.55 | 28.66 | 7.27 | 6.14 |

S0=100%FC (field capacity) moisture, S1= 70% of the FC moisture, S2= 40% of the FC moisture. Values followed by same letter(s) did not differ significantly at 5% level of probability.

There were remarkable differences on the panicle length among the varieties under water stress condition. The panicle length was decrease under water stress treatment and this might be due to decrease in different morphological and physiological processes (described above) under water stress treatment which ultimately affects the shoot length as well as the panicle length. It was reported that the decreased panicle length under water stress condition might be due to the fact that moisture stress slowed down the number of cell division and or reduced the length of the individual cell (Hussain *et al.,* 2008). Bakul *et al.* (2009) found that the panicle length was decreased due to water stress at tillering to panicle initiation (vegetative) and panicle initiation to grain filling stage (reproductive stage). They also found that the water stress at reproductive phase caused more decreased in length of panicle. Similar result was also observed by Islam *et al.* (1994a).

Considering all the varieties and soil moisture treatments, the filled spikelet weight of main stem recorded was the highest (3.81 g) in BRRI dhan56 at S0 treatment which was statistically similar to S0 treatment of BRRI dhan49 (Table 14). The filled spikelet weight of main stem recorded was the lowest (1.92 g) in BRRI dhan57 at S2 treatment which was statistically similar to S2 treatment of both BRRI dhan56 and Binadhan-7. In all the varieties, S0 treatment produced the highest weight of filled spikelet of main stem and the weight was gradually decreased with the increasing water stress. But the reduction in grain weight due to water stress treatments was much lower in BRRI dhan49 than the other varieties.

In the present experiment, decreased filled grain weight per main stem under lower soil moisture levels might be due to decrease in LA, SLA, RWC, chlorophyll content under water stress treatment that affected the stomatal conductance, gas exchange, RGR, AGR and grain filling duration which ultimately affected the grains weight of main stem. But the SRT towards grain was increased in all the varieties under water stress treatment compared to control, which is desirable. In BRRI dhan49, water stress had little effect on grain weight of main stem and this might be due to lower reduction in LA, RWC, SPAD, stomatal conductance, lower injury and leaf rolling, higher proline content under stress, the highest chlorophyll content at S1 treatment, lower reduction in RGR, sugar-starch, AGR, vegetative dry matter and grain filling duration. All of these contributed to perform better under water stress condition in BRRI dhan49. But the SRT was lower in this variety compared to other varieties under water stress treatment. Islam *et al.*

(1994b), RRDI (1999), O'Toole *et al.* (1979) and Tsuda and Takami (1991) also stated that water stress reduced grain weight.

Among the varieties and soil moisture treatments, the weight of unfilled spikelet of main stem recorded was the highest (0.24 g) in BRRI dhan56 at S2 treatment (Table 14) which was statistically similar to S2 treatment of BRRI dhan57 and with S1 treatment of Binadhan-7. The weight of unfilled spikelet of main stem recorded was the lowest (0.105 g) in BRRI dhan56 at S0 treatment which was statistically similar to S1 treatment of the same variety, with S0 and S1 treatments of BRRI dhan57, with S0 and S2 treatments of Binadhan-7 and with all the treatments of BRRI dhan49. In all the varieties, S2 treatment produced the highest weight of unfilled spikelet with an exception in BRRI dhan49 where S1 treatment produced the highest weight of unfilled spikelet. This might be due to decrease in different morphological and physiological processes (described above) under water stress treatment. But in all the varieties, S0 or control treatment produced the lowest weight of unfilled spikelet.

Considering all the varieties and soil moisture treatments, the total weight of grain of main stem recorded was the highest (3.915 g) in BRRI dhan56 at S0 treatment which was statistically similar to S0 treatment of BRRI dhan49 and the lowest (2.132 g) recorded was in BRRI dhan57 at S2 treatment which was statistically similar to S2 treatment of BRRI dhan56 and Binadhan-7 (Table 14). In all the varieties, the total weight of grain of main stem gradually decreased with the increasing water stress from S0 treatment to S2 treatment. This might be due to decrease in different morphological and physiological processes (described above) under water stress treatment. Widiati *et al*. (2014) stated that the average weight of the grain per plant declined with decreasing soil water content.

**Table 14**. Effect of different soil moisture levels on panicle length, filled spikelet weight, unfilled spikelet weight and total spikelet weight of main stem of transplanted *aman* rice varieties

| Varieties | Soil moisture Levels | Panicle length per main stem (cm) | Filled spikelet weight per main stem (g) | Unfilled spikelet weight per main stem (g) | Total spikelet weight per main stem (g) |
|---|---|---|---|---|---|
| BRRI dhan56 | S0 | 26.375 a | 3.810 a | 0.105 c | 3.915 a |
| | S1 | 25.925 ab | 3.300 bc | 0.135 bc | 3.435 bc |
| | S2 | 25.225 ab | 1.925 g | 0.240 a | 2.165 hi |
| BRRI dhan57 | S0 | 26.675 a | 2.775 e | 0.117 c | 2.893 ef |
| | S1 | 24.925 ab | 2.370 f | 0.125 bc | 2.495 gh |
| | S2 | 25.425 ab | 1.920 g | 0.212 ab | 2.132 i |
| Binadhan-7 | S0 | 26.125 ab | 2.915 de | 0.132 bc | 3.047 de |
| | S1 | 25.375 ab | 2.375 f | 0.214 ab | 2.590 fg |
| | S2 | 26.050 ab | 2.005 g | 0.145 bc | 2.140 i |
| BRRI dhan49 | S0 | 25.875 ab | 3.530 ab | 0.116 c | 3.648 ab |
| | S1 | 25.350 ab | 3.235 bcd | 0.127 bc | 3.362 bcd |
| | S2 | 24.400 bc | 2.980 cde | 0.121 c | 3.100 cde |
| CV (%) | | 4.35 | 8.32 | 34.65 | 7.94 |

S0=100%FC (field capacity) moisture, S1= 70% of the FC moisture, S2= 40% of the FC moisture. Values followed by same letter(s) did not differ significantly at 5% level of probability.

### 4.3.3. The seed size and shoot dry matter of main stem

The seed size is one of the major yield determinants. In the present experiment, considering all the varieties and soil moisture treatments, the largest (22.825 mg) seed recorded was in BRRI dhan56 at S1 treatment which was statistically similar to seed size of S0 treatment of the same variety and with S0 treatment of Binadhan-7 (Table 15). The smallest (16.293 mg) seed was found in BRRI dhan57 at S2 treatment which was statistically similar to other treatments of the same variety and with all the treatments of BRRI dhan49. Seed size was little affected due to water stress in BRRI dhan57 and in BRRI dhan49 (compared to control). Considering all the varieties, the seed size gradually decreased with the decreasing soil moisture level except in BRRI dhan56 where S1 treatment produced the largest seed.

The smallest seed was recorded at S2 treatment in all the varieties. This might be due to decrease in different morphological and physiological processes like LA, SLA, plant height, RWC, chlorophyll content, stomatal conductance, gas exchange, RGR, NAR and AGR. Lower soil moisture might have decreased the translocation of assimilates to the grain and affected the grain size. But the degree of reduction was different in different varieties. Islam *et al*. (1994b), RRDI (1999), O'Toole *et al*. (1979), Tsuda and Takami (1991) also stated that water stress reduced grain weight. Widiati *et al*. (2014) found that the average weight of grain per plant declined with decreasing soil water content

Among the varieties and soil moisture treatments, the shoot dry matter of main stem recorded was the highest (6.59 g) in BRRI dhan56 at S0 treatment which was statistically similar to all the treatments of BRRI dhan49 (Table 15). The lowest (4.293 g) shoot dry matter was recorded in BRRI dhan57 at S2 treatment which was statistically similar to S1 treatment of the same variety, with S2 treatment of BRRI dhan56 and withS1 and S2 treatments of Binadhan-7. Considering all the varieties, the shoot dry matter gradually decreased from S0 to S2 treatment. This might be due to the reduction in different morphological and physiological processes (described above). The shoot dry matter of main stem was also affected lower under water stress in BRRI dhan49 and this might be due to lower reduction of different morphological and physiological processes (described

**Table 15**. Effect of different soil moisture levels on seed size and shoot dry matter content of main stem of transplanted *aman* rice varieties

| Varieties | Soil moisture Levels | Seed size (mg) | Shoot dry matter per main stem (g) |
|---|---|---|---|
| BRRI dhan56 | S0 | 22.090 a | 6.590 a |
| | S1 | 22.825 a | 5.797 bcd |
| | S2 | 19.000 cd | 4.333 fg |
| BRRI dhan57 | S0 | 17.133 de | 5.085 def |
| | S1 | 17.085 de | 4.663 efg |
| | S2 | 16.293 e | 4.293 g |
| Binadhan-7 | S0 | 21.715 ab | 5.272 cde |
| | S1 | 19.760 bc | 4.607 efg |
| | S2 | 19.517 c | 4.380 fg |
| BRRI dhan49 | S0 | 17.080 de | 6.558 a |
| | S1 | 16.553 e | 6.155 ab |
| | S2 | 16.358 e | 5.865 abc |
| CV (%) | | 7.69 | 9.02 |

S0=100%FC (field capacity) moisture, S1= 70% of the FC moisture, S2= 40% of the FC moisture. Values followed by same letter(s) did not differ significantly at 5% level of probability.

before) compared to other varieties. It has been documented that biomass production (plant height and number of tillers per plant) is more affected under vegetative stage stress whereas severe effects on sink size (spikelet fertility, 1000 grain weight and seed yield) under reproductive stage stress would be resulted (Guan *et al.,* 2010).

#### 4.3.4. Relationship between seed size and grain yield

As the seed size is one of the major yield determinants, the grain yield must be influenced by seed size. It was also found in the present experiment. There was a positive correlation ($r = 0.409$) between seed size and grain yield in the present experiment have been shown in the figure 23. The grain yield recorded was the lowest when the seed size was the lowest. The grain yield was recorded the highest when the seed size was the highest also. Sabar and Arif (2014) found a positive correlation of 1000 grain weight with paddy yield indicates that the decrease in paddy yield was contributed due to the shrinkage of grain size and loss of spikelet fertility under water stress treatments. Kumar *et al.* (2011) studied 40 rice varieties under different environments and found a positive strong association between grain yield per plant and 1000 grain weight.

#### 4.3.5. Grain yield per plant and harvest index

Finally yield per plant and harvest index was calculated from different yield components. In the present experiment, the grain yield per plant differed significantly among the treatments by the interaction effect of soil moisture levels and the varieties. Considering all the varieties and soil moisture treatments the grain yield per plant recorded was the highest (31.68 g plant$^{-1}$) in BRRI dhan56 at S0 treatment which was significantly higher than any other treatments. The lowest (14.12 g plant$^{-1}$) grain yield per plant was recorded in BRRI dhan57 at S2 treatment which was statistically similar to S2 treatment of BRRI dhan56 (Table 16). In all the varieties, the highest grain yield recorded was at S0 (control) treatment which was gradually decreased from S0 to S2 treatment.

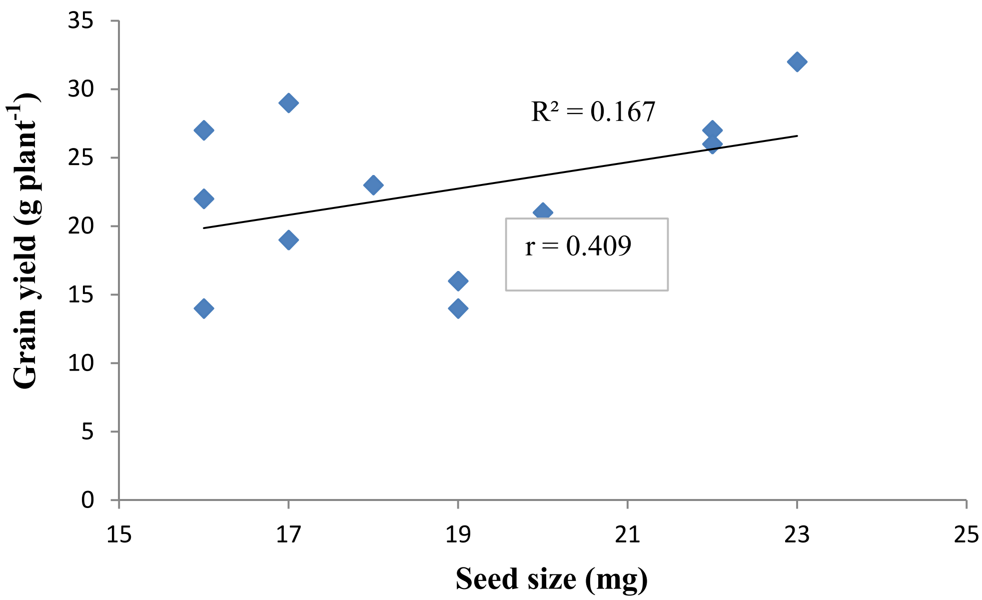


**Figure 23**. Relationship between seed size and grain yield of different rice varieties under various soil moisture treatments.

The lowest grain yield per plant was recorded at S2 treatment in all the varieties. This might be due to reduction in LA, SLA, plant height, effective tiller, RWC, chlorophyll content and increased leaf rolling under S2 treatment. As a result, the stomatal conductance, gas exchange, RGR, AGR and grain filling duration were decreased. All of these ultimately affected the grain yield under S2 treatment. Considering the relative value, it was clear that S1 treatment had little effect on grain yield compared to control. At S1 treatment, the highest relative yield (91.7%) was recorded in BRRI dan49, which was followed by BRRI dhan56 (86.6%) and above 80% in rest of the varieties. In all the varieties the S1 treatment did not differ significantly with control treatment (S0) considering different parameters such as plant height, number of effective tiller, LA, RGR, relative injury, NAR and grain filling duration. Considering the relative value of yield, it was recorded that the reduction was much lower in BRRI dhan49 compared to other varieties. This might be due to lower reduction in LA and vegetative dry matter, higher proline content, RWC and chlorophyll content, lower injury and leaf rolling, lower reduction in stomatal conductance, gas exchange, RGR, NAR, AGR, soluble sugar and starch content and higher SRT under moisture stress. The results also agree with Castilo *et al*. (1987) and Hossain (2001). Prasad *et al*. (2012) found a significance differences in grain yield per plant in rice varieties under control and drought treatment and also stated that drought stress reduced the grain yield in different rice varieties. It was reported that the grain yield is a multigenic factor. The susceptible varieties produced high grain yield under normal condition but it also showed high percent reduction over control during stress treatment. The yield stability in resistant varieties was due to specific adaptive feature that make it able to produce stable grain yield even in stress treatment (Van Heerden and Laurie, 2008; Liu *et al.*, 2008). Water deficit during vegetative, flowering and grain filling stages reduced mean grain yield by 21, 50 and 21% respectively in comparison to control (Sarvestani *et al*., 2008). Sokoto and Muhammad (2014) found that water stress at flowering and grain filling resulted to significant ($P < 0.05$) reduction in grain yield. Yield reduction due to water stress could be as a result of reduction in photosynthesis and translocation. They also reported that there was a linear relationship between available water and yield, where reduction in available water limits evapotranspiration and consequently reduced yield. Drought reduces grain yield probably by shortening the grain filling period (Shahryari *et al*., 2008), disrupting leaf gas exchange properties, limiting the size of the source and sink tissues, impaired phloem loading and assimilate translocation (Farooq *et al*., 2009b). It was also reported that the decline in yield may also be due to drought induced reduction in $CO_2$ assimilation rates, reduced stomatal conductance, photosynthetic pigments, small leaf size, reduced stem extension, disturbed plant water relations, reduced WUE, reduced activities of sucrose and starch synthesis enzymes and reduced assimilate partitioning, leading to a reduction in plant growth and productivity (Anjum *et al*., 2011). The magnitude of grain yield loss depends on the duration of drought, the stage of crop growth (Gana, 2011) and the severity of drought stress (Kumar *et al*., 2014).

Considering all the varieties and soil moisture treatments, the highest (0.59) harvest index recorded was in BRRI dhan49 at S0 (control) treatment which was statistically similar to S0 treatment of BRRI dhan56 and BRRI dhan57 and with S1 treatment of BRRI dhan56. The lowest (0.40) harvest index recorded was in Binadhan-7 at S2 treatment which was statistically similar to S2 treatment of BRRI dhan56 (Table 16). In every variety, the highest harvest index was recorded at S0 or control treatment which was gradually decreased from S0 to S2 treatment. Under water stress treatment the harvest index was much better in BRRI dhan49 compare to other varieties as same as grain yield. So, decreased harvest index in all the varieties, indicated that the vegetative dry matter was less affected and the reproductive dry matter was more affected due to water stress. But the degree of reduction was different in different varieties. Rahman *et al*. (2002) reported that harvest index was decreased with water stress. Lauteri *et al*. (2014) found a positive correlations between plant height and both yield and HI both under well watered and drought treatments. Sokoto and Muhammad (2014) stated that water stress at flowering and grain filling resulted in lower HI than water stress at tillering and no stress control which are statistically similar to higher HI. Kumar *et al*. (2014) found that drought stress at reproductive stage caused reduction in harvest index (29.2%). Sellammal *et al*. (2014) found genotypic variation in harvest index under control and water stress environments in rice.

**Table 16**. Effect of different soil moisture levels on grain yield per plant and harvest index of transplanted *aman* rice varieties

| Varieties | Soil moisture Levels | Grain yield per plant (g) | | Harvest index |
|---|---|---|---|---|
| | | Actual | Relative (%) | |
| BRRI dhan56 | S0 | 31.68 a | 100 | 0.590 ab |
| | S1 | 27.44 c | 86.6 | 0.550 abcd |
| | S2 | 14.15 k | 44.7 | 0.410 gh |
| BRRI dhan57 | S0 | 23.07 f | 100 | 0.560 abc |
| | S1 | 19.25 i | 83.4 | 0.480 ef |
| | S2 | 14.12 k | 61.2 | 0.460 fg |
| Binadhan-7 | S0 | 25.92 e | 100 | 0.500 def |
| | S1 | 21.12 h | 81.5 | 0.480 ef |
| | S2 | 15.90 j | 61.3 | 0.400 h |
| BRRI dhan49 | S0 | 29.35 b | 100 | 0.591 a |
| | S1 | 26.90 d | 91.7 | 0.510 cde |
| | S2 | 22.48 g | 76.6 | 0.540 bcd |
| CV (%) | | 0.13 | | 5.64 |

S0=100%FC (field capacity) moisture, S1= 70% of the FC moisture, S2= 40% of the FC moisture. Values followed by same letter(s) did not differ significantly at 5% level of probability.

#### 4.3.6. Drought susceptibility index (DSI)

Higher DSI value indicates less tolerant to drought. The drought susceptibility index also indicates the tolerant level of a variety under a specific stress. This value was based on the production of grain yield under normal and stress treatment. Higher the DSI indicates greater susceptibility. It is clear from the figure 24 that drought susceptibility index was much lower (0.594) in BRRI dhan49 compare to other varieties. So, it can be said that BRRI dhan49 is more drought tolerant under limited soil moisture condition compared to other three varieties. The highest (0.9846) drought susceptibility index recorded was in BRRI dhan57 which was followed by Binadhan-7, indicated the susceptibility of these varieties to water stress. The heat susceptibility index of different wheat varieties was described by Hasan (2009).

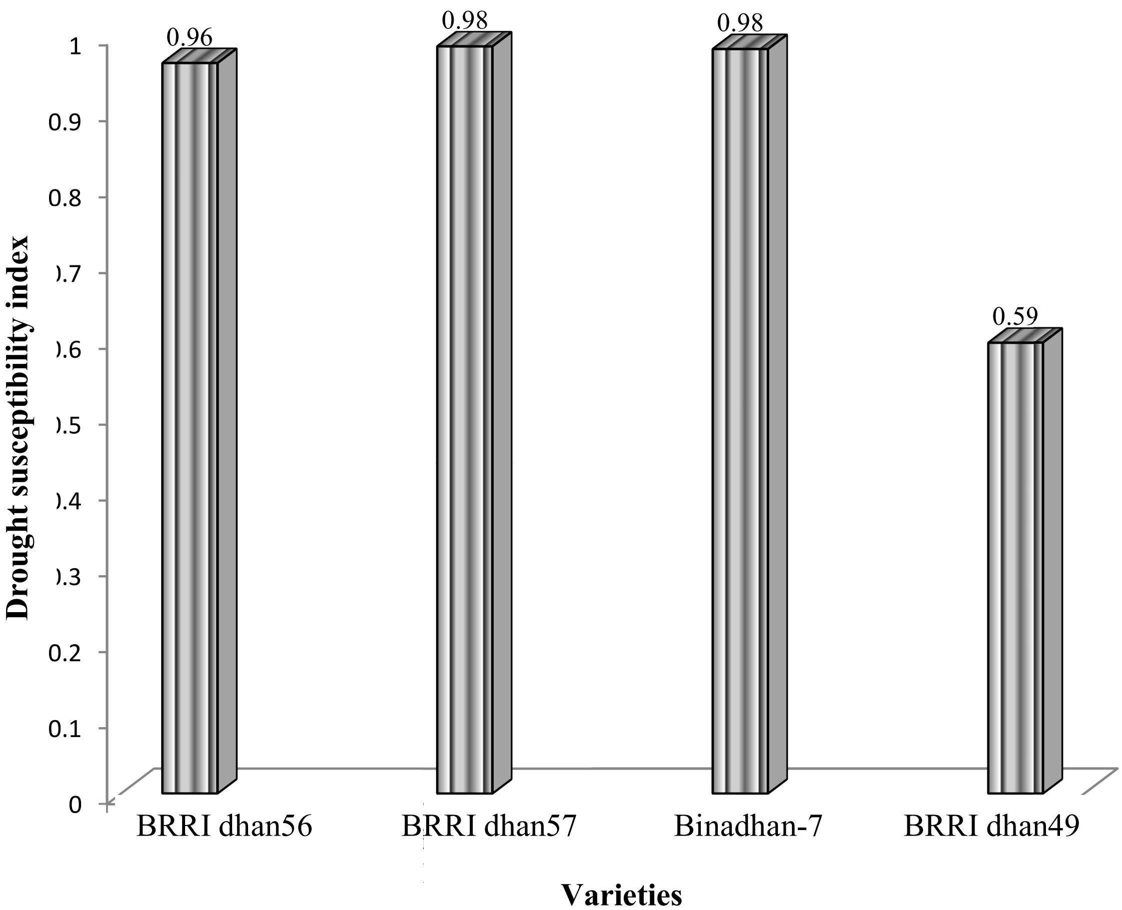


**Figure 24**. Drought susceptibility index of different rice varieties.

### 4.3.7. Relationship between grain filling duration and grain yield

In the present experiment, the relationship between grain filling duration and grain yield was also observed and the results indicated that there was a positive correlation (r = 0.111) between grain filling duration and grain yield (Figure 25). Grain yield was found lowest when the duration of grain filling was the lowest. The highest grain yield recorded was when the grain filling duration was the highest also. Higher grain filling duration indicates the higher translocation of assimilates towards grain. In the present experiment it was recorded that the grain yield was higher in those treatments where the duration of gain filling period was higher (as in BRRI dhan49 and BRRI dhan56) under both normal and water stress condition. Sellammal *et al*. (2014) found a positive correlation between grain filling duration and grain yield in rice.

### 4.3.8. Relationship between grain filling duration and harvest index

The relationship between grain filling duration and harvest index was also recorded in the present experiment. The results indicated that there was a positive linear relation (r = 0.375) between grain filling duration and harvest index (Figure 26). Harvest index was found lowest when the duration of grain filling was the lowest. The highest harvest index was recorded when the grain filling duration was the highest also. Higher grain filling duration indicates the higher translocation of assimilates towards grain and the grain yield became higher. As a result, in the present experiment, it was recorded that the harvest index was higher in those treatments where the duration of grain filling was higher. Kumar *et a*l. (2014) found that the grain yield was significantly and positively correlated with harvest index under both drought stress and non-stress irrigated treatment. In the present experiment, the relationship between grain filling duration and harvest index was stronger than the relationship between grain filling duration and grain yield.

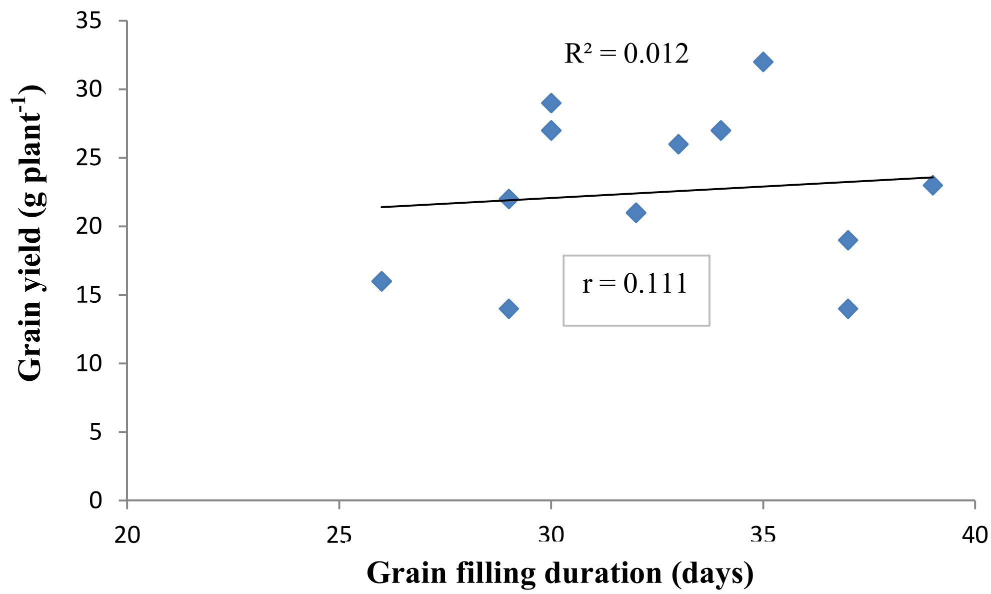


**Figure 25**. Relationship between grain filling duration and grain yield of different rice varieties under various soil moisture treatments.

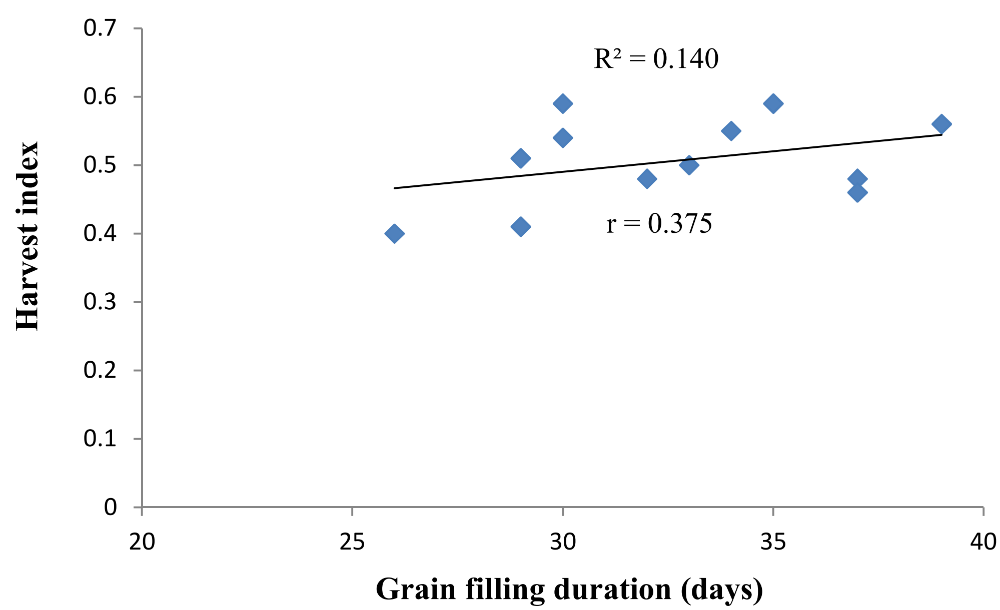


**Figure 26**. Relationship between grain filling duration and harvest index of different rice varieties under various soil moisture levels.

#### 4.3.9. Relationship between plant height and grain yield

In the present experiment, the relationship between plant height and grain yield was also estimated and it was found that there was a strong positive correlation (r = 0.837) between plant height and grain yield (Figure 27). Grain yield was found lowest when the plant height was the lowest. The grain yield gradually increased with the increasing plant height. The grain yield was recorded the highest when the plant height was also the highest. Higher plant height indicates the higher dry matter content of the plant. When the dry matter content remain higher the translocation of assimilates towards grain remains higher. Finally, the grain yield becomes higher with higher plant height. In the present experiment, it was also recorded that the grain yields were higher in those varieties where the plant height was also higher. The correlation between plant height and grain yield was highly significant in the present experiment. Kumar *et al*. (2014) also observed that grain yield was positively correlated with plant height under drought stress at reproductive stage.

#### 4.3.10. Relationship between plant height and harvest index

The relationship between plant height and harvest index was also recorded in the present experiment and it was found that the relation was highly significant. There was a positive correlation (r = 0.734) between plant height and harvest index (Figure 28). Harvest index was found lowest when the plant height was the lowest also. The harvest index was increased with the increasing plant height in all the treatments of every variety. The harvest index was recorded the highest when the plant height was also the highest. In the varieties where the plant height was higher, the dry matter content of those plants recorded was also higher. Higher dry matter supplied more assimilates towards grain. Consequently the grain yield as well as harvest index were also higher. So, in the present experiment, increased plant height contributed to grain yield rather than total dry matter content of the plant. Rahman *et al*. (2002) found a positive correlation between plant height and harvest index.

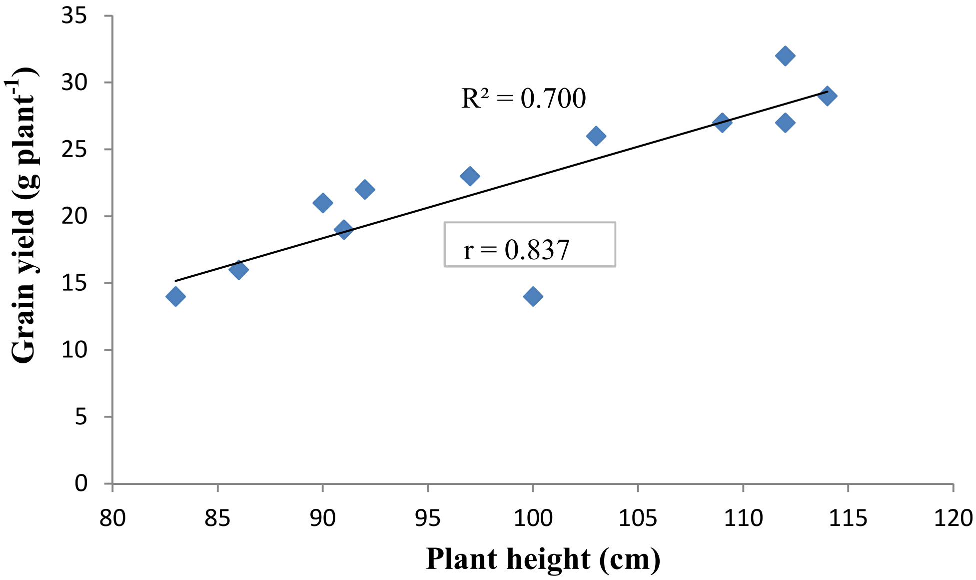


**Figure 27**. Relationship between plant height and grain yield of different rice varieties under various soil moisture levels.

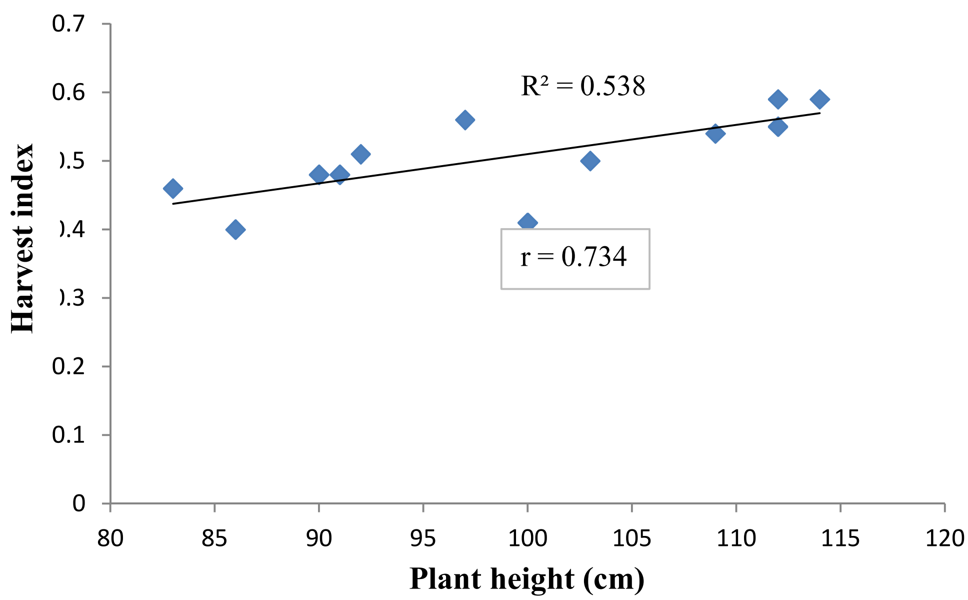


**Figure 28**. Relationship between plant height and harvest index of different rice varieties under different soil moisture levels.

### 4.4. Experiment-IV: Examining the biochemical and anatomical characters response of transplanted *aman* rice varieties to different soil moisture levels.

In the present experiment, different biochemical and anatomical parameters of four transplanted *aman* rice varieties under three soil moisture levels were studied to assess the biochemical and anatomical responses under those treatments.

#### 4.4.1. Relative membrane leakage of leaf tissue

In the present experiment, the relative membrane leakage of leaf tissue was determined from each treatment. The relative membrane leakage was determined by the amount of cell electrolytes leakage through the cell membrane. The membrane leakage of leaf tissue due to soil moisture stress was different from the membrane leakage in other stress treatment. The results of the present experiment indicated that the soil moisture stress decreased the leakage of cell electrolytes from the leaf tissue. As the membrane leakage level was determined by the leakage of cell electrolytes, so more leakage of electrolytes indicated more injury. Percent injury of leaf tissues was recorded during grain filling period. Considering all the varieties and soil moisture treatments, percent membrane leakage of leaf tissues recorded was the highest (50.95%) in BRRI dhan49 at S0 or control treatment which was significantly higher than any other treatment (Figure 29). The lowest (6.7%) injury recorded was in BRRI dhan56 at S2 treatment which was significantly lower than any other treatment. The relative membrane leakage gradually decreased with decreasing soil moisture level from S0 to S2 in all the varieties. The results indicated increased membrane stability and changes in aquaporin function of leaf membrane under water stress condition (Cattivelli *et al*. 2008). Under S2 treatment, the lowest injury was recorded in BRRI dhan56 which was followed by BRRI dhan49. The results indicated that leaf membrane leakage was much lower in BRRI dhan56 and BRRI dhan49 compared to other varieties under water stress treatment. Cell wall thickening under moisture stress in these two varieties might help them to perform better as well as produced better yield (have been shown in experiment III). Liu *et al*. (2000) reported that plants grown in drying soil had increased leaf membrane stability. But it was also

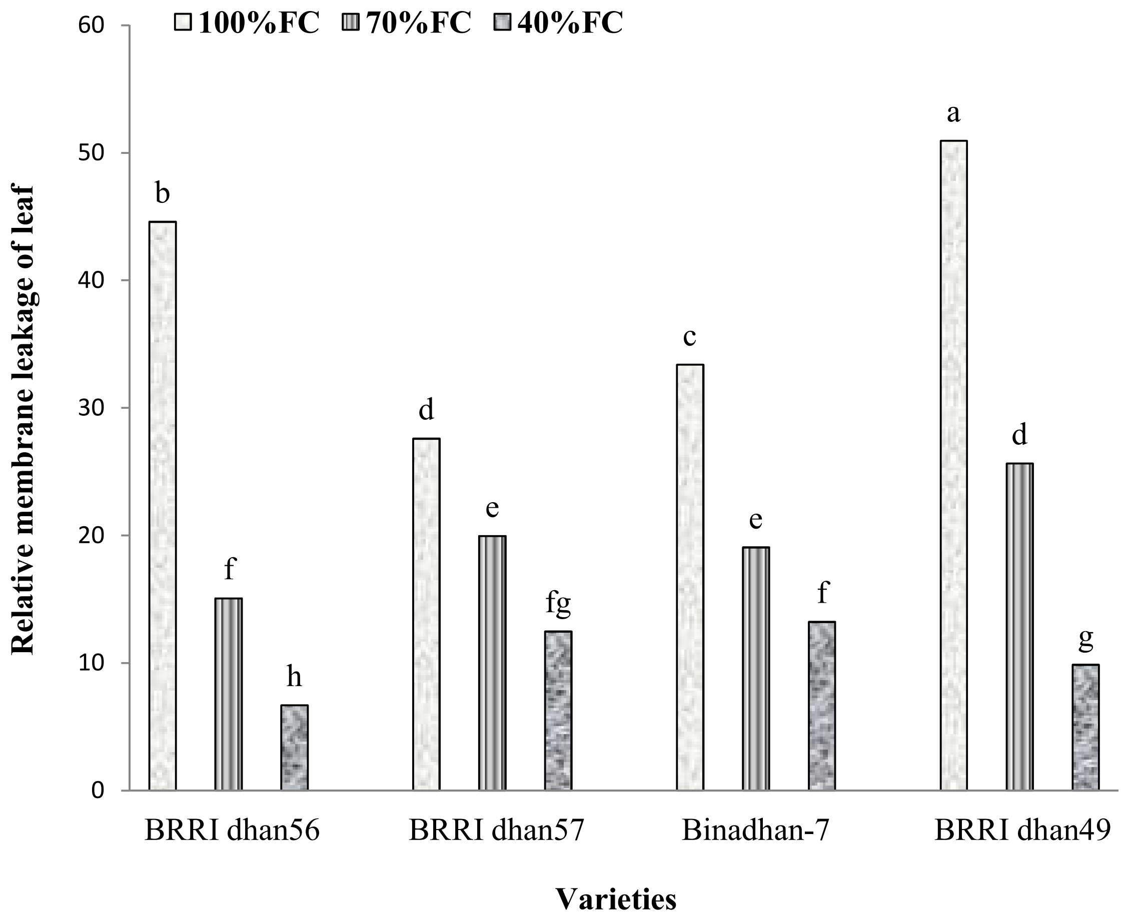


**Figure 29**. Relative membrane leakage of leaf tissue of different rice varieties under various soil moisture levels. S0=100%FC (field capacity) moisture, S1= 70% of the FC moisture, S2= 40% of the FC moisture. Values followed by same letter(s) did not differ significantly at 5% level of probability.

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recorded that membrane stability index was reduced under water stress in other rice varieties (Farooq *et al*., 2010; Akram *et al.,* 2013; Ding *et al*., 2014). Kumar *et al.* (2014) stated that membrane stability index (MSI) is a widely used criterion to assess crop drought tolerance, since water stress caused water loss from plant tissues, which seriously impairs both membrane structure and function. They found a significant differences in membrane stability index between the drought tolerant and susceptible rice varieties.

**4.4.2. Chlorophyll content**

The chlorophyll content of leaf was estimated during anthesis period from the flag leaf of main tiller. Chlorophyll is one of the important pigments of photosynthetic apparatus which absorbs light and transfers light energy to the reaction center of the photosystem. Both chlorophyll a and b are prone to soil drying (Pandey and Shukla, 2015). In the present experiment, the chlorophyll a content was found the highest (4.32 mg/g) in S1(70% FC) treatment of BRRI dhan49 which was significantly higher than any other treatment and the lowest (2.44 mg/g) content of chlorophyll a recorded was in BRRI dhan57 (Figure 30) at S1 treatment which was statistically similar to S1 treatment of BRRI dhan56. In all the varieties, S0 (control or 100% FC) treatment produced the higher chlorophyll a than S2 (40% FC). In BRRIdhan49, S1 treatment produced the highest chlorophyll a and S2 treatment produced the significantly lower chlorophyll a than control. The activation of chlorophyllase enzyme might play the major role in chlorophyll degradation under stress treatment. It was reported that the reduction in chlorophyll content due to water deficit is loss of chlorophyll by the action of increased chlorophyllase enzyme activity and inactivation of photosynthesis in many crops (Mudrik *et al*., 2003). In BRRI dhan56, control treatment produced the higher chlorophyll a compare to other treatments but was statistically similar S2 treatment of the same variety. The S1 treatment of BRRI dhan56 produced significantly lower chlorophyll a than the other treatments of the same variety. In BRRI dhan57, control treatment also produced the higher chlorophyll a compare to other treatments which was significantly higher than other treatments of the same variety. In Binadhan-7, there was no significant difference among the treatments considering their chlorophyll a content. It was also reported that

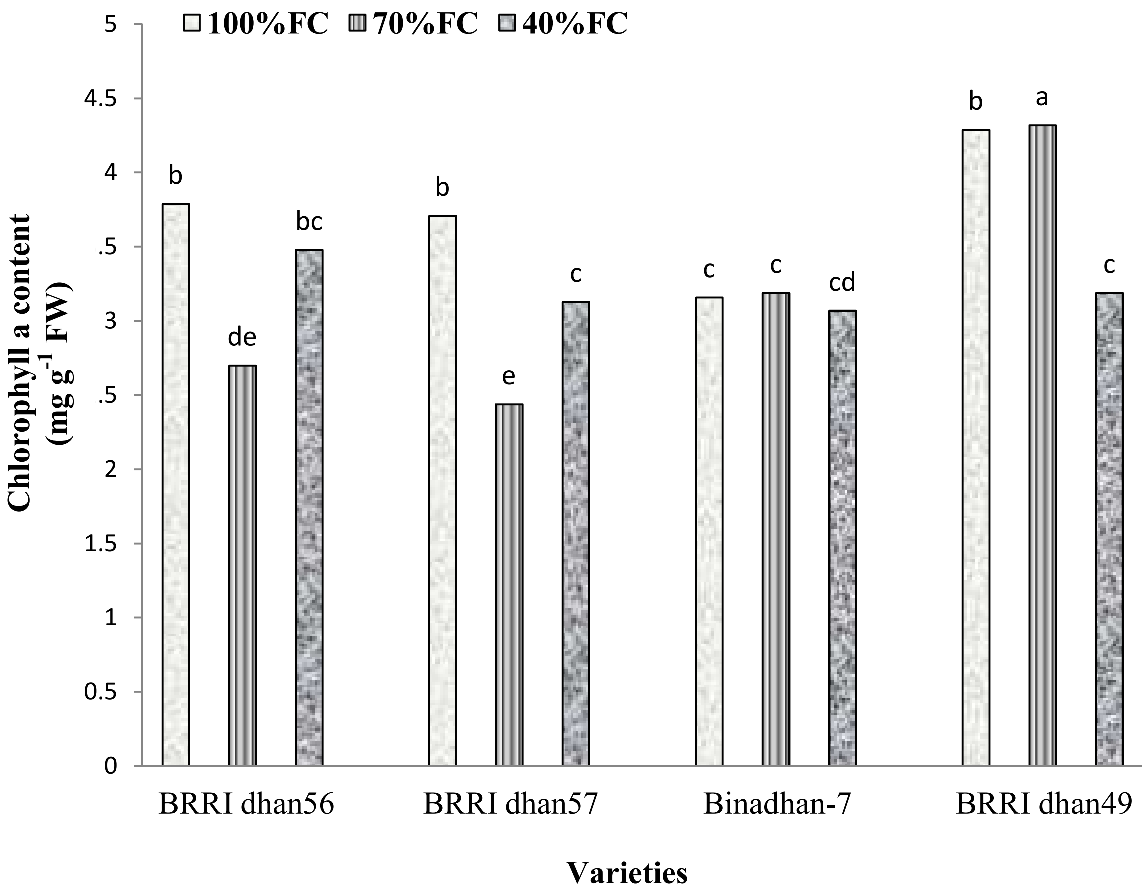



**Figure 30**. Chlorophyll a content of leaf of different rice varieties under various soil moisture levels. S0=100%FC (field capacity) moisture, S1= 70% of the FC moisture, S2= 40% of the FC moisture. FW=Fresh weight. Values followed by same letter(s) did not differ significantly at 5% level of probability.

the reduction in chlorophyll content may occur due to stress-induced impairment in pigment biosynthetic pathways or in pigment degradation, loss of the chloroplast membrane, and increased lipid peroxidation (Pandey and Shukla, 2015). Maisura *et al.* (2014) stated that a decrease in chlorophyll a content has the ability to change the reaction energy of light radiation decreases such that photosynthesis is inhibited. Farooq *et al*. (2009) stated that chlorophylla is susceptible to dehydration.

In the present experiment, chlorophyll b content was also estimated during chlorophyll a estimation. Considering all the varieties and soil moisture treatments the chlorophyll b content found the highest (1.68 mg/g) in BRRI dhan49 at S1 treatment which was statistically similar to the control treatment of the same variety and the lowest (0.9 mg/g) chlorophyll b recorded was in BRRI dhan57 (Figure 31) at the same treatment (S1) which was statistically similar to S1 treatment of BRRI dhan56. In all the varieties, S0 or control treatment produced the highest chlorophyll b than S2 treatment. It was reported that the reduction in chlorophyll content due to the action of increased chlorophyllase enzyme activity and inactivation of photosynthesis in many crops (Mudrik *et al*., 2003). Chlorophyll b serves as an antenna that collects light and transfers to the reaction center. Light energy is converted into chemical energy in the reaction center which can then be used in the reduction process of photosynthesis (Taiz and Zeiger, 1991). Under water stress treatment as the content of chlorophyll b found to be decreased, the harvesting of light also became lower, which ultimately affected the photosynthetic assimilation. Under water limiting condition, the chlorophyll b content was comparatively higher in BRRI dhan49 compared to other varieties. Farooq *et al*. (2009) stated that chlorophyll b is susceptible to dehydration.

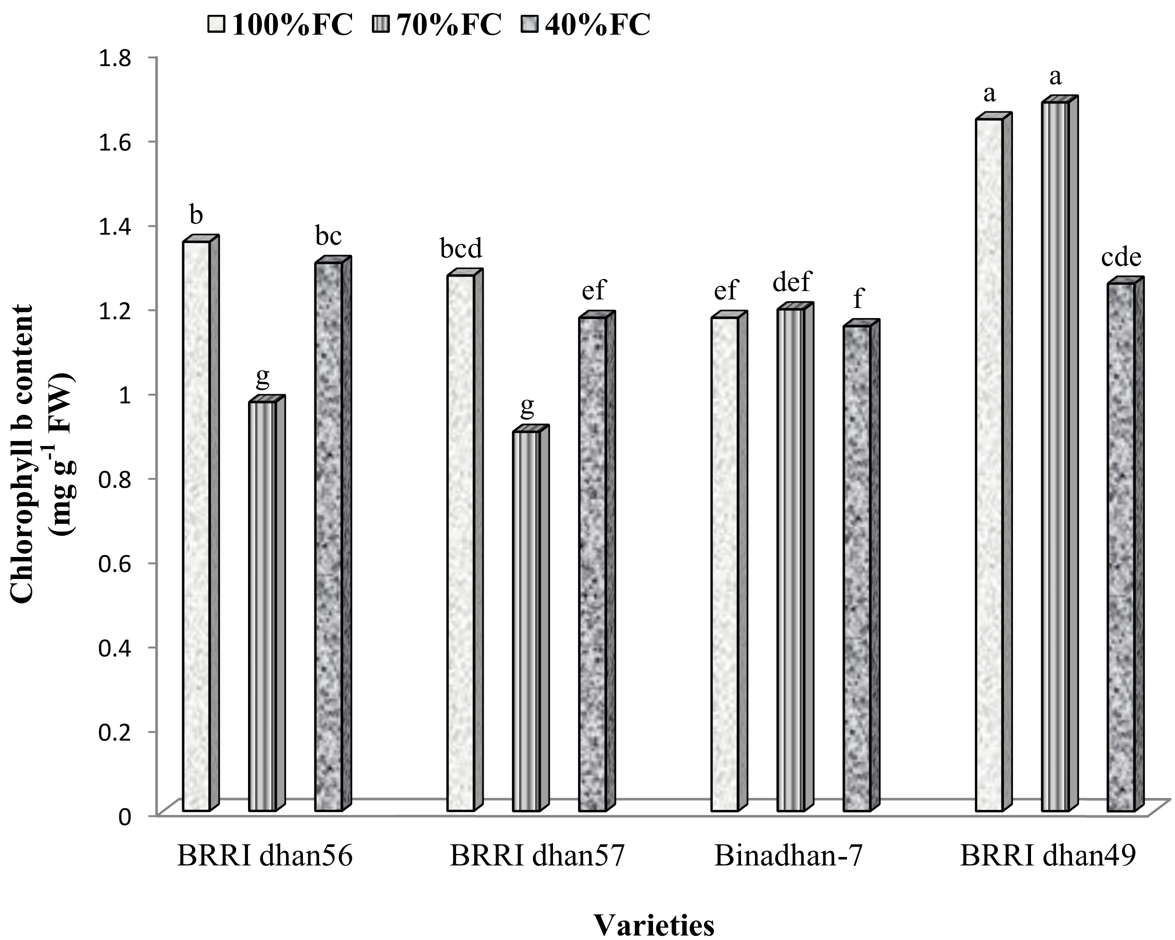



**Figure 31**. Chlorophyll b content of leaf of different rice varieties under various soil moisture levels. S0=100%FC (field capacity) moisture, S1= 70% of the FC moisture, S2= 40% of the FC moisture. Values followed by same letter(s) did not differ significantly at 5% level of probability.

In the present experiment, the total chlorophyll content was also affected by water stress treatment. Considering all the varieties and soil moisture treatments, the total chlorophyll content was also found the highest (8.71 mg/g) in BRRI dhan49 at S1 treatment which was statistically similar to control treatment of the same variety and the lowest (4.89 mg/g) total chlorophyll content recorded was in BRRI dhan57 (Figure 32) at S1 treatment also, which was significantly lower than any other treatments. In all the varieties, S0 treatment produced the higher total chlorophyll content than S2 treatment. In BRRI dhan56 and in BRRI dhan57, the highest total chlorophyll recorded was at control treatment and the lowest total chlorophyll recorded was at S1 treatment. But all the treatments of those varieties differed significantly among them. In Binadhan-7, all the treatments produced statistically similar total chlorophyll. In BRRI dhan49, the total chlorophyll content of control and S1 treatments were statistically similar but the total chlorophyll content in S2 treatment was significantly lower than any other treatments of the same variety.

Lower chlorophyll content at S2 treatment in all the varieties might contribute in lower harvesting of light and finally grain yield. Relatively higher chlorophyll content in BRRI dhan49 might contribute to perform better under S2 treatment and to give better yield. The reduction in chlorophyll content under water stress was due to the action of increased chlorophyllase enzyme activity and inactivation of photosynthesis in many crops (Mudrik et al., 2003). Drought causes many changes related to alter metabolic functions (described in experiment I) and one of those is the reduction of the synthesis of photosynthetic pigments (chlorophyll), recorded in the present experiment. This reduction in chlorophyll content decreases the harvesting of light as well as the generation of reducing power, which is a source of energy for dark reaction of photosynthesis. It was reported that these changes in the amounts of photosynthetic pigments are closely associated to plant biomass and yield (Jaleel *et al.,* 2009). It was also reported that chlorophyll a and chlorophyll b are susceptible to dehydration (Farooq *et al*., 2009). Kumar *et al*. (2014) found that chlorophyll content of drought tolerant varieties as well as check varieties was higher under normal (irrigated) treatment compared to water stress treatment. It was also observed by Zain *et al*. (2014) that

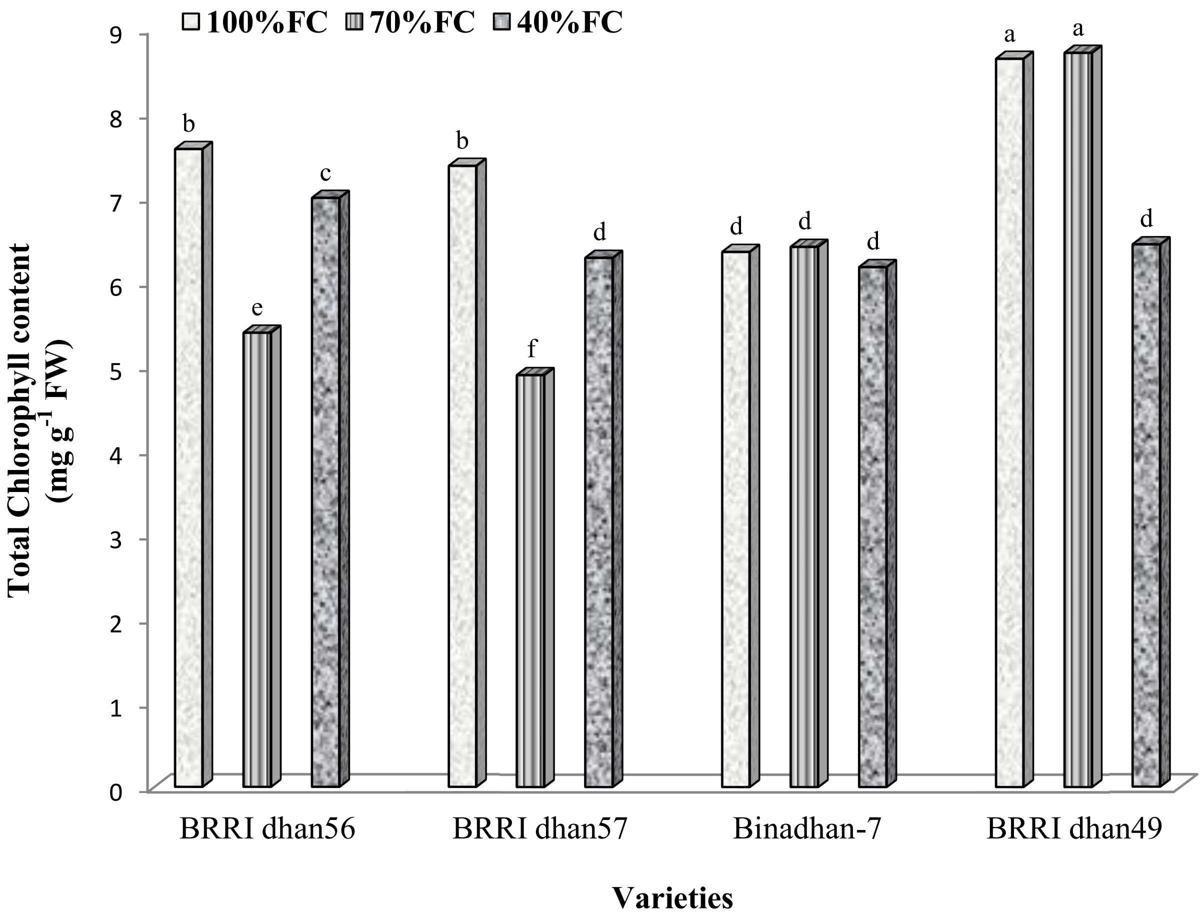



**Figure 32**. Total chlorophyll content of leaf of different rice varieties under various soil moisture levels. S0=100%FC (field capacity) moisture, S1= 70% of the FC moisture, S2= 40% of the FC moisture. Values followed by same letter(s) did not differ significantly at 5% level of probability.

increased duration of water stress can significantly reduced the total chlorophyll content as well as chlorophyll a/b ratio.

### 4.4.3. Proline content

The proline content of flag leaf was estimated during anthesis period. The results of proline content indicated that water stress significantly increased the proline content in all the varieties. The proline content gradually increased with the increasing water stress. The proline content was much higher at S2 (40% FC) treatment in all the varieties compared to their control treatment (Figure 33). Considering all the varieties and soil moisture treatments, the proline content recorded was the highest (8.69 µmole/g FW) in BRRI dhan56 at S2 treatment which was significantly higher than any other treatment and was followed by S2 treatment of BRRI dhan49 and the lowest (1.29 µmole/g FW) proline content in BRRI dhan49 at S0 treatment which was statistically similar to S1 treatment of the same variety and with S1 and S0 treatments of BRRI dhan57 and Binadhan-7. There were little differences in proline content among the varieties under normal or control treatment (Figure 33). Drought stress increased the proline accumulation in every rice cultivars.

Under S2 treatment, the proline content of BRRI dhan56 and BRRI dhan49 was comparatively higher than that in other two varieties. Higher proline content in BRRI dhan49 and in BRRI dhan56 under S2 treatment helped them to maintain higher RWC by osmotic regulation or adjustment with lower leaf rolling. For these reasons, under water stress treatment, lower reduction of different physiological processes such as RGR, NAR, AGR etc occurred in those varieties, which ultimately contributed in better yield of those varieties under water stress treatment. Increased proline content under water stress treatment in BRRI dhan56 and BRRI dhan49, indicated that these two rice varieties were more drought tolerant than the other varieties. It was reported that as the water deficit occurs, plants accumulate different types of organic and inorganic solutes in the cytosol to lower osmotic potential, thereby maintaining cell turgor (Rhodes and Samaras, 1994). This biochemical process is known as osmotic adjustment which strongly depends on the rate of plant water stress. Osmotic adjustment is achieved by the accumulation of proline, sucrose, glycinebetaine and other solutes in cytoplasm, improving water uptake from drying soil. Under water limiting condition, proline act as a osmolytic substance and help in absorption of water from the surrounding environment. Mostajeran and Rahimi (2009) stated that the amount of proline in both young and old leaves substantially increased in plants under drought effect. They also found that there was a significant variation in proline content among different rice cultivars and between different ages and different parts of the leaves. Zulkarnain *et al.* (2009) found that the accumulation of proline was high under water stress treatment than the well-watered treatment for different rice varieties. It was also reported that the leaves of the unstressed plants have been reported to be free from proline contents, which are very small and its accumulation increases 100-folds when the plants are subjected to drought stress (Widyasari and Sugiyarta, 1997). Thus, proline is known to play an important role as an osmoprotectant in plants subjected to hyperosmotic stresses such as drought and soil salinity. Kumar *et al.* (2014a) found that drought stress treatment caused an average increase of 61.45 % in proline content across the varieties as compared to irrigated treatment. It was also reported that the water stress has significant effect on free proline and maliondialdehyde content in rice and the proline and MDA content increased with the increased duration of water stress (Zain *et al*., 2014).

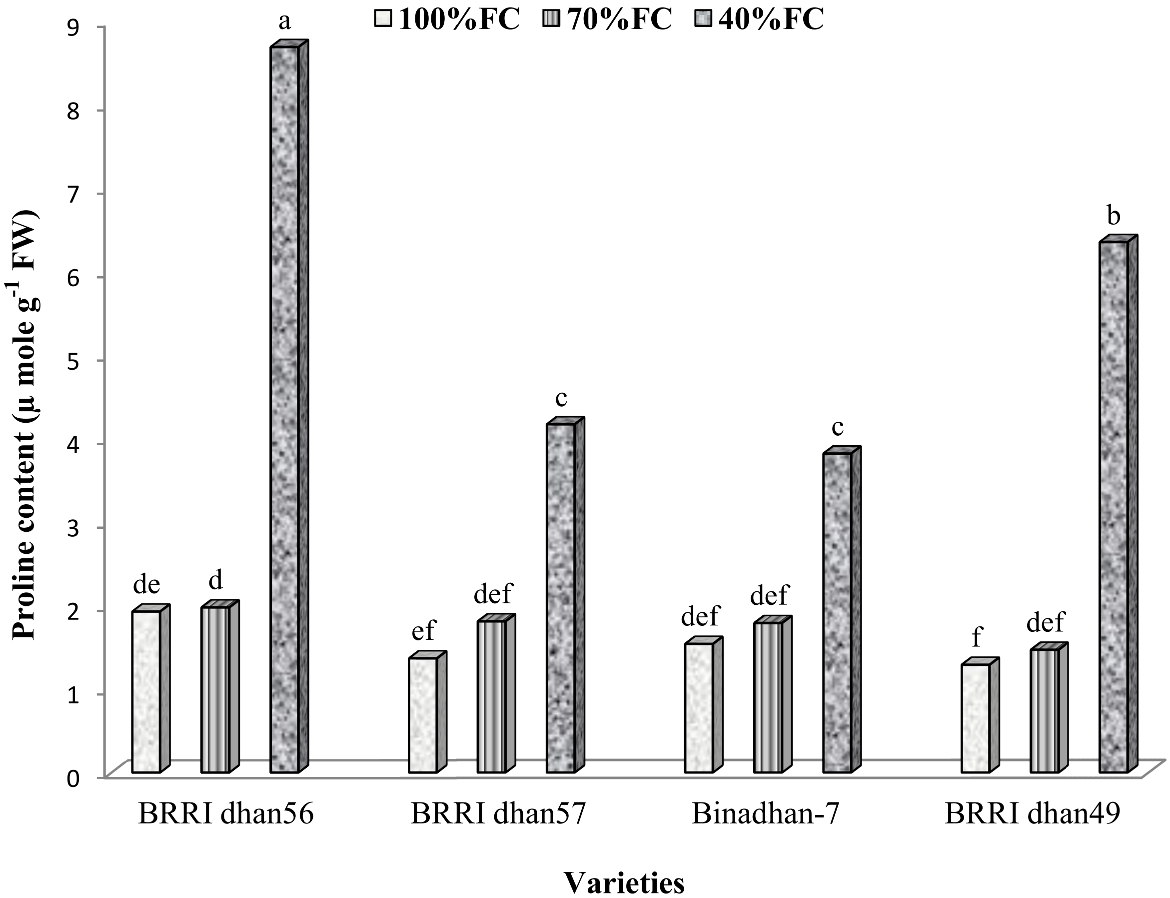



**Figure 33**. Proline content of leaf of different rice varieties under various soil levels. S0=100%FC (field capacity) moisture, S1= 70% of the FC moisture, S2= 40% of the FC moisture. Values followed by same letter(s) did not differ significantly at 5% level of probability.

### 4.4.4. Relationship between relative water content and proline content

In the present experiment, the relationship between relative water content and proline content was also recorded and the results indicated that there was a negative correlation (r = -0.778**) between relative water content and proline content (Figure 34). The relationship was highly significant. When the relative water content of leaf was higher, the little proline content was found in leaf under that situation. It was reported that the osmotic adjustment is an adaptive process, which can reduce some of the harmful effects of water deficits (Sellammal *et al*., 2014). Under water stress condition, proline act as an osmolytes and proline helps to accumulates more water. Kumar *et al.* (2014) found that drought stress at reproductive stage caused reduction in relative water content (31.57%) and increase in proline content (55.9%).

### 4.4.5. Relationship between membrane leakage and proline content

The relationship between relative injury and proline content was also estimated in this experiment. It was found that there was a negative correlation (r = -0.664**) between relative injury and proline content (Figure 35). The relationship was highly significant. When the relative injury of the plant tissues were higher, little proline content was found in that situation. In the present experiment, relative injury was estimated by the leakage of different electrolytes through the cell membrane. But the cell wall became thicker under water stress condition in all the varieties. As a results, the membrane leakage was lower as well as the relative injury was also lower under water stress condition. But the proline content was recorded higher under water stress condition compared to control treatment. So, the relationship between relative injury and proline content found to be negative. Kumar *et al.* (2014b) found that drought stress treatment caused an average increase of 61.45 % in proline content across the varieties as compared to irrigated treatment. It was reported that the proline accumulation might promote plant damage repair ability by increasing antioxidant activity during drought stress (Pandey and Shukla, 2015).

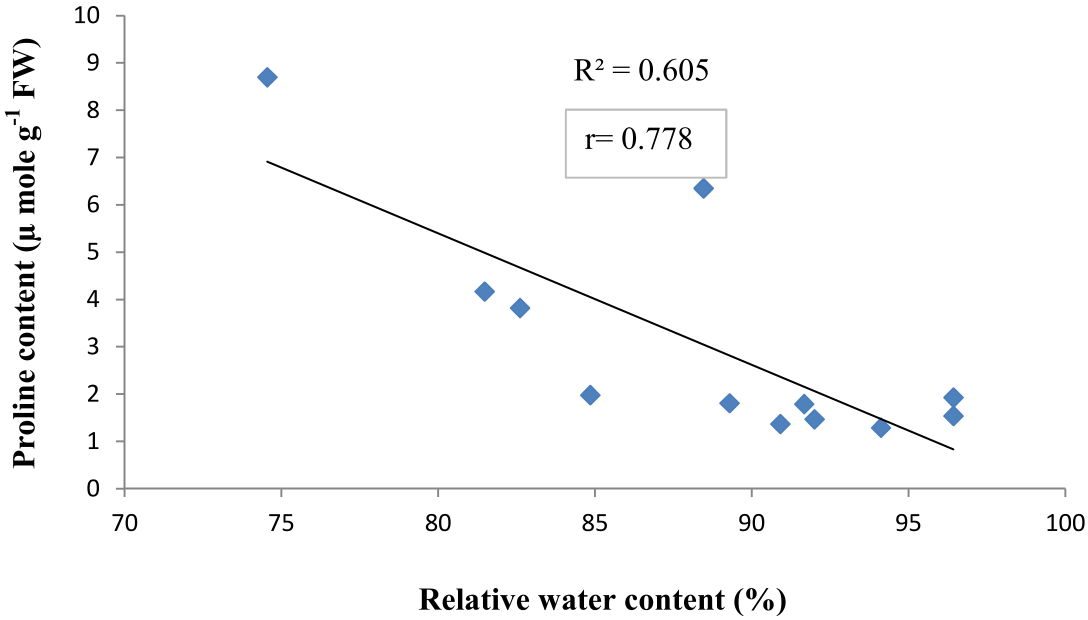



**Figure 34**. Relationship between relative water content and proline content of leaf of different rice varieties under various soil moisture levels.

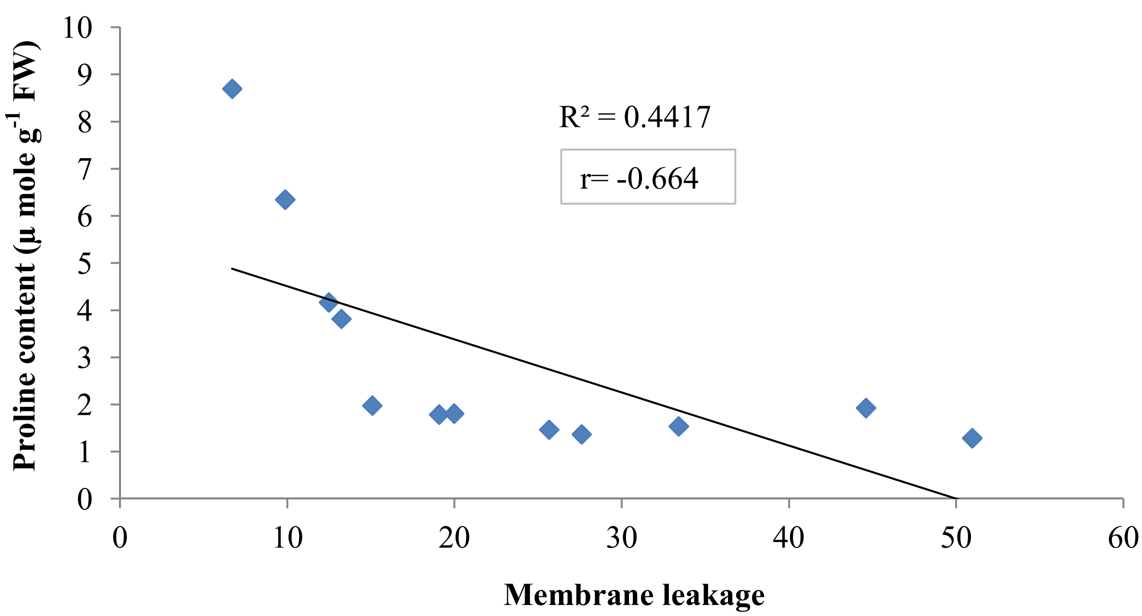



**Figure 35**. Relationship between relative membrane leakage and proline content of leaf of different rice varieties under various soil moisture levels

#### 4.4.6. Soluble sugar content

The soluble sugar is a product of photosynthesis. Under water stress condition, the photosynthesis become lower due to lack of water. Because water is the main substrate for photosynthesis. As a results, the soluble sugar content become lower under water stress condition compare to control. In the present experiment, the soluble sugar content of the flag leaf was estimated during anthesis. The soluble sugar content was found the highest at S0 (100% FC) or control treatment in all the varieties which was gradually decreased with decreasing the moisture content of the soil from S0 to S2 treatment (Figure 36). Among the varieties and soil moisture treatments, the highest (51 mg/g) sugar content was recorded in BRRI dhan56 at S0 treatment which was statistically similar to S1 treatment of the same variety, with control treatment of other three varieties and with S1 treatment of Binadhan-7 and BRRI dhan49 and the lowest (22 mg/g) soluble sugar recorded was in BRRI dhan57 at S2 treatment which was statistically similar to S2 treatment of BRRI dhan56 and Binadhan-7.

In the present experiment, the soluble sugar content at S1 treatment was statistically similar to control treatment (S0) in all the varieties; this might be due to the chlorophyll content, SPAD value, RWC and stomatal conductance at S1 treatment were statistically similar to control treatment in all the varieties. But at S2 treatment, the soluble sugar content was much lower compared to control and this might be due to lower RWC, chlorophyll content, stomatal conductance and higher leaf rolling. As a result, the harvesting of light and gas exchange was lower at S2 treatment, which ultimately affected the soluble sugar content. But the soluble sugar content in BRRI dhan49 was less affected due to water stress treatment compared to other varieties; this might be due to lower reduction in RWC, chlorophyll content, stomatal conductance and lower leaf rolling under water stress treatment compared to other varieties. It was reported that the sugar accumulation in rice varieties is severely affected by drought stress (Prasad *et al*., 2012). It was also reported that the drought stress generally accelerates senescence and reduce photosynthesis in susceptible varieties while tolerant varieties maintains its water balance and keep pace with photosynthetic activity and carbohydrate metabolisms (Watanbe *et*

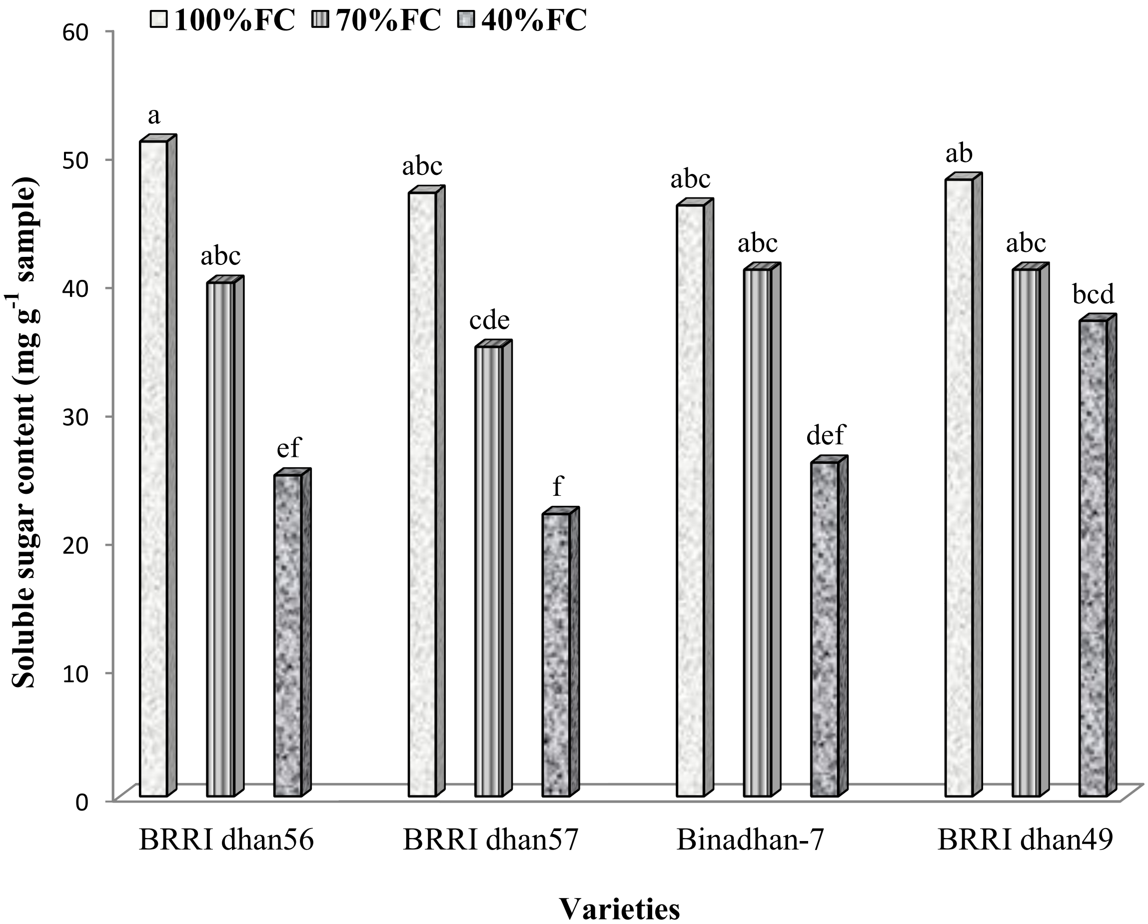



**Figure 36**. Soluble sugar content of leaf of different rice varieties under various soil moisture levels. S0=100%FC (field capacity) moisture, S1= 70% of the FC moisture, S2= 40% of the FC moisture. Values followed by same letter(s) did not differ significantly at 5% level of probability.

*al.*, 2000). On the other hand it was also recorded that drought induces the accumulation of soluble sugars (Shehab *et al*., 2010; Usman *et al*., 2013; Maisura *et al*., 2014).

### 4.4.7. Starch content

Starch is the stored form of photosynthetic carbohydrate. Under limited soil moisture content, as the photosynthesis become lower, the starch content of the plant also become lower compare to control treatment. In the present experiment, the starch content was also estimated with soluble sugar estimation from the sample. The starch content was also found the highest in S0 (100% FC) or control treatment which was gradually decreased with the increasing water stress treatment from S0 to S2 treatment in all the varieties (Figure 37). Considering all the varieties and soil moisture levels the highest (50.6 mg/g) starch content was recorded in S0 or control treatment of BRRI dhan56, which was statistically similar to S1 treatment of the same variety and with control and S1 treatments of rest of the varieties and the lowest (23.6 mg/g) starch content was recorded in S2 treatment of Binadhan-7 which was statistically similar to S2 treatment of rest of the varieties.

The starch content of different rice varieties at various soil moisture levels were more or less similar to that of soluble sugar content and the reasons might be the same as described in soluble sugar part. Abebe *et al.* (2003) found decreased starch content under drought. They also stated that the decreased starch content under water stress might due to a shift in C-partitioning from non-soluble carbohydrates (starch) to soluble carbohydrates, which can help maintain turgor for a longer period during drought and also participate in stress-protective functions. Under drought stress the accumulation of soluble carbohydrates can maintain plant turgor and contribute to stress-protective functions, such as maintenance of RWC in HYR lines due to osmotic adjustment, a mechanism of drought tolerance (Babu *et al*., 2004). It was also reported that the non-structural carbohydrate (total soluble sugar and starch) concentrations in shoot have long been associated with genotypic differences in ability to water deficit stress tolerance in rice and in general the starch concentration was the highest under control treatment than drought stress (Kumar *et al*., 2014b).

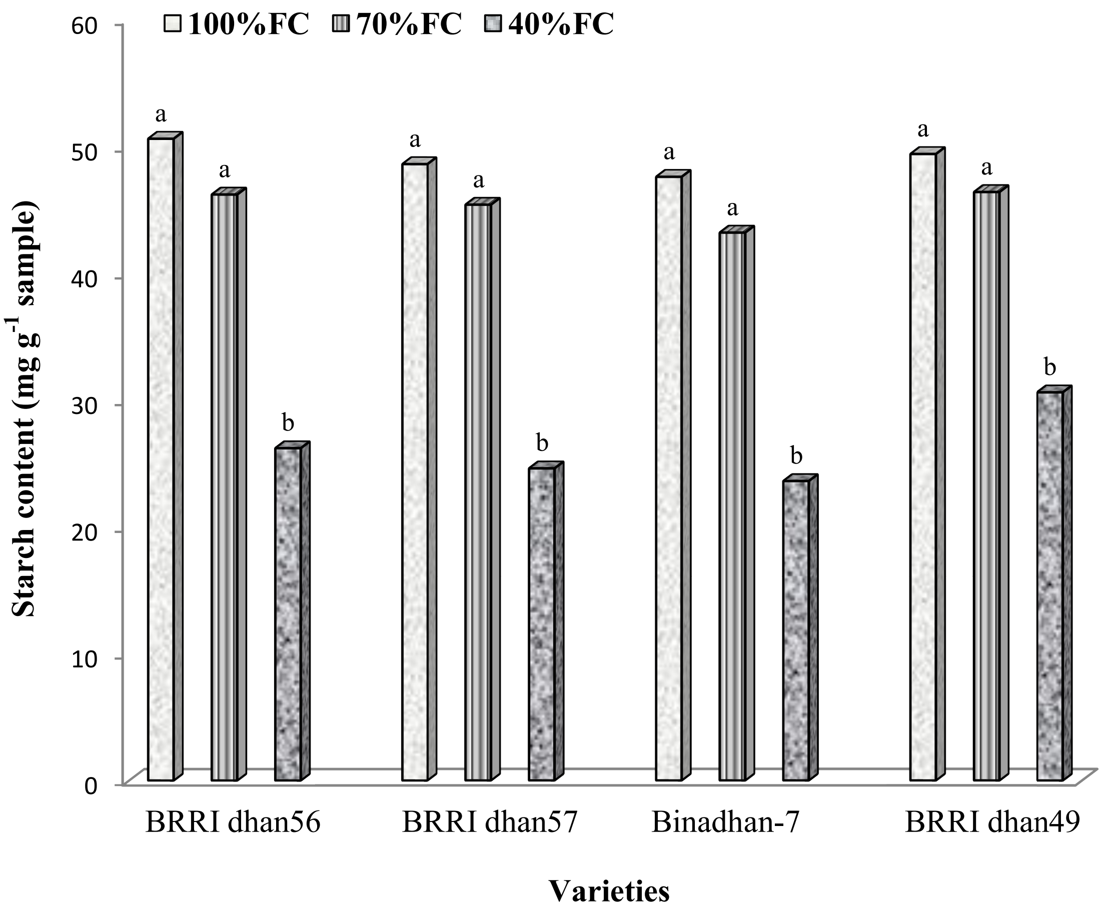



**Figure 37**. Starch content of leaf of different rice varieties under various soil moisture levels. S0=100%FC (field capacity) moisture, S1= 70% of the FC moisture, S2= 40% of the FC moisture. Values followed by same letter(s) did not differ significantly at 5% level of probability.

#### 4.4.8. Leaf anatomy under soil moisture stress

Different anatomical changes also occur due to soil moisture stress. In the present experiment, the anatomical changes of leaves due to soil moisture stress were observed under photomicroscope and the photographs have been taken. The anatomical changes of mid rib area of leaf due to moisture stress (S2) treatment of four rice varieties were observed under microscope and it was recorded that there were genotypic variations in this regard (Figure 38). Larger air space in leaf of Binadhan-7 under limited soil moisture condition is not desirable. The transpiration rate becomes higher when the air spaces inside the leaf increased. But in the other three varieties, the midrib air space of leaf was lower compared to Binadhan-7. Different drought tolerant varieties showed little air space inside the leaf due to minimize the transpiration loss under limited soil moisture condition.

In the present experiment, different anatomical changes occurred under various soil moisture treatments. But in those varieties where these types of changes are little, suppose to be more drought tolerant as in BRRI dhan56 and BRRI dhan49 in the present experiment (Figure 39). Little changes in leaf air space in these varieties helped them to decrease transpiration rate under water limiting condition (as shown in experiment I). The anatomical changes of these varieties under water limiting condition also indicate their drought tolerant character.

Higher leaf air space was formed through schizogenous process (via separation of cells during tissue development) due to soil moisture stress in BRRI dhan57 and in Binadhan-7 (Figure 40). The transpiration rate was also recorded higher in those varieties under water stress treatment. In BRRI dhan57, the highest transpiration rate was recorded at S2 treatment (have been shown in experiment I). In Binadhan-7, the transpiration rate was comparatively higher than in BRRI dhan49 and BRRI dhan56 under water stress treatment. Under water limiting condition, increase in transpiration is not desirable. So, higher leaf air space under water limiting condition in BRRI dhan57 and in Binadhan-7 indicated that these two varieties were less drought tolerant compared to other varieties.

Under S2 (40% FC) treatment, the thickening of the epidermal cell was much higher and have been shown in figure 41. As a result, the membrane leakage was comparatively lower under S2 treatment compared to control treatment in all the varieties. The protoplasm of the thicken cell was much smaller at S2 treatment compared to control protoplasm.

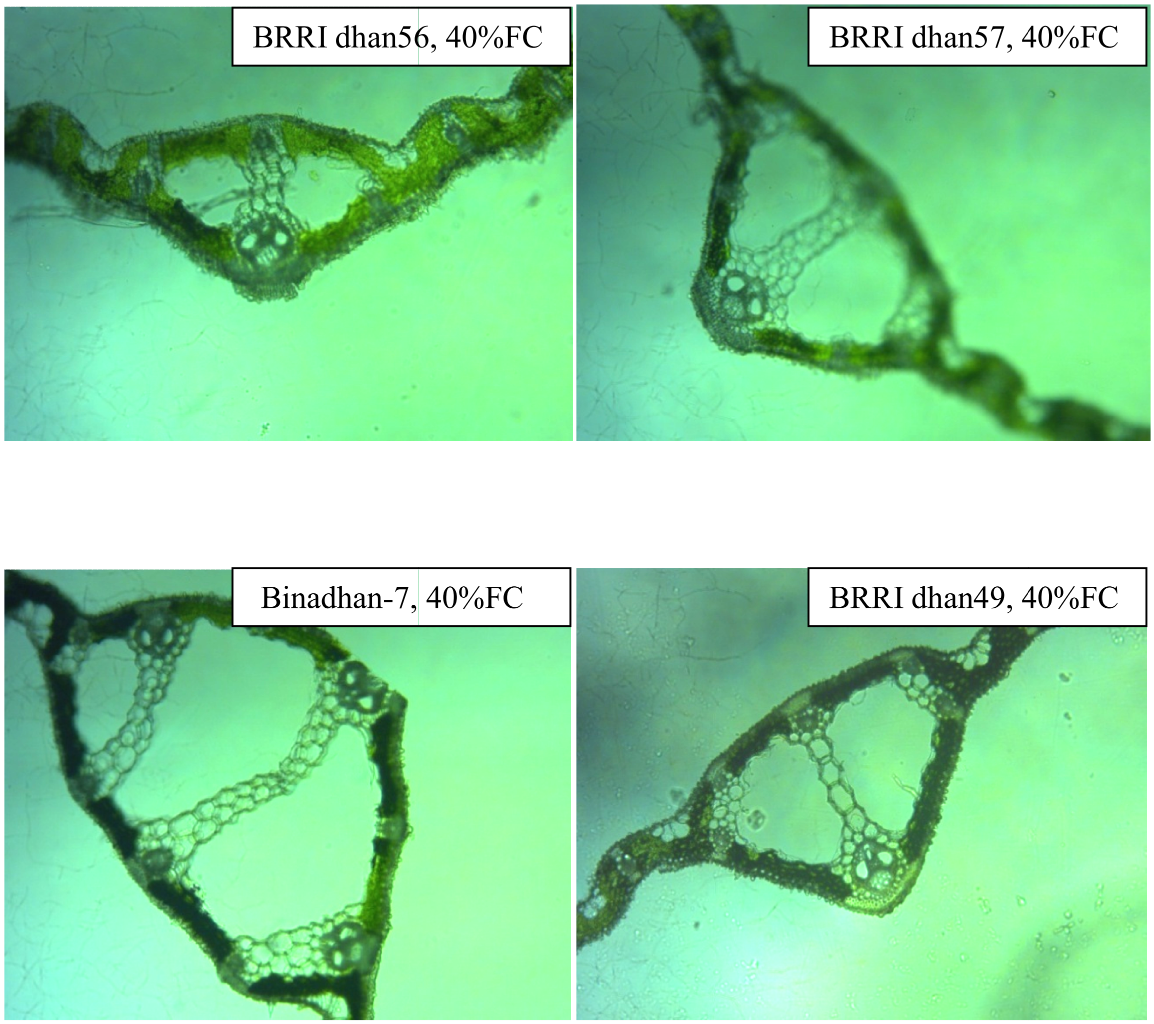



**Figure 38**. Leaf anatomy of four rice varieties under S2 (40% FC) treatment.

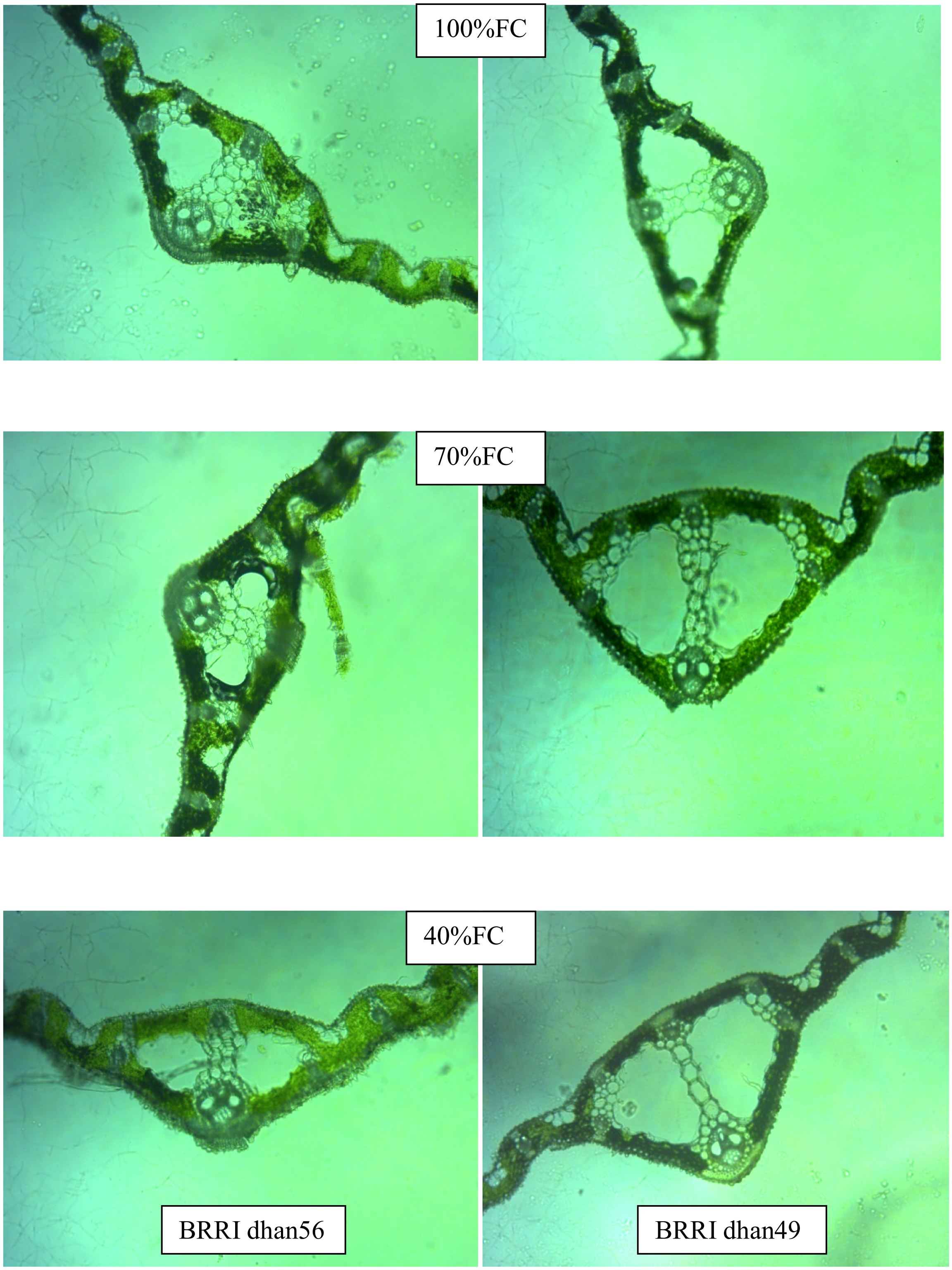



**Figure 39**. Gradual changes in leaf air spaces under three soil moisture regimes in BRRI dhan56 and BRRIdhan49.

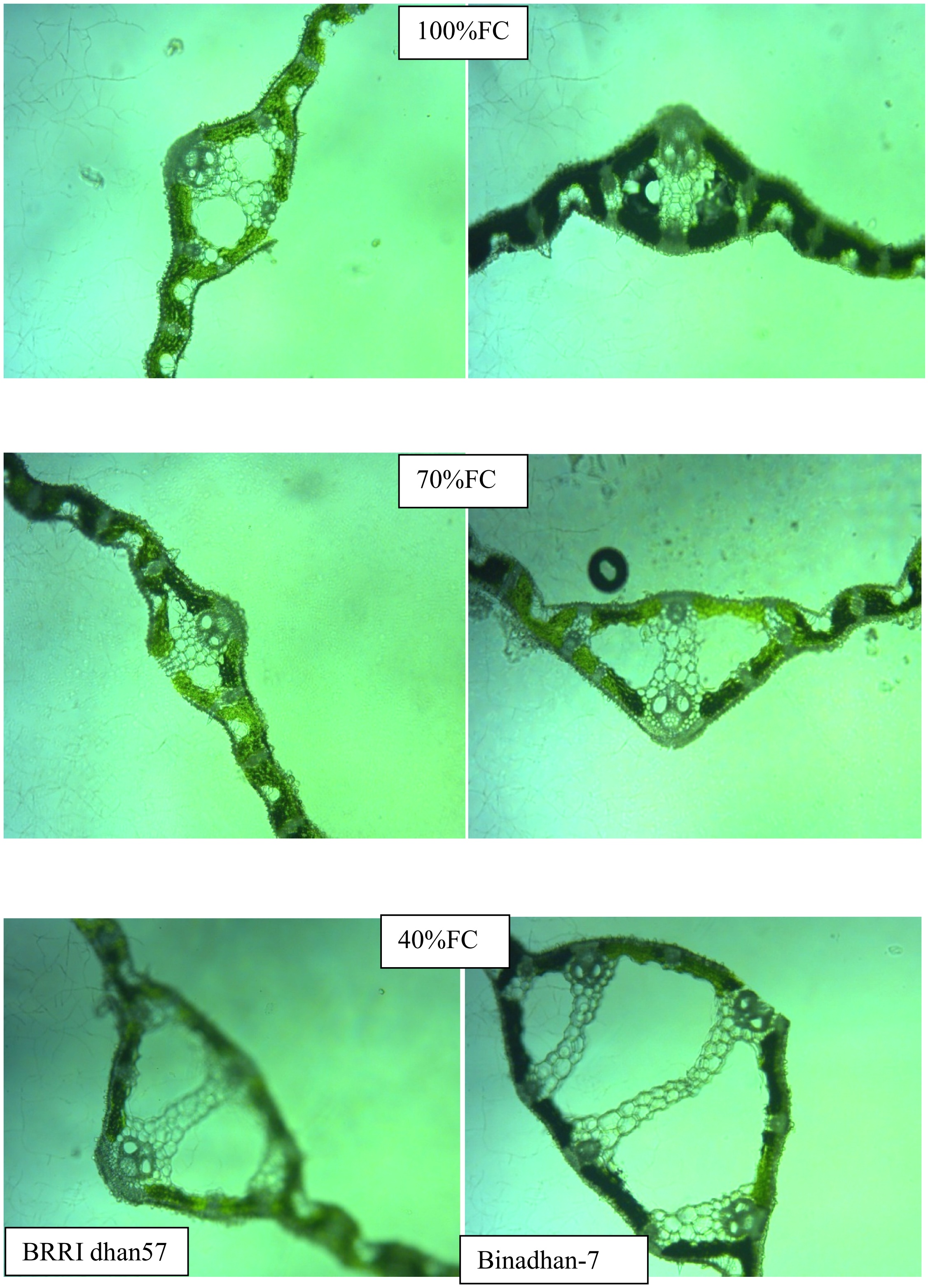
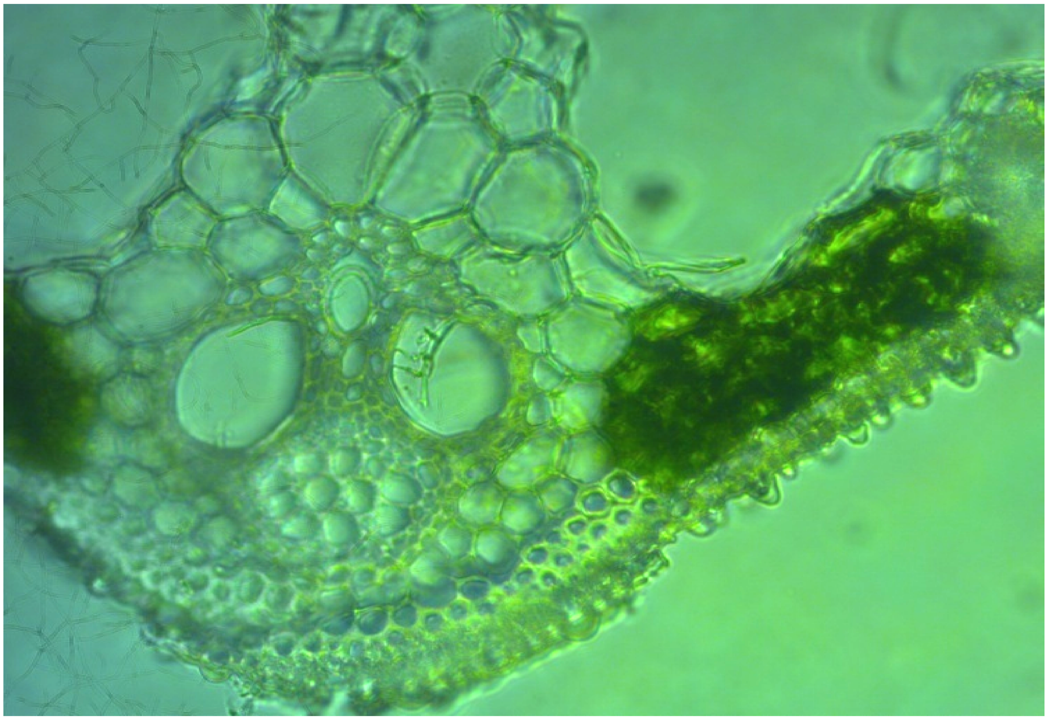
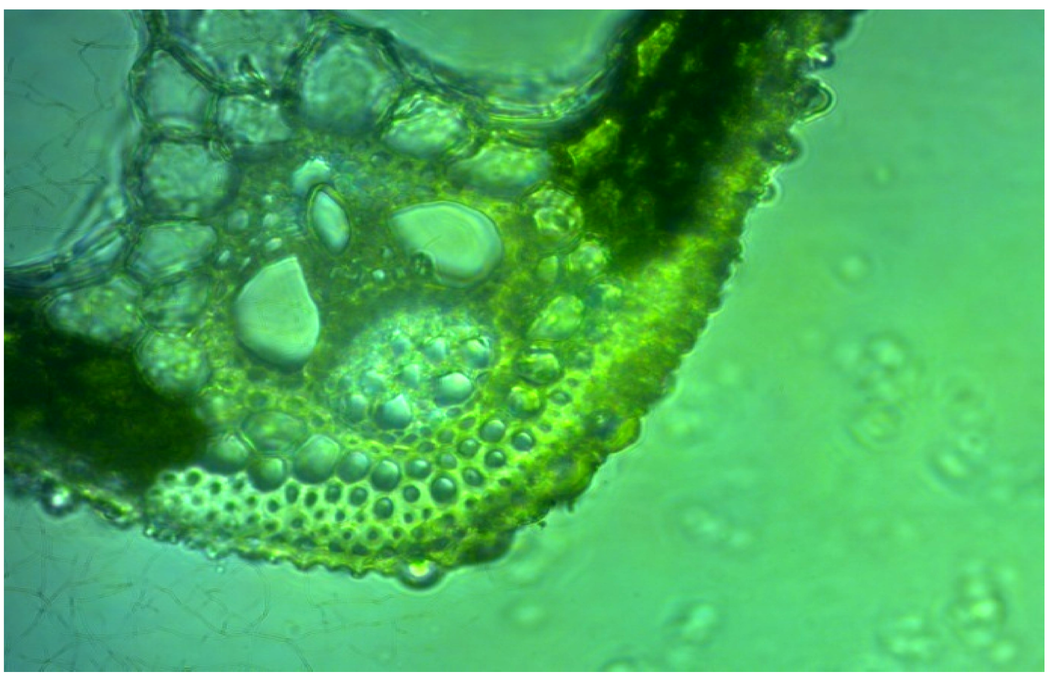



**Figure 40**. Gradual changes in leaf air spaces under three soil moisture levels in BRRI dhan57 and Binadhan-7.



BRRI dhan49 at control treatment



BRRI dhan49 at 40%FC treatment

**Figure 41**. Major vein vascular bundle and epidermal area of BRRI dhan49 grown under control and S2 (40%FC) treatment.

# CHAPTER 5

# SUMMARY AND CONCLUSION

Different morphological, physiological and biochemical processes of rice plant are affected by limited soil moisture content. In the present experiment the seedling of BRRI dhan49 was stronger than any other genotypes. 100%FC condition produced the longest plant height which was gradually decreased with decreasing the moisture level of the supplied soil. Different genotypes and different field capacity did not differ significantly among them in the number of effective and total tiller. There was no so much difference among the FC condition in a specific genotype regarding day to anthesis. It is clear that the genotype in which the anthesis become earlier get longer grain filling period and the genotype in which the anthesis was delayed get shorter grain filling period. Considering all the genotypes 40%FC possess the lowest and 100%FC possesses the highest life duration in all the genotypes except in Binadhan-7 in which the life durations was same both in 100%FC and 40%FC condition. In all the genotypes, 40%FC condition produced the less umber of leaf and dry weight than100%FC. The root dry matter was higher at 40%FC compare to control in all the genotypes and in BRRI dhan49 the root DM was much higher under water stress condition. In all the genotypes, 100%FC condition produced the highest leaf area. The specific leaf area (SLA) was recorded highest at 100%FC in all the genotypes except BRRI dhan57 and 40%FC produced the lowest SLA in all the genotype except BRRI dhan57. The specific leaf weight (SLW) gradually increased in Binadhan-7 and BRRI dhan49 from 100% to 40%FC condition. In both BRRI dhan56 and BRRI dhan57 the SLW was found maximum at 70%FC condition. The leaf and stem investment increased under limited soil moisture condition compare to control in all the genotypes. In all the genotypes the panicle investment was found lower at 40%FC compare to 100%FC condition. In all the genotypes the root investment was found higher at 40%FC compare to 100%FC condition. In BRRI dhan49 the root investment was found almost double at 40%FC condition than that in 100%FC condition. The root:shoot ratio was recorded higher at 40%FC condition compare to 100%FC condition in all the genotypes except in BRRI dhan56. Considering all the genotypes the highest TDM was produced at 100%FC condition and lowest TDM was produced at 40%FC condition. No leaf rolling was yet observed at 100%FC condition. The highest leaf rolling was observed at 40%FC condition. There was a highly negative linear relation between leaf rolling and grain yield. The relative water content of leaf gradually decreased with increasing water stress from 100%FC to 40%FC condition. The lowest stomatal conductance was recorded at 40%FC condition in all the rice genotypes. The highest transpiration rate was observed at 100%FC condition in all the rice genotypes. The internal $CO_2$ concentration was decreased with increasing water stress. The net assimilation rate was also decreased under water stressed condition. The SPAD value gradually decreased from anthesis to maturity. Considering all the genotypes, BRRI dhan49 maintained a strong SPAD values throughout it grain filling period. The stomatal conductance was much higher during anthesis in all the genotypes and was gradually decreased from anthesis to maturity. The decreasing rate was very sharp at 40%FC treatment. Under water stress condition, BRRI dhan49 showed much better conductance near maturity compare to others. The absolute grain growth rate gradually decreased with increasing water stress condition in all the genotypes. Under water stress condition the AGR was much better in BRRI dhan56 and BRRI dhan49. In all the genotypes, the 40%FC treatment showed the highest SRT.

The shoot and root length was found highest at 0%PEG treatment in all the genotypes and was gradually decreased with increasing PEG concentrations. Considering all the genotypes the leaf area was recorded highest at 0%PEG concentration and lowest at 10% PEG concentration and 5%PEG concentration in Binadhan-7. The fresh weight of plants was found highest at 0%PEG concentration in all the genotypes. A remarkable reduction in plant fresh weight was recorded in 5% and 10%PEG concentration in all the genotypes. But the reduction was relatively lower (52% of the control) in BRRI dhan49 compare to other genotypes at 5%PEG concentration. Percent dry matter distribution to shoot decreased and percent dry matter distribution to root increased with increasing PEG concentration in all the genotypes. The relative water content of leaf, SPAD reading and stomatal conductance was recorded highest at 0% PEG concentration which was gradually decreased with increasing PEG concentration. Under water stress condition the SPAD value and the stomatal conductance was much higher in BRRI dhan49. There is a positive linear relation between stomatal conductance and SPAD value.

In all the genotypes, the 100%FC condition produced the highest number and weight of filled spikelet percentage which was gradually decreased from 100% to 40%FC condition. Under water stress condition, BRRI dhan49 produced relatively higher filled spikelet percentage than the other genotypes. The panicle length decreased under water stress condition. Considering all the genotypes, the seed size gradually decreased with decreasing moisture level except in BRRI dhan56 where 70%FC condition produced the large seed. Seed size was little affected by water stress in BRRI dhan57 and in BRRI dhan49. There was a positive linear relation between seed size and grain yield. It was observed that grain yield per hill decreased with decreasing soil moisture level. But the reduction was relatively lower in BRRI dhan49 and in BRRI dhan56 than the other genotypes. In every genotype the highest harvest index were recorded at 100%FC condition which were gradually decreased from 100%FC to 40%FC condition. Under water stress condition the harvest index was much better in BRRI dhan56 and BRRI dhan49 compare to other genotypes. The drought susceptibility index was much lower in BRRI dhan49 which was followed by in BRRI dhan56. There was a positive linear relation between grain filling duration and grain yield, between grain filling duration and harvest index. There was a strong positive linear relation between plant height and grain yield. There was a positive linear relation between plant height and harvest index.

The relative injury gradually decreased with decreasing field capacity from 100% to 40% in all the genotypes. In all the genotypes 100%FC produced the higher chlorophyll a, chlorophyll b and chlorophyll a+b than 40%FC. Proline content gradually increased with increasing water stress condition in all the genotypes. The proline content was found highest in BRRI dhan56 at 40%FC condition which was followed by BRRI dhan49 in the same condition. There was a negative linear relation between relative water content and proline content. The soluble sugar and starch content was found highest at 100%FC condition in all the genotypes which was gradually decreased with decreasing field capacity of the soil.

## CONCLUSION

Considering the above statement, it may be concluded that-

i) Lower soil moisture content (40% of the field capacity) affected different morpho-physiological and biochemical processes and drastically reduced the grain yield.
ii) It was revealed that the drought resistant varieties showed lower leaf rolling and lower amount of membrane injury, increased amount of proline and better stem reserve utilization, less affected chlorophyll content which helped plants to provide better harvest

under water stress condition. The genotypes BRRI dhan49 and BRRI dhan56 showed more drought tolerance compared to other genotypes.

iii) It was found that *aman* rice (e.g. BRRI dhan49, BRRI dhan56) could be cultivated under low soil moisture level (under 70% of the field capacity moisture) without any remarkable effect on grain yield.

iv) During a*man* season, the farmers could provide irrigation water of 70%FC moisture, cultivating those genotypes without remarkable yield reduction but saving a large amount of irrigation water.

This study was conducted in pot under a partially controlled environment and further extensive research with more parameters of rice plants is suggested to have more information.

# CHAPTER 6

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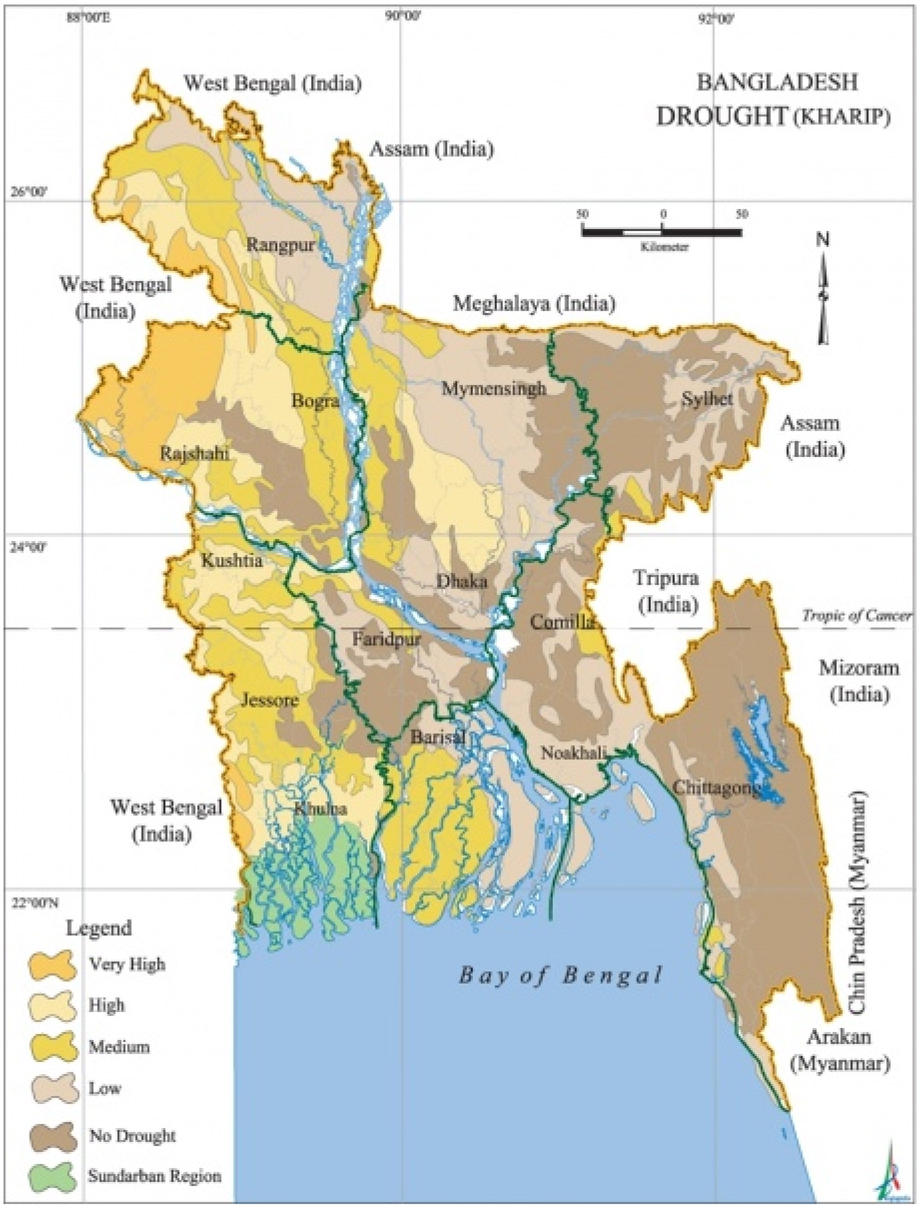
# APPENDICES

**Appendix 1**. Kharif drought-prone areas of Bangladesh



(Source: http://en.banglapedia.org/index.php?title=Drought)

**Appendix 2**. Morphological characteristics of the experimental field

| Morphological features | Characteristics |
|---|---|
| 1. Location | : Agricultural Botany field, SAU, Dhaka-1207 |
| 2. AEZ | : Madhupur Tract (28) |
| 3. General soil type | : Shallow red brown terrace soil (cultivated long time) |
| 4. Land type | : High land |
| 5. Soil series | : Tejgaon |
| 6. Topography | : Fairly leveled |

**Appendix 3**. Different meteorological data during the crop growth period

| **Months** | **2012** | | | | **2013** | | | | **2014** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Temperature(ºC) | | MRH | R | Temperature(ºC) | | MRH | R | Temperature(ºC) | | MRH | R |
| | Max. | Min. | | (cm) | Max. | Min. | | (cm) | Max. | Min. | | (cm) |
| July | 33 | 26.72 | 77 | 16.7 | 30.33 | 27.11 | 71.42 | 23.9 | 32.40 | 24.6 | 83 | 56.3 |
| August | 34 | 27.05 | 78.55 | 35.0 | 29.67 | 26.97 | 74.91 | 30.0 | 33.73 | 23.6 | 81 | 31.9 |
| September | 32.85 | 26.15 | 79.05 | 16.5 | 29.97 | 26.88 | 73.20 | 21.0 | 30.72 | 26.72 | 75.80 | 18.6 |
| October | 33.20 | 25.50 | 75.5 | 17.0 | 28.88 | 25.14 | 67.82 | 14.0 | 30.98 | 25.13 | 72.08 | 16.3 |
| November | 30 | 20.90 | 69.30 | 0 | 26.50 | 21.13 | 58.18 | 4.2 | 28.12 | 21.02 | 65.74 | 3.1 |
| December | 22.4 | 13.5 | 66.4 | 0 | 24.25 | 19.68 | 54.30 | 0 | 23.62 | 17.32 | 61.43 | 0 |

MRH=Mean relative humidity (%), R=Rainfall (mm), Source: 1) Mini weather station, Sher-e-Bangla Agricultural University and 2) Bangladesh Meteorological Department, Climate and Weather Division, Sher-e-Bangla Nagar, Dhaka-1207.

**Appendix 4**. Different physical and chemical properties of soil

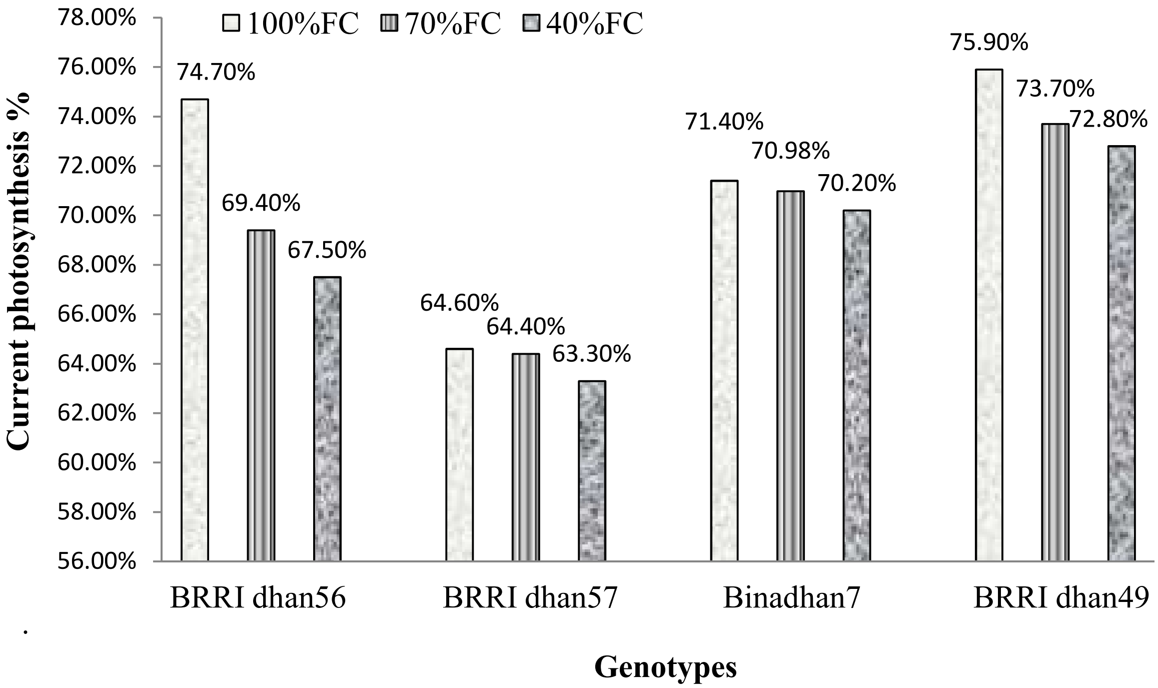
| *Physical features* | *Characteristics* |
|---|---|
| 1. Sand (2.0-0.02 mm) | : 22.26% |
| 2. Silt (0.02-0.002 mm) | : 56.72% |
| 3. Clay (< 0.002 mm) | : 20.75% |
| 4. Textural class | : Silt loam |
| *Chemical features* | *Characteristics* |
| 1. $P^H$ | : 5.9 |
| 2. Organic matter | : 1.09% |
| 3. Total N | : 0.07% |
| 4. Available P | : 8.5 ppm |
| 5. Exchangeable K | : 28 ppm |
| 6. Available S | : 8 ppm |

Source: Department of Soil Science, Sher-e-Bangla Agricultural University, Dhaka-1207.

**Appendix 5**. Germination percentage of the rice varieties that used in the present experiment

1. BRRI dhan56 = 81.30%
2. BRRI dhan57 = 80.70%
3. BINA Dhan7 = 79.40%
4. BRRI dhan49 = 80.80%

**Appendix 6**. Percent contribution of current photosynthesis towards grain yield of different transplanted *aman* rice varieties under various soil moisture levels



**Appendix 7**. Composition of Yoshida Culture Solution

| Macro elements for 2 liter solution | | |
|---|---|---|
| Element | Amount in gram | |
| N | 182.80 | |
| P | 80.60 | |
| K | 142.80 | |
| Ca | 177.20 | |
| Mg | 684.00 | |
| Micro elements for 2 liter solution | | |
| Elements | Amount in gram | |
| Mn | 3.00 | With 100 ml $H_2SO_4$ |
| Mo | 0.148 | |
| B | 1.868 | |
| Zn | 0.070 | |
| Cu | 0.062 | |
| Fe | 15.40 | |
| Citric acid | 23.80 | |

**Appendix 8**. 7-days after transplanting some treatment plants looked strong and some plants looked about to die

**Appendix 9**. 50-days old seedlings in different treatment pots. More stronger plants were recorded in less concentration of PEG

**Appendix 10**. 50-days old seedlings at the end of the experiment