

**RESPONES OF SOME AROMATIC RICE VARIETIES TO SALT
STRESS**

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**RESPONES OF SOME AROMATIC RICE VARIETIES TO SALT
STRESS**

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CERTIFICATE

*This is to certify that thesis entitled, “**RESPONSES OF SOME AROMATIC RICE VARIETIES TO SALT STRESS**” submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (MS) IN AGRONOMY**, embodies the result of a piece of bona-fide research work carried out by **MD. HABIBUR RAHMAN**, Registration no. **09-03333** 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.

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RESPONES OF SOME AROMATIC RICE VARIETIES TO SALT STRESS

ABSTRACT

A pot experiment with six aman rice varieties *viz.* BRRI dhan34, BRRI dhan38, Binadhan-9, Binadhan-13, Kalijira and Rajbhogh was conducted at the experimental shed of the Department of Agronomy, Sher-e Bangla Agricultural University, Dhaka during the period of June- December, 2014 to investigate the effect of salinity on the growth, physiology and yield of some aromatic rice under different salt stress condition. The experiment was carried out with five salt stress treatments *viz.* control (N₀), 25 mM (N₂₅), 50 mM (N₅₀), 75 mM (N₇₅) and 100 mM (N₁₀₀). Salt stresses significantly reduced the plant height and tiller hill⁻¹ of six varieties at all growth duration. Leaf relative water content (RWC) and chlorophyll (chl) content also reduced due to salt stress. At harvest, salt stresses reduced the effective tiller hill⁻¹, number of filled grain panicle⁻¹, 1000 grain weight, grain yield and straw yield of six varieties. Binadhan-9 and BRRI dhan38 performed better under saline condition. On the other hand, local variety Kalijira did not perform well under saline condition.

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LIST OF ABBREVIATIONS

AO	Ascorbate oxidase
APX	Ascorbate peroxidase
AsA	Ascorbic acid (ascorbate)
BRRRI	Bangladesh Rice Research Institute
CAT	Catalase
Chl	Chlorophyll
DHA	Dehydroascorbate
DHAR	Dehydroascorbate reductase
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Corporate
GR	Glutathione reductase
GSH	Reduced glutathione
GSSG	Oxidized glutathione
GST	Glutathione <i>S</i> -transferase
MDA	Malondialdehyde
MDHA	Monodehydroascorbate
MDHAR	Monodehydroascorbate reductase
POD	Peroxidase
ROS	Reactive oxygen species
SOD	Superoxide dismutase
SRDI	Soil Resource Development Institute
USDA	United States Department of Agriculture

Chapter 1

INTRODUCTION

Rice (*Oryza sativa* L.) is the main source of food for more than one third of the world's population. It is the second most important crop in the world after wheat, more than 90 per cent of which is grown in Asia. Rice (*Oryza sativa* L.) is rated as one of the major food crops in the world, but is also considered extremely salt-sensitive (Maas and Hoffman, 1977). Salinity is a major threat to crop productivity in the southern and south-western part of Bangladesh, where it is developed due to frequent flood by sea water of the Bay of Bengal and on the other hand introduction of irrigation with saline waters. In Bangladesh out of 2.85 million hectares of the coastal and off-shore areas, about 0.833 million hectares are arable lands, which constitute about 52.8 percent of the net cultivable area in 13 districts (Karim *et al.*, 1990). The majority of the saline land (0.65 million ha) exists in the districts of Satkhira, Khulna, Bagerhat, Barguna, Patuakhali, Pirojpur and Bhola on the western coast and a smaller portion (0.18 million ha) in the districts of Chittagong, Cox's Bazar, Noakhali, Lakshmipur, Feni and Chandpur. According to the report of the Soil Resource Development Institute (SRDI, 2010) of Bangladesh, about 0.203 million ha of land is very slightly ($2-4 \text{ dSm}^{-1}$), 0.492 million ha is slightly ($4-8 \text{ dSm}^{-1}$), 0.461 million ha is moderate ($8-12 \text{ dSm}^{-1}$) and 0.490 million ha is strong ($>12 \text{ dSm}^{-1}$) salt affected soils in southwestern part of the coastal area of Bangladesh.

Among the environmental stresses soil salinity is a widespread environmental problem that has been found to affect over 77 million hectares or 5% of the arable land worldwide (Wang *et al.*, 2001; Athar and Ashraf 2009). Salinity adversely affects the plant growth and productivity. The yield reduction due to salt stress may account for substantial reduction of the average yield of major crops by more than 50% (Bray *et al.*, 2000). The nature of damages due to salt

stress is very complex because it causes both osmotic stress and ionic toxicity (Hasanuzzaman *et al.*, 2013). Plants can respond and adapt to salt stress by altering their cellular metabolism and invoking various defense mechanisms (Ghosh *et al.*, 2011). The survival of plants under this stressful condition depends on their abilities to perceive the stimulus, generate and transmit a signal, and initiate various physiological and biochemical changes (Tanou *et al.*, 2009; El-Shabrawi *et al.*, 2010). Molecular and biochemical studies of the salt stress responses of plants have demonstrated significant increases in reactive oxygen species (ROS), such as singlet oxygen (1O_2), superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^\cdot) (Mittler 2002; Tanou *et al.*, 2009; Pérez-López *et al.*, 2010).

Salinity affects one-fifth of the irrigated land worldwide (Koyama *et al.*, 2001). Reducing sodium and chloride uptake into rice while maintaining potassium uptake are characteristics that would aid growth under saline conditions. Salinity is a major constrain to irrigated rice production in river deltas and former floodplains, particularly in semi-arid and arid climates. Irrigated rice is well suited to controlling and even decreasing soil salinity (Wopereis *et al.*, 1998), but rice is a salt susceptible crop and yield losses due to salinity can be substantial (Asch *et al.*, 1997).

Aromatic rice's have gained significant market shares in the global rice trade. Consequently, efforts have been undertaken in many countries to increase or develop the production of this type of rice. Several studies have reported the Variability of aromatic quality of the grain when rice has been grown in traditional fragrant rice areas. Field observations and recent scientific studies support the hypothesis that osmotic stress salinity may have a positive effect on aromatic quality of rice. Thus, if osmotic stresses are likely to increase 2-acetyl-1-pyrroline (2AP) content of the grains, they are also likely to adversely affect the number and weight of grains and consequently the yield as assumed by Bradbury *et al.* (2008).

Taking the above mentioned points in view, the present study was undertaken with the following objectives:

- i. To investigate the effect of salinity on the growth and physiology of some aromatic rice Varieties
- ii. To investigate the yield incurred by salinity
- iii. To compare the relative performance of local and HYV rice under the same condition

Chapter 2

REVIEW OF LITERATURE

2.1 Rice

Rice (*Oryza sativa* L.) is one of the most important staple foods for more than half of the world's population (IRRI 2006) and influences the livelihoods and economies of several billion people. In 2010, approximately 154 million ha were harvested worldwide, of which 137 million ha (88 percent of the global rice harvested) were in Asia of which 48 million ha (31 percent of the global rice harvested) were harvested in Southeast Asia alone (FAOSTAT, 2012). It is the main source of calories for almost 40% of the world population (Haffman, 1991). It is grown in many countries of the world; most of them are in Asia. Rice cultivars are classified on morphological basis primarily into three types- *indica*, *japonica*, and *javanica* (Purseglove, 1985). *Indica* rice cultivars are generally adapted to areas with a tropical monsoon climate. The rice cultivar grown in Bangladesh belongs to the sub-species *indica* (Alim, 1982). Rice is the principal source of food for more than one third of the world's population. It is the second most important crop in the world after wheat, more than 90 per cent of which is grown in Asia. In 2014-15, the production of rice is about 494.9 million tons (FAO, 2015). Rice is one of the most widely grown crops in coastal areas inundated with sea water during high tidal period, although it is usually considered moderately susceptible to salinity (Akbar *et al.*, 1972; Korbe and Abdel-Aal, 1974; Mori and Kinoshita, 1987). Rice (*Oryza sativa* L.) is rated as one of the major food crops in the world, but is also considered extremely salt-sensitive (Maas and Hoffman, 1977). It provides about 22 per cent of the world's supply of calories and 17% of the proteins.

2.2 Abiotic stress

World agriculture is facing a lot of challenges like producing 70% more food for an additional 9.7 billion people by 2050 while at the same time fighting with poverty and hunger, consuming scarce natural resources more efficiently and adapting to climate change (Wilmoth, 2015). However, the productivity of crops is not increasing in parallel with the food demand. The lower productivity in most of the cases is attributed to various abiotic stresses. Curtailing crop losses due to various environmental stressors is a major area of concern to cope with the increasing food requirements (Shanker and Venkateswarlu, 2011). The complex nature of the environment, along with its unpredictable conditions and global climate change, are increasing gradually, which is creating a more adverse situation (Mittler and Blumwald, 2010). Plants can experience abiotic stress resulting from the high concentrations of toxic or antagonistic substance. In some cases, such as the supply of water, too little (drought) or too much flooding can both impose stress on plants. Abiotic stresses modify plant metabolism leading to harmful effects on growth, development and productivity. If the stress becomes very high and/or continues for an extended period it may lead to an intolerable metabolic load on cells, reducing growth, and in severe cases, result in plant death (Hasanuzzaman *et al.*, 2012).

Plant stress may vary depending on the types of stressor and on the prevailing period. In nature, plants may not be completely free from abiotic stresses. They are expected to experience some degree of stress by any factor(s). Some environmental factors, such as air temperature, can become stressful in just a few minutes; others, such as soil water content, may take days to weeks, and factors such as mineral deficiencies can take months to become stressful (Taiz and Zeiger, 2006).

According to Araus *et al.*, (2002), abiotic stresses not only limit crop productivity, but also influence the distribution of plant species in different types of environment. Wang *et al.* (2003) quoted that temperatures could rise by another 3-9⁰C by the end of the century with far-reaching effects. Increased drought and salinization of arable land are expected to have devastating global effects. There is also growing evidence that all of these stresses are interconnected, for instance during drought stress, plant also suffers nutrient deficiency as most of the nutrients in the soil are available to plant when dissolved in water. In case of heat stress drought stress occurred simultaneously. Ahmad and Prasad (2012) reported that abiotic stress cause changes in soil-plant-atmosphere continuum which is responsible for reduced yield in several of the major crops in different parts of the world. Abiotic stresses like heavy metals, drought, salt, low temperature, etc. are the major factors that limit crop productivity and yield. These stresses are associated with production of certain deleterious chemical entities called reactive oxygen species (ROS), which include hydrogen peroxide (H₂O₂), superoxide radical (O²⁻), hydroxyl radical (OH⁻), etc. (Choudhury *et al.*, 2013). In their review, Macedo (2012) concluded that plant abiotic stress has been a matter of concern for the maintenance of human life on earth and especially for the world economy. In their review, Keunen *et al.*, (2013) concluded that plants suffering from abiotic stress are commonly facing an enhanced accumulation of reactive oxygen species (ROS) with damaging as well as signaling effects at organellar and cellular levels. The outcome of an environmental challenge highly depends on the delicate balance between ROS production and scavenging by both metabolic and enzymatic antioxidants. To meet these challenges, genes, transcripts, proteins, and metabolites that control the architecture and/or stress resistance of crop plants in a wide range of environments will need to be identified, in order to facilitate the biotechnological improvement of crop productivity.

The crop losses due to abiotic stress are estimated by many researchers. As per the report of Bray *et al.* (2000), abiotic stress is already the primary reason of crop loss worldwide, reducing average yields for most major crop plants by more than 50%. Some recent reports showed that the major abiotic stresses negatively influence the survival, biomass production and yields of staple food crops up to 70% (Thakur *et al.*, 2010). However the loss due to abiotic stresses has been predicted to become even more severe as desertification will further increase and the current amount of annual loss of arable area may double by the end of the century because of global warming (Evans, 2005; Vinocur and Altman, 2005). Although all of the abiotic stresses which are devastating for crop production, dehydration stress imparted by drought, salinity and temperature severity has been reported as the most prevalent abiotic stress that limits plant growth and productivity (Jaleel *et al.*, 2009; Thakur *et al.*, 2010). Collins *et al.* (2008) reported that the tolerance to abiotic stress is multigenic and quantitative in nature and thus a massive challenge exists to understand the key molecular mechanisms for advanced selective breeding purposes. Similarly, Patakas (2012) reported that the understanding abiotic stress responses in plants is difficult due to the complexity, interrelationship, and variability of mechanisms and molecules involved a fact that consist their evaluation an important and challenging topic in plant research. Mantri *et al.* (2012) also reported that the yield of food crops worldwide become reduced severely because of drought, cold, high-salinity and heat which are major abiotic stresses. Traditional plant breeding approaches to improve abiotic stress tolerance of crops had limited success due to multigenic nature of stress tolerance.

2.3 Salt stress

Salinity is one of the most brutal environmental factors limiting the productivity of crop plants because most of the crop plants are sensitive to salinity caused by high concentrations of salts in the soil. A considerable

amount of land in the world is affected by salinity which is increasing day by day. More than 45 million hectares (M ha) of irrigated land which account to 20% of total land have been damaged by salt worldwide and 1.5 M ha are taken out of production each year due to high salinity levels in the soil (Pitman and Läuchli, 2002; Munns and Tester, 2008). On the other hand, increased salinity of agricultural land is expected to have destructive global effects, resulting in up to 50% loss of cultivable lands by the middle of the twenty- first century (Mahajan and Tuteja, 2005).

Most of Bangladesh's coastal region lies on the southwest coastal region of the country. Approximately 30% of the crops land of Bangladesh is located in this region (Mondal *et al.*, 2001) and continuous to support crops productivity and GDP growth. But in the recent past, the contribution of crops to GDP has decreased because of salinity. In total, 52.8% of the cultivable land in the coastal region of Bangladesh was affected by salinity in 1990 (Karim *et al.*, 1990) and the salt affected area has increased by 14600 ha per year (SRDI, 2001). SRDI had made a comparative study of the salt affected area between 1973 to 2009 and showed that about 0.223 million ha (26.7%) of new land has been affected by varying degrees of salinity during the last four decades and that has badly hampered the agro-biodiversity (SRDI, 2010). Farmers mostly cultivate low yielding, traditional rice varieties. Most of the land kept fallow in the summer or pre-monsoon hot season (March-early June) and autumn or post-monsoon season (October- February) because of soil salinity, lack of good quality irrigation water and late draining condition. In the recent past, with the changing degree of salinity of southwest coastal region of Bangladesh, crop production becomes very risky and crop yields, cropping intensity, production levels of crop and people's quality of livelihood are much lower than that in the other parts of the country. Cropping intensity in saline area of Bangladesh is relatively low, mostly 170% ranging from 62% in Chittagong coastal region to 114% in Patuakhali coastal region (FAO, 2007).

In most of the cases, the negative effects of salinity have been attributed to increase in Na⁺ and Cl⁻ ions in different plants hence these ions produce the critical conditions for plant survival by intercepting different plant mechanisms. Although both Na⁺ and Cl⁻ are the major ions produce many physiological disorders in plant, Cl⁻ is the most dangerous (Tavakkoli *et al.*, 2010). Salinity at higher levels causes both hyperionic and hyperosmotic stress and can lead to plant demise. The outcome of these effects may cause membrane damage, nutrient imbalance, altered levels of growth regulators, enzymatic inhibition and metabolic dysfunction, including photosynthesis which ultimately leading to plant death (Mahajan and Tuteja, 2005; Hasanuzzaman *et al.*, 2012).

The available literature revealed the effects of salinity on the seed germination of various crops like *Oryza sativa* (Xu *et al.*, 2011), *Triticum aestivum* (Akbarimoghaddam *et al.*, 2011), *Zea mays* (Carpici *et al.*, 2009; Khodarahampour *et al.*, 2012), *Brassica spp.* (Ibrar *et al.*, 2003; Ulfat *et al.*, 2007) and *Helianthus annuus* (Mutlu and Bozcuk, 2007). It is well established that salt stress has negative correlation with seed germination and vigor (Rehman *et al.*, 2000). Higher level of salt stress inhibits the germination of seeds while lower level of salinity induces a state of dormancy (Khan and Weber, 2008).

Hasanuzzaman *et al.* (2009) observed a significant reduction in germination rate of 4 rice cultivars when exposed to various concentration of salt (30-150 mM). However, the sensitive cultivars were more prone to germination reduction under salt stress. In *Vigna radiata*, germination percentage decreased up to 55% when irrigated with 250 mM NaCl (Nahar and Hasanuzzaman, 2009). In a recent study, Khodarahampour *et al.* (2012) observed drastic reduction in germination rate (32%), length of radicle (80%) and plumule (78%), seedling length (78%) and seed vigour (95%) when *Zea mays* seeds were exposed to 240 mM NaCl.

One of the most initial effects of salt stress on plant is the reduction of growth rate. Salinity can affect growth of plant in various ways. First, the presence of salt in the soil reduces the water uptaking capacity of the plant, and this quickly causes reduction in the growth rate. This first phase of the growth response is due to the osmotic effect of the soil solution containing salt, and produces a package of effects similar to water stress (Munns, 2002a, b).

Some crops are most sensitive under saline condition during vegetative and early reproductive stages, less sensitive during flowering and least sensitive during the seed filling stage. Seed weight is the yield component in all these studies, but similar conclusions regarding growth stage sensitivity were obtained with both determinate crops (the grain crops) and indeterminate (cowpea) crops. Dolatabadian *et al.* (2011) observed that salinity stress significantly decreased shoot and root weight, total biomass, plant height and leaf number but not affected leaf area while studying with *Glycine max.*

A high concentration of Na^+ and/or Cl^- accumulation in chloroplasts is also inhibited photosynthesis. As photosynthetic electron transport is relatively insensitive to salts, either carbon metabolism or photophosphorylation may be affected due to salt stress (Sudhir and Murthy, 2004). In fact, the effect of salinity on photosynthetic rate depends on salt concentration as well as plant species or genotypes.

Fisarakis *et al.* (2001) reported a positive growth inhibition caused by salinity associated with a marked inhibition of photosynthesis. There is evidence that at low salt concentration salinity sometimes stimulate photosynthesis. For instance, in *B. parviflora*, Parida *et al.* (2004) observed that rate of photosynthesis increased at low salinity while decreased at high salinity, whereas stomatal conductance remained unchanged at low salinity and decreased at high salinity.

The alteration of photosynthetic pigment biosynthesis is one of the most notable effects of salt stress (Hasanuzzaman *et al.*, 2012). The decrease in chlorophyll (chl) content under salt stress is a commonly reported phenomenon and in various studies and the chl concentration were used as a sensitive indicator of the cellular metabolic state (Chutipaijit *et al.*, 2011).

Saha *et al.* (2010) observed a linear decrease in the levels of total Chl, Chl *a*, Chl *b* Car and xanthophylls as well as the intensity of Chl fluorescence in *Vigna radiata* under increasing concentrations of NaCl treatments. Compared to control, the pigment contents decreased on an average, by 31% for total Chl, 22% for Chl *a*, 45% for Chl *b*, 14% for carotene and 19% for xanthophylls (Saha *et al.*, 2010). Associated with the decline in pigment levels, there was an average 16% loss of the intensity of Chl fluorescence as well. In the study of Hasanuzzaman *et al.* (2011) observed that a higher chlorosis in wheat and rapeseed leaves when subjected to salt stress.

In *O. sativa* leaves, the reduction of Chl *a* and *b* contents of leaves was observed after NaCl treatment (200 mM NaCl, 14 d) where reduction of the Chl *b* content of leaves (41%) was affected more than the Chl *a* content (33%) (Amirjani, 2011). In another study, *O. sativa* exposed to 100 mM NaCl showed 30, 45 and 36% reduction in Chl *a*, Chl *b* and carotenoids (Car) contents compared to control (Chutipaijit *et al.*, 2011) which retarded the growth efficiency.

According to Romero-Aranda *et al.* (2006) increase of salt in the root medium can lead to a decrease in leaf water potential and, hence, may affect many plant processes. Osmotic effects of salt on plants are the result of lowering of the soil water potential due to increase in solute concentration in the root zone. At very low soil water potentials, this condition interferes with plants' ability to extract water from the soil and maintain turgor. However, at low or moderate salt

concentration (higher soil water potential), plants adjust osmotically (accumulate solutes) and maintain a potential gradient for the influx of water. Salt treatment caused a significant decrease in relative water content (RWC) in sugar beet varieties (Ghoulam *et al.*, 2002).

A decrease in RWC indicates a loss of turgor that results in limited water availability for cell extension processes (Katerji *et al.*, 1997). Steudle (2000) reported that in transpiring plants, water is thought to come from the soil to the root xylem through apoplastic pathway due to the hydrostatic pressure gradient. However, under salt stressed condition, this situation changes because of the restricted transpiration. Under these situations, more of the water follows the cell-to-cell path, flowing across membranes of living cells (Vysotskaya *et al.*, 2010).

Salt stress significantly reduced the yield of crops as indicated by many researchers. As reported by Greenway and Munns (1980), after some time in 200 mM NaCl, a salt-tolerant species such as sugar beet might have a reduction of only 20% in dry weight, a moderately tolerant species such as cotton might have a 60% reduction, and a sensitive species such as soybean might be dead. On the other hand, a halophyte such as *Suaeda maritime* might be growing at its optimum rate (Flowers *et al.*, 1986).

Murty and Murty (1982) reported that the severe inhibitory effects of salts on fertility may be due to the differential competition in carbohydrate supply between vegetative growth and constrained supply of these to the developing panicles. Grain yield reduction of rice varieties due to salt stress is also reported earlier by Linghe and Shannon (2000) and Gain *et al.* (2004). In *O. sativa* varieties, grain yield, which is the ultimate product of yield components greatly influenced by salinity levels. The loss of grain yield due to 150 mM salinity are 50%, 38%, 44% and 36% over control for the cultivars BR11,

BRRRI dhan41, BRRRI dhan44 and BRRRI dhan46, respectively (Hasanuzzaman *et al.*, 2009).

Nahar and Hasanuzzaman (2009) also reported that different yield components of *V. radiata* were significantly affected by salinity stress. Numbers of pods per plant, seeds per pod and seed weight were negatively correlated with salinity levels. The reproductive growth of *V. radiata* was also affected by salinity as the number of pods per plant substantially decreased with increasing salinity levels. An application of 250 mM NaCl reduced 77%, 73% and 66% yield in *V. radiata* cv. BARI mung-2, BARI mung-5 and BARI mung-6, respectively over control (Nahar and Hasanuzzaman, 2009).

2.4 Effect of salinity on rice

Rice is relatively tolerant during germination, becomes very sensitive during early seedling stage, gains tolerance during active tillering, but becomes sensitive during panicle initiation, anthesis and fertilization and finally relatively more tolerant at maturity (Makihara *et al.*, 1999 and Singh *et al.*, 2004).

Studies have shown that a very poor correlation exists between tolerances at seedling stage with that during reproduction, suggesting that tolerance at these two stages is regulated by a different set of genes (Moradi *et al.*, 2003)

The reproductive stage is crucial as it ultimately determines the grain yield. However, the importance of the seedling stage cannot be undermined as it affects crop establishment. Salinity reduces the growth of plant through osmotic effects, reduces the ability of plants to take up water and this causes reduction in growth. There may be salt specific effects. If excessive amount of salt enters the plant, the concentration of salt will eventually rise to a toxic level in older transpiring leaves causing premature senescence and reduces the

photosynthetic leaf area of a plant to a level that cannot sustain growth (Munns, 2002).

Alam *et al.* (2004) attributed the possible reasons for decrease in the shoot and root growth in salinized plants as reduction of photosynthesis, which in turn limits the supply of carbohydrates needed for growth and reduction of turgor in expanding tissues resulting from lowered water potential in root growth medium.

Rice cultivars differ substantially in their growth rate with the most vigorous lines being the traditional varieties. Naturally occurring salt resistant varieties invariably belong to these traditional tall varieties. The high vigour of land races may enable them to tolerate growth reduction. Vigorous growth also has a dilution effect. The Na⁺ uptake of the salt tolerant land race 'Pokkali' is not less than the salt sensitive dwarf IR-28 but the low Na⁺ concentration in Pokkali is attributed to the diluting effect of its rapid vegetative growth (Yeo and Flowers, 1984 and Bohra and Doerffling, 1993).

Plant roots experience the salt stress when Na⁺ and Cl⁻ along with other cations are present in the soils in varying concentration (1 to 150 mM for glycophytes and more for halophytes). The toxic ions sneak into the plant along with the water stream which moves from soil to the vascular system of the root by different pathways like symplastic and apoplastic. Na⁺ and K⁺ are mediated by different transporters which are clearly demonstrated by Garciadebleas *et al.* (2003).

Ion homeostasis in cell is taken care of by the ion pumps like antiporters, symporters and carrier proteins on membranes (plasma membrane or tonoplast membrane). Salt Overly Sensitive (*SOS*) regulatory pathway is one good example of ion homeostasis. This pathway is activated after the receptor

perceives the salt stress to alter protein activity and gene transcription by signaling intermediate compounds. Guo *et al.* 2004)

Addition of salt induces the Na⁺/H⁺ antiporter activity but it increases more in salt tolerant than salt sensitive species (Staal *et al.*, 1991).

Na⁺ which enters leaf cells is pumped into vacuole before it reaches to toxic level for enzymatic activities. This pumping activity is controlled by vacuolar Na⁺/H⁺ antiporters (Blumwald *et al.*, 2000)

Dubey and Sharma (1989) reported delayed differentiation of root and shoot and reduction in seedling vigour index with increase in salt concentration.

Shereen *et al.* (2005) and Haq *et al.* (2009) screened seven rice cultivars at 100 mM of salt concentration and reported that with increase in salinity, a significant reduction was observed in shoot dry weight; shoot fresh weight and number of tillers plant⁻¹ after 42 days of salt stress.

Hakim *et al.* (2010) studied the response of twelve rice varieties against six salinity levels (0, 4, 8, 12, 16 and 20 dS m⁻¹) at germination and early seedling stages and found that salinity decreased the final germination per cent and led to reduction in shoot and root length and dry weight in all varieties. Further they noticed that magnitude of reduction increased with increasing salinity stress.

Zeng and Shannon (2000) observed significant reduction in root dry weight of rice genotypes at 1.9 and 3.4 dS m⁻¹ of salinity. While Ali *et al.* (2004a) emphasized the importance of root shoot ratio in screening the rice germplasm against salinity as the lines with higher root shoot ratio recorded lower visual score of 4-5.

Growth reduction immediately after the application of 12.5 dS m⁻¹ of EC was observed by Alam *et al.* (2004) in rice, but no significant variation was seen at lower levels (8.5 and 4.5 dS m⁻¹). They observed severe effects on leaf area, shoot and root fresh weight besides effect on all plant parts.

Rahmanzadeh *et al.* (2008) evaluated four rice cultivars both in pots and under *in vitro* conditions at various levels of salinity and found Tichung-65 as most sensitive cultivar, based on reduction in seedling dry weight, wet weight, shoot length and root length.

The researchers Awala *et al.* (2010) screened 54 genotypes of *Oryza glaberrima*, NERICA (21) and *O. sativa* (41) and grown in pots by irrigating with NaCl (80 mM) solution. They observed that relative root biomass was significantly lower in *Oryza glaberrima* than others.

Lee *et al.* (2003) observed significantly lower reduction of all growth parameters of tolerant *indica* varieties than *japonica* varieties. They further observed that tolerant *indica* cultivars were good Na⁺ excluders with high K⁺ absorption and maintained a low Na⁺/K⁺ ratio in shoot, and indicated that tolerance level of *indica* was higher than that of *japonica*. They also observed that the cultivar with low Na⁺/K⁺ ratio was highly tolerant and the susceptible one had high Na⁺/K⁺ ratio.

Walia *et al.* (2007) revealed in their study on both *indica* (IR 6373 and IR-29) and *japonica* (Agami and M-103) varieties of rice and found that tolerant genotypes maintained much lower shoot Na⁺ than sensitive genotypes under salinity stress.

Haq *et al.* (2009) reported significant variation in leaf Na⁺ under salt stress but not in control. The tolerant variety (CO-34) accumulated lower Na⁺ (14.9 mol m⁻³) while susceptible variety (Monoberekan) accumulated 52.9 mol m⁻³ in the leaf sap. They further reported larger reduction in K⁺/Na⁺ ratio under salt stress

compared to control. They revealed that the key feature of plant salt tolerance was the ability of plant cells to maintain optimum K^+/Na^+ ratio in the control.

Ali *et al.* (2004b) observed significant reduction of yield in many rice genotypes at a salinity level of 8.5 dS m^{-1} besides the reduction of many yield contributing parameters *viz.*, chlorophyll content, productive tillers plant^{-1} , and panicle length and fertility percentage.

Uddin *et al.* (2007) stated that salinity reduced the number of effective tillers plant^{-1} , number of grains panicle^{-1} , 100-grain weight and yield plant^{-1} of rice. Hasamuzzaman *et al.* (2009) reported that 1000-grain weight and grain yield decreased with increase in levels of salinity in rice.

Govindaraju and Balakrishnan (2002) indicated that plant height, number of productive tillers hill^{-1} , 1000-grain weight, grain yield, straw yield, chlorophyll content and photosynthetic ability of rice decreased with increase of salinity.

Khatun *et al.* (1995) reported that salinity delayed flowering, reduced the productive tillers plant^{-1} , fertile florets panicle^{-1} , seed set (weight grain^{-1}), 1000-seed weight and overall grain yield Khatun *et al.* (1995)

Zeng and Shannon (2000a) revealed that tiller production gradually decreased with increased levels of salinity. In case of variety BR11, more than 30 per cent reduction of effective tillers was observed at 150 mM NaCl treatment compared to control.

Misra *et al.* (1996) observed the effect of salinity on seed germination, shoot and root length, seedling vigor index (SVI) and increase in the root: shoot length ratio in the laboratory was relatively more in cv. Jaya than in cv. Damodar. The relative susceptibility to salinity was more in cv. Jaya than in cv. Damodar in the field also. However, their responses varied with growth period. The root: shoot length and fresh and dry weight ratios increased with salinity at 15 days in cv. Jaya. The root: shoot fresh and dry weight ratio decreased with

salinity at 15 days in cv. Damodar. However, the root: shoot fresh weight ratio decreased with salinity at 25 days in the susceptible cv. Jaya. The root and shoot length fresh and dry weight of cv. Damodar was enhanced at 0.5% (w/v) NaCl treatment compared to the control seedlings at 25 days. SVI in cv. Jaya decreased with salinity in the laboratory and field conditions. SVI showed little change at 15 days but decreased with salinity of 1-3% NaCl with an enhancement at 0.5% NaCl level in the laboratory and at 25 days in field conditions.

The possible involvement of activated oxygen species in the mechanism of damage by NaCl stress was studied in leaves of four varieties of rice (*Oryza sativa* L.) exhibiting different sensitivities to NaCl (Maribel *et al.* 1998). The 3-week-old rice seedlings were subjected to 0, 6 and 12 dS m⁻¹ salinity levels for 1-week after which differences in antioxidant capacities and possible correlation, growth rate and Na₊ uptake of the leaves were analyzed. High salinity treatment caused a decrease in growth rate in all the varieties tested except Pokkali. The salt-sensitive varieties, Hitomebore and IR28, exhibited a decrease in superoxide dismutase activity and an increase in peroxidase activity under high salinization. These varieties also exhibited increase in lipid peroxidation and electrolyte leakage as well as higher Na⁺ accumulation in the leaves under salt stress. The salt-tolerant variety Pokkali however, showed only slight increase and decrease in superoxide dismutase and peroxidase activity, respectively, and virtually unchanged lipid peroxidation, electrolyte leakage and Na⁺ accumulation upon salinization. On the other hand, the putative salt-tolerant Bankat variety, which showed a slight stimulation in growth rate similar to Pokkali at moderate salinity level, exhibited Na₊ accumulation and symptoms of oxidative damage during salt stress similar to the salt-sensitive varieties rather than the salt-tolerant one. These results indicate that free radical-mediated damage of membrane may play an important role in the cellular toxicity of NaCl in rice seedlings and that salt-tolerant varieties exhibit

protection mechanism against increased radical production by maintaining the specific activity of antioxidant enzymes

The effect of varying (0–200 mM NaCl) salt stress on two popular scented non basmati type *indica* rice cultivars, namely Indrayani and Ambemohar on germination and growth and biochemical parameters was observed by Tambhale *et al.* (2011). Salt stress-induced proline accumulation was observed in both the cultivars, however, with much higher extent of proline accumulation in Ambemohar than Indrayani. A salinity stress of 200 mM NaCl resulted into 305% higher proline content than the control plants of Ambemohar against 222% higher proline in Indrayani at the same stress level. Similarly protein content was also higher in Ambemohar than Indrayani at the highest stress level used in this study. Contrasting results were seen in terms of starch content amongst both the cultivars, where continuous decrease with increasing salt stress was observed in Indrayani, on the other hand, an increase in starch content was evident in Ambemohar under the influence of NaCl-induced salt stress. These finding clearly indicates the comparably higher salt tolerant nature of Ambemohar than Indrayani which might be attributed to higher proline, protein and starch content than Indrayani cultivar under salt stress.

Aref (2013) studied on the effect of different growth stages on all yield components except number of tillers was significant. Different growth stages showed different sensitivity to salinity. In fact, the primitive growth stages, that is, tillering and panicle initiation showed more sensitivity to salinity than final growth stages (panicle emergence and ripening). Therefore, irrigation with saline water at the early growth stages has more negative effect on yield and its components.

The results revealed that the studied morphological traits such as plant height, tillers plant⁻¹, leaves plant⁻¹, leaf length and plant dry and the physiological attributes, chlorophyll *a*, *b*, total chlorophyll contents, photosynthetic rates,

stomatal conductance and transpiration rate were reduced significantly with increasing saline condition in both of varieties. The transpiration rate was also reduced in both varieties, which showed less intercellular CO₂ at higher salinity. Identical findings were also noted for the vapor pressure deficit in leaves (VPDL). MR219 showed more salt affected than Pokkali in some parameters but the saline effects alleviated when GA3 applied. The present study concludes that GA3, a safe plant growth regulator, could be effectively sprayed on rice variety MR219 in saline belts as it adequately proved its unique salinity alleviating role (Khadija *et al* 2013).

There was significant variation between genotypes for all the characters of 4 commercial varieties and 17 breeding lines of Basmati rice (*Oryza sativa* L.) studied. On an average, plant height, number of tillers per plant, panicle length, number of grains per panicle, shoot dry weight, grain straw ratio, grain yield per plant, K content of shoot and K/Na ratio were reduced linearly while grain sterility and Na content of shoot were increased with increasing soil salinity. With increased salinity, reduced number of grains per panicle was mainly found responsible for reduction in grain yield. Generally genotypes having ability to exclude Na from shoot were found salt tolerant in respect of grain yield and *vice versa*. Na contents of shoot and shoot dry weight 45 days after sowing (DAS) showed significant correlations with grain yield. It is suggested that selection for salinity tolerance in rice can be carried out at an early stage of growth (Mahmood *et al.*, 2009).

Jamil *et al.* (2012) observed that there was a regular decrease in seed germination and seedling growth raised in Petri dishes for ten days with increasing salt concentration. Highest germination was observed in NIAB-IR 9 and Shaheen Basmati as compared to Basmati-385. A marked reduction in the protein content of the rice plants under stress was observed with increasing salt concentrations. The effect was more prominent in Shaheen Basmati as compared to Basmati-385 and NIAB-IR 9. It was concluded on the basis of

physiological and biochemical characteristics that Shaheen Basmati is more sensitive to salt stress as compared to Basmati-385 and NIAB-IR 9.

Rice varieties MR211, IR20, BR40 and MR232 showed greater salt tolerance during germination (germinated at 12 dS m⁻¹ salinity). However, MR211, MR232 and IR20 performed better based on dry matter yield reduction. The result suggested that MR211, MR232 and IR20 might be used for further study of salinity effect on growth processes and physiological consequences at advanced stage of growth, since salt tolerance of a crop at germination and early seedling stage may not correspond to that at advanced stage (Hakim *et al.*, 2010)

Anbumalarmathi and Preeti (2013) reported that the response of eight *indica* rice varieties against six salinity levels (0, 4, 8, 12, 16 and 20 dS m⁻¹) was studied at germination and early seedling growth stage. Germination was completely arrested in six varieties at 20 dS m⁻¹ salt concentration. Rice varieties ADT43, IR50, and MDU5 showed greater salt tolerance during germination (germinated at 12 dS m⁻¹ salinity).

The contents of polyamines, especially supermidine, were high in the pre-stressed leaf blades of NERICA rice seedlings. After the salt-stress treatment, the polyamine content of leaf blades differed with the degree of salt tolerance of the NERICA rice seedlings. These results suggested that the salt tolerance of NERICA rice seedlings might be associated not only with the regulation of Na absorption and translocation but also with their ability to maintain leaf polyamine levels under salt-stress conditions (Yamamoto *et al.*, 2011)

Kazemi and Eskandari stated that the three rice cultivars (Anbar, LD and Hamar) were significantly ($P \leq 0.05$) affected by salt stress, where germination, plumule and radicle length and weight were decreased with increasing in salt concentration. The extent of these reductions was related with the variations in rice cultivar under different salt stress condition. By increasing NaCl

concentration, seed germination delayed and decreased in all cultivars. Regarding the relationship between speed of germination and seed vigor, salt stress decreased seed vigor of rice cultivars LD a superior cultivar under all salt stress which can be suggested for cultivation under salinity condition.

BRRRI dhan47 and Binadhan-10 were treated with five concentrations of NaCl, viz., 0, 4, 8, 12, and 16 dSm⁻¹. Result indicated that plant height, number of effective tiller hill⁻¹, number of non effective tiller hill⁻¹, number of field grain panicle⁻¹, number of unfilled grain panicle⁻¹, panicle length and grain yield hill⁻¹ were influenced at different levels of salinity. The number of effective tiller hill⁻¹, panicle length, number of filled grain panicle⁻¹ and grain yield hill⁻¹ were significantly decreased with the increased levels of salinity. It was found that the K content in shoot was decreased with the increased levels of salinity. The highest K content (1.77 %) in shoot was found in Binadhan-10 at 0 dSm⁻¹. The highest Na content (1.69 %) in shoot was found in BRRRI dhan47 at 12 dSm⁻¹. Between these two varieties Binadhan-10 showed better performance at salinity stress up to a certain level except plant height (Sultana *et al.*, 2014)

Hasamuzzaman *et al.* (2009) observed that seed germination, plant height, tiller number and leaf area index are negatively influenced by different salinity levels in all the rice varieties. All the yield components that are number of panicles, panicle length, spikelets per panicle, filled grain and grain weight also significantly decrease with the increased salinity stress. An increase of NaCl concentration up to 150 mM decreased 36-50% of the grain yield of all the four rice varieties (BR11, BRRRI dhan41, BRRRI dhan44 and BRRRI dhan46). Among the varieties BRRRI dhan41 showed better performance at salinity stress up to a certain level.

An experiment showed that increase in salinity levels of irrigation water significantly decreased length of filled panicle, number of filled grains per filled panicle, number of spikelets per filled panicle and total number of

spikelets per panicles but effect of different salinity levels on percentage of ratio of filled panicle number to tiller number and percentage of ratio of yield to straw weight was not significant. The least of these yield components were observed at the highest salinity level (8 dS/m). In different growth stages of rice, all yield components were different. Final growth stages, i.e., panicle emergence and ripening showed less sensitivity to salinity but primary stages, i.e., tillering and panicle initiation were more sensitive to salinity. Therefore, irrigation with saline water can be used in the final stages of plant growth, i.e. panicle emergence and ripeness. (Rad *et al.*, 2012).

According to Kapoor (2011), 50 and 100 mM salt solution significantly decreased seed germination, seedling length, vigour index and biomass of *Oryza sativa* in comparison to control. A significant reduction in chlorophyll and protein contents was also observed in seedlings of *Oryza sativa* with 100 mM NaCl concentration at 72 h. The degree of toxicity was proportional to the NaCl concentration and exposure period. Higher concentration of NaCl (100 mM) exhibited 81.75% and 79.78% reduction in chlorophyll and protein contents in the leaves of *Oryza sativa* at 72 h.

The response of rice to salinity varies with growth stage. In the most commonly cultivated rice cultivars, young seedlings were very sensitive to salinity (Pearson and Bernstein, 1959; Kaddah, 1963; Flowers and Yeo, 1981; Heenan *et al.*, 1988; Lutts *et al.*, 1995). Yield components related to final grain yield were also severely affected by salinity. Panicle length, spikelet number per panicle, and grain yield were significantly reduced after salt treatments. (Sajjad, 1984; Heenan *et al.*, 1988; Cui *et al.*, 1995; Khatun *et al.*, 1995). Salinity also delayed the emergence of panicle and flowering (Khatun *et al.*, 1995) and decreased seed set through reduced pollen viability (Khatun and Flowers, 1995; Khatun *et al.*, 1995). In contrast, rice was more salt tolerant at germination than at other stages (Narale *et al.*, 1969; Heenan *et al.*, 1988; Khan *et al.*, 1997). Seed germination was not significantly affected up to 16.3 dS m⁻¹,

but was severely inhibited when salinity increased to 22 dS m⁻¹ (Heenan *et al.*, 1988). The suppression of germination at high salt levelst might be mainly due to osmotic stress (Narale *et al.*, 1969; Heenan *et al.*, 1988).

Dry weight of root, shoot and yield significantly decreased with the increase of salinity levels, while MR232 and MR211 were less affected. Na⁺ ions accumulations increased in the root and shoot with the increase of salinity, while the lowest accumulation was in MR211. Na⁺/ K⁺ ratio sharply increased in the root with increasing the salinity. Whereas, Ca⁺⁺/Na⁺ and Mg⁺⁺/Ca⁺⁺ ratio showed decreasing trend with increasing salinity level. The maximum amount of nitrogen and phosphorous accumulation was observed in the shoot of MR211, while Na⁺ in BRRI dhan29, K⁺ in Pokkali. The highest accumulation of Na⁺ and K⁺ observed in the root of MR219. The maximum Ca⁺⁺ and Mg⁺ were found in MR33 and MR211, respectively. Considering all, genotypes MR211 andMR232 were found to be relatively tolerant to salt than the other genotypes (Hakim *et al.*, 2014).

Chapter 3

MATERIALS AND METHODS

This chapter presents a brief description about experimental period, site description, climatic condition, crop or planting materials, treatments, experimental design and layout, crop growing procedure, fertilizer application, uprooting of seedlings, intercultural operations, data collection and statistical analysis.

3.1 Location

The experiment was conducted at the Experimental shed of the Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka during the period from June to December, 2014. The location of the experimental site has been shown in Appendix I.

3.2 Soil

The soil of the experimental area belonged to the Modhupur tract (AEZ No. 28). It was a medium high land with non-calcareous dark grey soil. The pH value of the soil was 5.6. The physical and chemical properties of the experimental soil have been shown in Appendix II.

3.3 Climate

The experimental area was under the subtropical climate and was characterized by high temperature, high humidity and heavy precipitation with occasional gusty winds during the period from April to September, but scanty rainfall associated with moderately low temperature prevailed during the period from October to March. The detailed meteorological data in respect of air temperature, relative humidity, rainfall and sunshine hour recorded by the

meteorology center, Dhaka for the period of experimentation have been presented in Appendix III.

3.4 Materials

3.4.1 Plant materials

Six rice varieties BRRRI dhan34, BRRRI dhan38, Binadhan-9, Binadhan-13, Kalijira and Rajbhogh were used in the experiment. The features of six varieties are presented below:

BRRRI dhan34: BRRRI dhan34 is an aromatic rice variety. It released by Bangladesh Rice Research Institute (BRRRI) in 1997. It completes its life cycle in 135 DAS. It attains a plant height 117 cm. 1000-seed weight is 24 g and yield is 3.5 t ha⁻¹.

BRRRI dhan38: BRRRI dhan38 is an aromatic rice variety. It released by Bangladesh Rice Research Institute (BRRRI) in 1998. It completes its life cycle in 140 DAS. It attains a plant height 125 cm. 1000-seed weight is 24 g and yield is 3.5 t ha⁻¹.

Binadhan-9: It released by Bangladesh Institute of Nuclear Agriculture (BINA) in 2012. It completes its life cycle in 118-123 DAS. It attains a plant height 100-110 cm. 1000-seed weight is 13 g and yield is 3.5-4.15 t ha⁻¹.

Binadhan-13: It released by Bangladesh Institute of Nuclear Agriculture (BINA) in 2014. It completes its life cycle in 138-142 DAS. It attains a plant height 100-110 cm. 1000-seed weight is 13.2 g and yield is 3.2-3.6 t ha⁻¹.

Kalijira: It was collected from mymensingh region. It is popular local aromatic rice. It completes its life cycle in 138-142 DAS. It attains a plant height 100-110 cm. 1000-seed weight is 14 g and yield is 3.2-3.6 t ha⁻¹.

Rajbhogh: It was collected from mymensing region. It is popular local aromatic rice. It completes its life cycle in 138-142 DAS. It attains a plant height 130 cm. 1000-seed weight is 20 g and yield is 3.2-3.6 t ha⁻¹.

3.4.2 Earthen pot

Empty earthen pots with 18 inch depth were used for the experiment. Twelve kilogram sun-dried soils were put in each pot. After that, pots were prepared for seed sowing.

3.5 Salinity treatment

The salinity treatments were applied on 30 DAT, 45 DAT, 60 DAT and 75 DAT. There were five salinity levels including control where developed by adding respected amount commercial NaCl salt to the soil/pot as water dissolved solution. The salinity levels were C (control), S25 (25 mM), S50 (50 mM), S75 (75 mM) and S100 (100 mM). When no salt added in control (C) while 87.5g salts in S25, 175.5g salts in S50, 263.5.5g salts in S75 and 351g salts in S100 added in each pot. In order to spread homogenously in each pot. The salts were dissolved in 60 liter water and were added to pots for proper salinity imposition.

3.6 Treatments

The experiment consisted of two factors as mentioned below:

- a) Factor A: varieties
 - i. BRRI dhan34
 - ii. BRRI dhan38

- iii. Binadhan-9
- iv. Binadhan-13
- v. Kalijira
- vi. Rajbhogh

b) Factor B: Salinity level

- vii. Control (C)
- viii. 25 mM NaCl (N25)
- ix. 50 mM NaCl (N50)
- x. 75 mM NaCl (N75)
- xi. 100 mM NaCl (N100)

3.7 Design and layout

The experiment was laid out in Randomized Completely Block Design (RCBD) with three replications. There were 90 pots all together replication with the given factors. Two separate sets of pots were palced for growth and yield analysis.

Conduction of the experiment

3.8 Seed collection

Seeds of BRRI dhan34 and BRRI dhan38 were collected from Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur. Binadhan-9 and Binadhan-13 were collected from Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. Seeds of kalijira and Rajbhog were collected from Mymensingh region.

3.9 Pot Preparation

The collected soil was sun dried, crushed sand sieved. The soil and fertilizers were mixed well before placing the soils in the pots. Soils of the pots were poured in polythene bag. Each pot was filled up with 12 kg soil. Pots were placed at the net house of Sher-e-Bangla Agricultural University. The pots were pre-labeled for each variety and treatment. Finally, water was added to bring soil water level to field capacity.

3.10 Fertilizer Application

The nitrogenous, phosphatic, potassic and sulphur fertilizer were applied in the experimental pots @ 250 kg ha⁻¹, 110 kg ha⁻¹, 140 kg ha⁻¹, 50 kg ha⁻¹ in the form of urea, triple super phosphate, muriate of potash and gypsum, respectively. One-third of urea and the whole amount of other fertilizers were incorporated with soil at final pot preparation before sowing. Rest of the nitrogen were applied in two equal splits one at 30 days after transplanting (DAT) and second at 45 days after transplanting (DAT).

3.11 Sowing of seeds in seedbed

Previously collected seeds were soaked for 48 hours and then washed thoroughly in fresh water and incubated for sprouting, the sprouted seeds sown in the wet seedbed on June 23, 2014.

3.12 Uprooting and transplanting of seedlings

Seedlings of thirty days old were uprooted carefully from the seedbed and transplanted in the respective pots at the rate of single seedling hill⁻¹ on July 23, 2014.

3.13 Intercultural operations

3.13.1 Weeding and irrigation

Sometimes there were some small aquatic weeds observed in pots that were uprooted by hand pulling. About 3-4 cm depth of water was maintained in the pot until the crop attained maturity although a 2-3 days drying period was provided after tillering stage to suppress the weeds.

3.13.2 Plant protection measures

Before heading green leafhopper infestations were observed in the crop and they were successfully controlled by applying Durshban two times on 42 DAT and 52 DAT at 20ml/10L of water. Rice stem borer also attacked and it was controlled by the application of Furadan 5G at 2.5 g/pot. From heading onwards, the pots were netted to protect the rice grain from the attack of birds.

3.14 General observation of the experimental pots

Observations were made regularly and the plants looked normal green. No Lodging was observed at any stage. The maximum tillering, panicle initiation, and flowering stages were not uniform.

3.15 Detecting maximum tillering and panicle initiation stages

Maximum tillering and panicle initiation stages were detected through field observations. When the number of tiller hill⁻¹ attained the highest number and there after decreasing in trend, was indicated as maximum tillering stage. When a small growth at the top of upper most nodes of main stem was noted like a

dome indicated the beginning of panicle initiation stage. These stages were not uniform. These were varied with varieties as well as salt treatments.

3.16 Collection of data

Data were recorded on the following parameters:

1. Phenological parameters

- Days to flowering
- Days to grain formation
- Days to maturity

2. Crop growth parameters:

- Plant height (cm) at 15 days interval up to harvest
- Tiller no.plant⁻¹ at 15 days interval up to harvest

3. Physiological parameters:

- Chlorophyll content
- Relative water content (RWC)

4. Yield contributing parameter:

- Panicle length,
- Number of grains per panicles,
- Number of filled grains per panicle,
- number of unfilled grains per panicle,
- 1000-grain weight,
- Number of effective tiller per plant
- Number of non-effective tiller per plant

5. Yields:

- Grain yield pot⁻¹
- Straw yield pot⁻¹

3.17 Procedure of sampling for growth study during the crop growth period

3.17.1 Plant height

The height of the rice plants was recorded from 30 days after transplanting (DAT) at 15 days interval up to 90 DAT, beginning from the ground level up to tip of the leaf was counted as height of the plant. The average height of three plants was considered as the height of the plant for each pot.

3.17.2 Tiller no. plant⁻¹

Total tiller number was taken from 30 DAT at 15 days interval up to 90 DAT. The average number of tillers of three plants was considered as the total tiller no plant⁻¹.

3.18 Procedure of sampling phonological parameters

3.18.1 Chlorophyll content

Three leaflets were randomly selected from each pot. The top and bottom of each leaflet were measured with atLEAF as atLEAF value. Then it was averaged and total chlorophyll content was measured by the conversion of atLEAF value into SPAD units and then totals chl content.

3.18.2 Relative water content (RWC)

Three leaflets were randomly selected from each pot and cut with scissors. Relative water content (RWC) was measured according to Barrs and Weatherley (1962). Leaf laminas were weighed (fresh weight, FW) and then immediately floated on distilled water in a petridish for 4 h in the dark. Turgid weights (TW) were obtained after drying excess surface water with paper

towels. Dry weights (DW) were measured after drying at 80⁰C for 48 h. Then calculation was done using the following formula:

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

3.19 Procedure of sampling yield contributing parameter

3.19.1 Plant height

Plant height was measured from the soil level to the apex of the leaf in randomly 5 plants of each pot.

3.19.2 Effective tillers plant⁻¹

The total number of tillers plant⁻¹ was counted from selected samples and were grouped in effective and non-effective tillers plant⁻¹.

3.19.3 Panicle Length

Panicle length was recorded from the basal nodes of the rachis to apex of each panicle.

3.19.4 Number of total grain per panicle

Grains of 10 randomly selected panicle of each replication were counted and then the average number of grains for each panicle was determined.

3.19.5 Grain yield per pot

The grains were separated by threshing per plant and then sun dried and weighed.

3.19.6 Straw yield per pot

The straw were separated by threshing per plant and weighed.

3.19.7 1000-grain weight

One thousand clean sun dried grains were counted from the seed stock obtained from the sample plants and weighed by using an electronic balance.

3.20 Statistical analysis

The data obtained for different parameters was statistically analyzed following computer based software XLSTAT 2014 (AddinSoft, 2014) and mean separation will be done by LSD at 5% level of significance.

Chapter 4

RESULTS AND DISCUSSION

4.1 Crop growth parameters

4.1.1 Plant height

4.1.1.1 Effect of variety

Plant height of the varieties was measured at different growing period (Table 1). The highest plant height was found in BRRI dhan38 at all growth duration except 90 DAT (97.98 cm at 30 DAT, 108.41 cm at 45 DAT, 110.51 cm at 60 DAT and 113.71 cm). However, highest plant height also observed in BRRI dhan34 at 75 DAT (112.33 cm) and at 90 DAT (119.05 cm); in Rajbhog at 30 DAT (97.98 cm). The plant height of BRRI dhan34 at 90 DAT and Rajbhog at 60 DAT, 75 DAT, and 90 DAT were statistically similar. This result was supported by Hossain and Sikdar (2009).

4.1.1.2 Effect of salinity level

Plant height differed significantly at different salinity treatments (Table 1). It was significantly greater at control than the other salinity level. At 30 DAT, plant height was the highest at control (93.10 cm) which was statistically similar to that of 25 mM salinity (90.53 cm). The lowest plant height (82.42 cm) was recorded at 100 mM salinity which was statistically to that of 75 mM (86.16 cm) salinity. The decreasing trends of the plant height were found with increasing salinity at 45 DAT and 90 DAT. Gradual decreased in plant height might be due to the nutrients availability caused by increased salinity or plant height might be decreased in the salt stress due to the inhibition of cell division

or cell enlargement. Salinity has direct effect in plant height. Choi *et al.* (2003) observed that the plant height decreased in the 0.5% saline water in the soil.

Table 1. Effect of variety and salinity level on plant height of rice at different days after transplanting.

Variety	Plant Height (cm)				
	30 DAT	45 DAT	60 DAT	75 DAT	90 DAT
BRR1 dhan34	87.59bc	98.95bc	104.82bc	112.33a	119.05a
BRR1 dhan38	97.98a	108.41a	110.51a	113.71a	118.31ab
Binadhan-9	88.79bc	98.77bc	104.19bc	111.00a	113.91bc
Binadhan-13	85.56 c	95.90 c	100.32c	105.69b	110.27c
Kalijira	77.84d	87.20d	92.55d	99.41c	103.16 d
Rajbhogh	97.98a	101.88 b	108.77ab	109.34ab	117.85ab
LSD_(0.05)	4.18	4.90	5.11	5.06	5.01
Salinity level					
C	93.10a	103.11a	108.85a	114.49a	119.38a
N ₂₅	90.53ab	100.94a	105.6a	111.24a	116.28ab
N ₅₀	89.02bc	98.73ab	104.27ab	110.35a	113.97bc
N ₇₅	86.16cd	96.41bc	100.93bc	103.66 b	111.46cd
N ₁₀₀	82.42d	93.41c	97.96c	103.16b	107.70d
LSD_(0.05)	3.82	4.4776	4.6733	4.6273	4.5811
CV (%)	6.49	6.81	6.77	6.39	6.04

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.1.1.3 Interaction effect of variety and salinity level

The combined effect of salinity and varieties on plant height was significant at $p \leq 0.05$ (Table 2). Plant height generally decreased with the increasing level of salinity. In interaction effects plant height was highest at control in BRR1 dhan38 and lowest plant height was recorded at 100 mM salinity levels distinctly influenced plant height in Kalijira. Similar results were recorded by Khandakar and Alim (2004). Chen *et al.* (1989) reported that the plant height seriously decreased by salinity.

Table 2. The combined effects of salinity and Aman rice varieties on plant height

Variety	Salinity treatment	Plant height (cm)				
		30 DAT	45 DAT	60 DAT	75 DAT	90 DAT
BRRi dhan34	C	93.07a-g	103.07abc	109.83a-f	114.43a-e	122.73abc
	N ₂₅	89.90c-h	100.63bc	106.50a-f	112.67a-f	122.07abc
	N ₅₀	89.13 c-h	99.13bcd	106.13a-f	112.43a-f	119.73a-e
	N ₇₅	83.80g-k	96.40cde	101.87d-i	111.60a-f	118.30a-e
	N ₁₀₀	82.07 h-l	95.53c-f	99.77 f-j	110.50a-g	112.40c-i
BRRi dhan38	C	101.90a	112.00a	116.73a	118.10a	124.50a
	N ₂₅	101.00ab	112.30a	113.70ab	118.10a	120.60a-d
	N ₅₀	97.60abc	107.63ab	111.27a-e	116.53abc	117.30a-f
	N ₇₅	96.63a-d	105.77abc	106.53a-f	109.50a-h	115.30a-g
	N ₁₀₀	92.80a-g	104.33abc	104.30 b-g	106.23b-i	113.83a-h
Binadhan-9	C	91.80b-g	101.90abc	107.03a-f	114.73a-d	118.40a-e
	N ₂₅	89.17c-h	99.37bcd	104.37b-g	114.93a-d	118.20a-e
	N ₅₀	88.83c-h	98.40bcd	104.60 b-g	114.34a-e	116.53a-f
	N ₇₅	88.43c-h	98.47bcd	103.90b-h	106.53b-i	109.50d-j
	N ₁₀₀	85.73f-j	95.73c-f	101.07d-i	104.47d-i	106.90f-k
Binadhan-13	C	90.43c-h	100.40bc	106.07a-f	116.97ab	116.03a-g
	N ₂₅	89.67c-h	99.63bc	103.70c-h	103.20e-i	113.83a-h
	N ₅₀	86.8e-i	97.33b-e	100.40e-j	110.23a-g	110.00d-j
	N ₇₅	85.47f-j	95.33c-g	98.90f-k	98.53hij	108.97e-j
	N ₁₀₀	75.43kl	86.80e-h	92.53h-k	99.53g-j	102.53ijk
Kalijira	C	85.70f-j	95.43c-g	100.83e-j	108.50a-h	105.27g-k
	N ₂₅	78.10 i-l	88.50d-h	93.17h-k	105.23c-i	102.97h-k
	N ₅₀	77.30j-l	85.03fgh	91.80ijk	95.87ij	99.60jk
	N ₇₅	74.77kl	84.53gh	89.4jk	90.97j	102.97h-k
	N ₁₀₀	73.27l	82.50 h	87.50k	96.47ij	97.47 k
Rajbhogh	C	95.73a-e	105.83abc	112.60abc	114.23a-e	124.13ab
	N ₂₅	95.37a-e	105.23abc	112.30a-d	113.20a-e	117.70a-f
	N ₅₀	94.40a-f	104.83abc	111.40 a-e	112.67a-f	117.27 a-f
	N ₇₅	87.87d-h	97.93bcd	104.97b-f	104.83 d-I	117.07a-f
	N ₁₀₀	85.23f-j	95.5c-f	102.57b-i	101.77f-j	113.07b-i
LSD (0.05)		9.36	10.96	11.44	11.33	11.22
CV (%)		6.49	6.81	6.77	6.39	6.04

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.1.2 Tillers hill⁻¹

4.1.2.1 Effect of variety

There was a significant variation found in number of tillers hill⁻¹ due to varietal variation (Table 3). The highest number of tillers hill⁻¹ found at 30 DAT (4.76), at 75 DAT (25.27) and at harvest (20.51) in case of Binadhan-9; at 45 DAT (10.13) and at 60 DAT (16.03) in case of BRRI dhan38 and at 45 DAT (10.03) in case of Binadhan-13. However, the lowest number of tillers hill⁻¹ observed at 30 DAT (3.61) in BRRI dhan34; 45 DAT (7.14), 60 DAT (12.59), 90 DAT (13.47) and harvest (12.02) in Kalijira; at 75 DAT (18.66) in Binadhan-13.

Table 3. Effect of variety and salinity level on number of tillers hill⁻¹ of rice at different days after transplanting.

Variety	Number of tillers hill ⁻¹					
	30 DAT	45 DAT	60 DAT	75 DAT	90 DAT	At Harvest
BRRI dhan34	3.61e	7.52c	14.22c	20.02d	16.28d	15.36d
BRRI dhan38	4.76c	10.13a	16.03a	21.78c	17.56c	17.38c
Binadhan-9	5.52a	9.51b	15.11b	25.27a	20.69a	20.51a
Binadhan-13	5.14b	10.03a	13.68c	18.66e	14.91e	14.07e
Kalijira	3.98d	7.14d	12.59d	23.54b	13.47f	12.02f
Rajbhogh	4.70c	9.57b	13.96c	20.02d	18.37b	19.31b
LSD (0.05)	0.20	0.41	0.65	0.77	0.73	0.67
Salinity level						
C	7.34a	14.52a	20.21a	26.64a	21.45a	19.54a
N ₂₅	6.32b	11.98b	18.37b	24.76b	19.53b	18.29b
N ₅₀	4.61c	8.96c	13.98c	20.58c	17.03c	16.41c
N ₇₅	2.98d	5.66d	10.50d	17.52d	14.21d	14.78d
N ₁₀₀	1.83e	3.79e	8.27e	14.60e	12.17e	13.20e
LSD (0.05)	0.19	0.38	0.59	0.708	0.66	0.61
CV (%)	6.21	6.38	6.25	5.11	5.92	5.62

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.1.2.2 Effect of salinity level

Application of saline decreased the number of tillers hill⁻¹ of rice varieties (Table 3). It was significantly greater at the control than in the other salinity level. The highest number of tillers hill⁻¹ was recorded in control at all growing period (7.34 at 30 DAT, 14.52 at 45 DAT, 20.21 at 60 DAT, 26.64 at 75 DAT, 21.45 at 90 DAT and 19.54 at harvest). After the increasing the salinity levels the number of tillers hill⁻¹ drastically reduced. So the lowest number of tillers hill⁻¹ found in 100 mM salinity level at all growth period. It was observed that number of productive tillers hill⁻¹ decreased with increase in salinity levels (Sajjad, 1984; Heenan and Lewin, 1998 and Hasamuzzaman *et al.*, 2009).

4.1.2.3 Interaction effect of variety and salinity level

The interaction effect of salinity levels and cultivars in relation to the number of tillers hill⁻¹ was found significant ($p \leq 0.05$) (Table 4) at all growing period. The highest number of tillers hill⁻¹ was obtained from Binadhan-9 at control condition with the increase of growing period (8.33 at 30 DAT, 17.30 at 45 DAT, 22.43 at 60 DAT, 31.55 at 75 DAT, 25.17 at 90 DAT and 23.02 at harvest). Sometimes BRRI dhan38 at 30 DAT and Binadhan-13 at 30 DAT obtained highest tillers hill⁻¹. The lowest number of tillers hill⁻¹ was obtained from Kalijira at 100 mM saline condition with the increase of growing period (1.17 at 30 DAT, 3.11 at 45 DAT, 7.51 at 60 DAT, 10.43 at 75 DAT, 8.45 at 90 DAT and 8.29 at harvest). Sometimes BRRI dhan38 at 30 DAT and Binadhan-13 at 30 DAT obtained highest tillers hill⁻¹. Islam *et al* (2007) found the same result that increasing salinity levels negatively effects on the number of tillers hill⁻¹.

Table 4. The combined effects of salinity and Aman rice varieties on plant height

Variety	Salinity treatment	Number of tillers hill ⁻¹					
		30 DAT	45 DAT	60 DAT	75 DAT	90 DAT	At Harvest
BRRRI dhan34	C	6.16c	13.30c	20.63b	25.85de	20.52de	18.97cd
	N ₂₅	5.28d	10.37efg	18.64cd	23.13f	18.98ef	16.33gh
	N ₅₀	3.15f	6.57j	13.45fg	19.8hi	17.183ghi	15.22hi
	N ₇₅	2.30g	4.04lm	11.40hi	17.50 j	13.43lm	13.43jkl
	N ₁₀₀	1.16h	3.25lmn	7.00k	13.70k	11.30no	12.88jkl
BRRRI dhan38	C	8.00a	14.70b	20.54b	27.92bc	22.40bc	20.32bc
	N ₂₅	7.08b	13.40c	19.50bc	25.63e	19.66ef	18.46def
	N ₅₀	5.11d	10.39ef	17.26de	21.80fg	18.46fg	17.52defg
	N ₇₅	2.26g	7.02j	12.28gh	18.61ij	14.56kl	16.22gh
	N ₁₀₀	1.33h	5.16k	10.59ij	14.95k	12.73mn	14.37ij
Binadhan-9	C	8.33a	17.30a	22.43a	31.55a	25.517a	23.02a
	N ₂₅	7.01b	11.54d	19.72bc	29.32b	23.63b	22.53a
	N ₅₀	5.18d	9.45ghi	13.57fg	25.51e	19.51ef	20.85b
	N ₇₅	4.05e	5.18k	10.37ij	22.53f	18.22f-i	18.88cde
	N ₁₀₀	3.04f	4.08l	9.46j	17.44j	16.60ij	17.29fg
Binadhan-13	C	8.29a	14.69b	20.23b	23.37f	18.55fg	17.21fg
	N ₂₅	7.17b	13.39c	17.52de	22.40f	16.82hij	16.66gh
	N ₅₀	5.12d	9.44hi	13.55fg	19.47hi	15.41jk	13.76ijk
	N ₇₅	3.10f	8.54i	9.56j	14.72k	13.31lm	12.37kl
	N ₁₀₀	2.05g	4.08l	7.53k	13.35k	10.50o	10.34m
Kalijira	C	6.13c	11.58d	17.71de	22.40f	18.67g	15.22hi
	N ₂₅	5.27d	9.57fgh	16.53e	20.54gh	16.65ij	14.13ij
	N ₅₀	5.10d	7.45j	11.47hi	13.49k	13.22lm	12.07l
	N ₇₅	2.28g	4.03lm	9.72j	11.40l	10.36o	10.33m
	N ₁₀₀	1.17h	3.11mn	7.51k	10.43l	8.45p	8.29n
Rajbhogh	C	7.17b	15.40b	19.73bc	28.73bc	23.08bc	22.49a
	N ₂₅	6.21c	13.67c	18.28cd	27.55cd	21.46cd	21.60ab
	N ₅₀	4.00e	10.50e	14.60f	23.33f	18.43fgh	19.01cd
	N ₇₅	3.90e	5.18k	9.66j	20.40gh	15.41jk	17.45efg
	N ₁₀₀	2.25g	3.06n	7.54k	17.72j	13.48lm	16.02gh
LSD (0.05)		0.46	0.93	1.45	1.73	1.63	1.51
CV (%)		6.21	6.38	6.25	5.11	5.92	5.62

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD

4.1.3 Number of Leaves hill⁻¹

4.1.3.1 Effect of variety

Number of leaves differed significantly due to the varietal modification (Table 5). BRRI dhan34 obtained the highest number of leaves at all growth duration except 30 DAT (155.03 at 45 DAT, 163.62 at 60 DAT, 145.18 at 75 DAT and 122.65 at 90 DAT). However, the lowest number of leaves found in Binadhan-9 at 30 DAT (77.09), at 45 DAT (91.83) and in Binadhan-13 at 75 DAT (94.57), at 90 DAT (94.32).

Table 5. Effect of variety and salinity level on number of leaves hill⁻¹ of rice at different days after transplanting

Variety	Number of Leaves hill ⁻¹				
	30 DAT	45 DAT	60 DAT	75 DAT	90 DAT
BRRI dhan34	111.84b	155.03a	163.62a	145.18a	143.08a
BRRI dhan38	116.95a	133.65b	147.66b	122.47c	122.65b
Binadhan-9	77.09 e	91.83e	118.63d	107.97d	116.92b
Binadhan-13	85.15d	116.05d	126.76c	94.57e	94.32c
Kalijira	92.35c	122.55c	113.62d	94.46e	99.31c
Rajbhogh	92.15c	112.97d	153.95b	136.30b	137.46a
LSD (0.05)	4.88	6.04	7.04	6.19	6.22
Salinity level					
C	114.64a	156.40a	183.22a	159.84a	156.92a
N ₂₅	105.30b	136.75b	158.83b	135.33b	135.80b
N ₅₀	95.24 c	120.29c	138.96c	116.96c	118.26c
N ₇₅	86.86d	89.17d	114.37d	92.68d	100.67d
N ₁₀₀	77.56 e	107.46e	91.47e	79.33e	83.14e
LSD (0.05)	4.45	5.52	6.43	5.65	5.68
CV (%)	6.97	6.78	7.02	7.26	7.16

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.1.3.2 Effect of salinity level

Different salinity treatments affected on the number of leaves hill⁻¹ significantly throughout the growing period. Salinity treatment reduced the number of leaves hill⁻¹ compared to control (Table 5). The highest number of leaves recorded at control from the beginning to end. (114.64 at 30 DAT, 156.40 at 45 DAT, 183.22 at 60 DAT, 159.84 at 75 DAT and 156.92 at 90 DAT). With increase of salinity level the number of leaves hill⁻¹ reduces. The lowest number of leaves hill⁻¹ found in 100 mM saline condition at all growing period. This result showed that number of leaves hill⁻¹ decreased gradually with the increasing salinity levels. Similar results was reported Dabnath (2003).

4.1.3.3 Interaction effect of variety and salinity level

Sharp decreases in number of leaves hill⁻¹ was observed in response to salt stress, compared to the control at 30, 45, 60, 75 and 90 DAT for six aman rice varieties (Table 6). The highest number of leaves hill⁻¹ was recorded in BRRI dhan34 at all days after transplanting (127.81 at 30 DAT, 194.74 at 45 DAT, 205.01 at 60 DAT, 183.06 at 75 DAT and 184.08 at 90 DAT) during control condition. But after the application of saline at different levels, the number of leaves hill⁻¹ was reduced. Kalijira showed lowest number of leaves hill⁻¹ at 100 mM saline condition (70.85 at 45 DAT, 68.57 at 60 DAT, 55.02 at 75 DAT and 62.28 at 90 DAT) which were similar in case of Binadhan-13 at 100 mM saline condition during 45 DAT and in case of Binadhan-9 at 100mM saline during 75 DAT. Alamgir and Ali (2006) found that increasing the salinity levels in different rice varieties reduces the number of leaves significantly.

Table 6. The combined effects of salinity and Aman rice varieties on number of leaves hill⁻¹

Variety	Salinity treatment	Number of leaves hill ⁻¹				
		30 DAT	45 DAT	60 DAT	75 DAT	90 DAT
BRRI dhan34	C	127.81a	194.74a	205.01a	183.06a	184.08a
	N ₂₅	125.59a	147.57cd	172.60bc	161.33bc	163.94b
	N ₅₀	108.12cd	147.23cd	166.36bc	148.25cd	137.36de
	N ₇₅	104.72c-f	144.06cd	163.71cd	121.34e	130.63efg
	N ₁₀₀	92.95g-k	141.54de	110.41jk	111.94e	99.41j
BRRI dhan38	C	131.79a	175.87b	178.91bc	165.23b	156.25bc
	N ₂₅	127.44a	140.44de	178.00bc	145.44d	153.54bc
	N ₅₀	121.58ab	128.66ef	173.28bc	142.17d	147.13cd
	N ₇₅	114.21bc	122.83ef	113.68ijk	86.90fg	84.24k
	N ₁₀₀	89.72h-l	100.44i-l	94.43lm	72.61h	72.09klm
Binadhan-9	C	86.59i-m	128.45ef	164.19cd	162.42b	161.72b
	N ₂₅	83.74j-n	108.15hij	145.84ef	117.16e	122.36fgh
	N ₅₀	83.32k-n	95.92jkl	124.43g-j	109.52e	117.68f-i
	N ₇₅	66.15pq	64.25m	87.89mn	78.92gh	113.73lm
	N ₁₀₀	65.64pq	62.36m	70.79o	71.80h	69.10ef
Binadhan-13	C	122.87ab	145.69cd	181.34b	142.39d	131.23ij
	N ₂₅	94.27f-j	140.18de	150.44de	115.55e	106.08kl
	N ₅₀	78.23mno	115.75fgh	119.65h-k	87.46fg	79.50k
	N ₇₅	67.67opq	109.53ghi	104.40kl	78.93gh	79.71l
	N ₁₀₀	62.72q	69.10m	77.95no	48.50i	75.11klm
Kalijira	C	105.72cde	157.06c	170.20bc	139.30d	146.71cd
	N ₂₅	99.96defgh	145.42cd	134.74e-h	112.86e	117.07ghi
	N ₅₀	96.20efghi	128.21ef	111.17ijk	93.07f	99.28j
	N ₇₅	85.03j-n	111.23ghi	83.43mno	72.07h	71.20kl
	N ₁₀₀	74.83nop	70.85m	68.57o	55.02i	62.28m
Rajbhogh	C	113.05bc	136.57de	199.69a	166.61b	161.52m
	N ₂₅	100.83d-g	138.71de	171.36bc	159.62bc	151.81b
	N ₅₀	83.97j-n	105.97h-k	138.89efg	121.27e	128.63bc
	N ₇₅	83.38j-n	92.88l	133.14fgh	117.92e	124.51efg
	N ₁₀₀	79.52lmn	90.72k	126.67ghi	116.09e	120.83e-h
LSD (0.05)		10.92	13.523	15.76	13.86	13.93
CV (%)		6.97	6.78	7.02	7.26	7.16

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD

4.2 Physiological parameters

4.2.1 Relative water content (%)

4.2.1.1 Effect of variety

There was significant variation in relative water content due to varietal diversity (Fig 1). Rajbhog showed the highest relative water content (82.80%) compared to other varieties. Although the relative water content of Binadhan-13 (80.44%) and Kalijira (80.84%) were statistically similar with the relative water content of highest one. However, the lowest relative water content was found in BRRI dhan34 (68.97%).

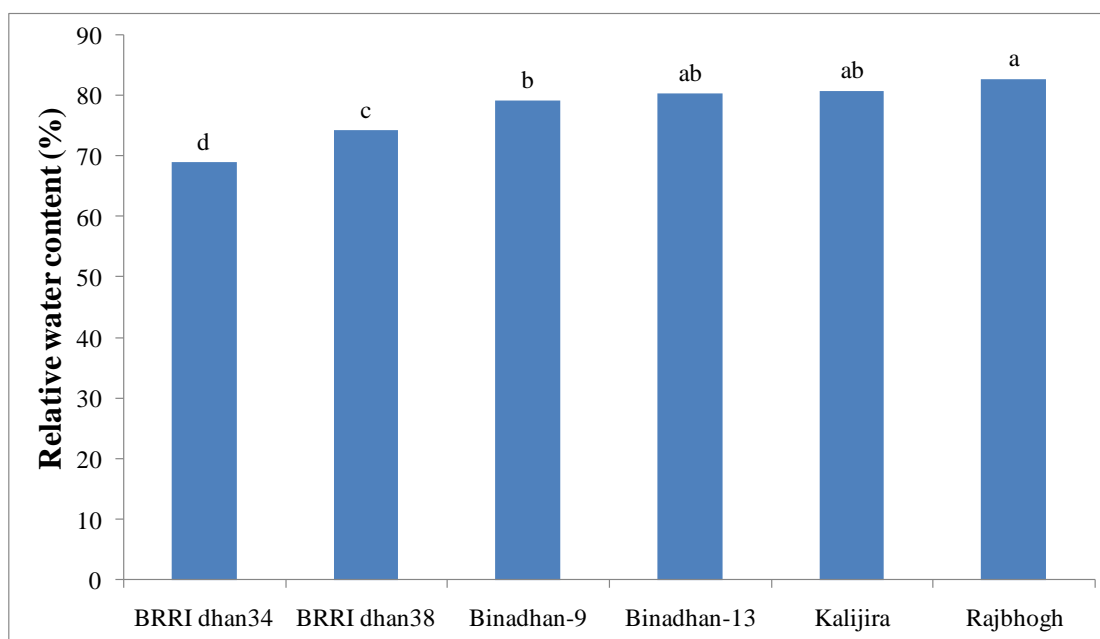


Figure 1. Effect of variety on relative water content in different aman rice cultivars.

(Bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

4.2.1.2 Effect of salinity level

The variation in relative water content among different rice cultivars was significant for different salinity levels (Fig 2). Sharp decreases in relative water content (81.44%, 80.06%, 78.58%, 76.11% and 72.59% at control, 25 mM, 50 mM, 75mM and 100mM saline condition). Similar decrease in relative water content due to salt stress was reported earlier (Vysotskaya *et al.*, 2010; Chaparzadeh and Mehrnejad, 2013). Decrease in RWC was due to loss of turgor that results in limited water availability for cell extension processes (Katerji *et al.*, 1997).

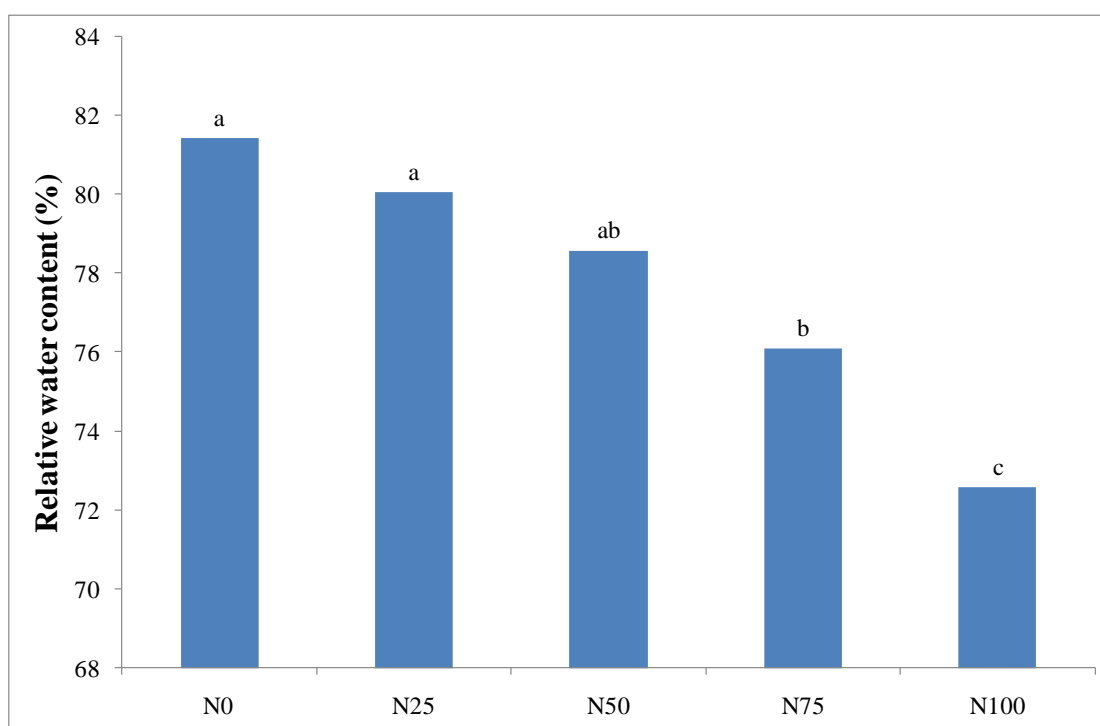


Figure 2. Effect of salinity levels on relative water content in different aman rice varieties.

Bars with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.2.1.3 Interaction effect of variety and salinity level

Upon exposure of salt stress, relative water content decreases in different rice varieties (Fig 3). The highest relative water content (87.62%) was recorded in Rajbhog which was statistically similar with Binadhan-9 (81.93%) and Binadhan-13 (84.35%). However decrease rate was lower in case of Rajbhog compared to other cultivars. Decrease in LAI might have been due to decrease in leaf expansion in salinity stress condition. This result corroborates with Bal and Dutt (1984).

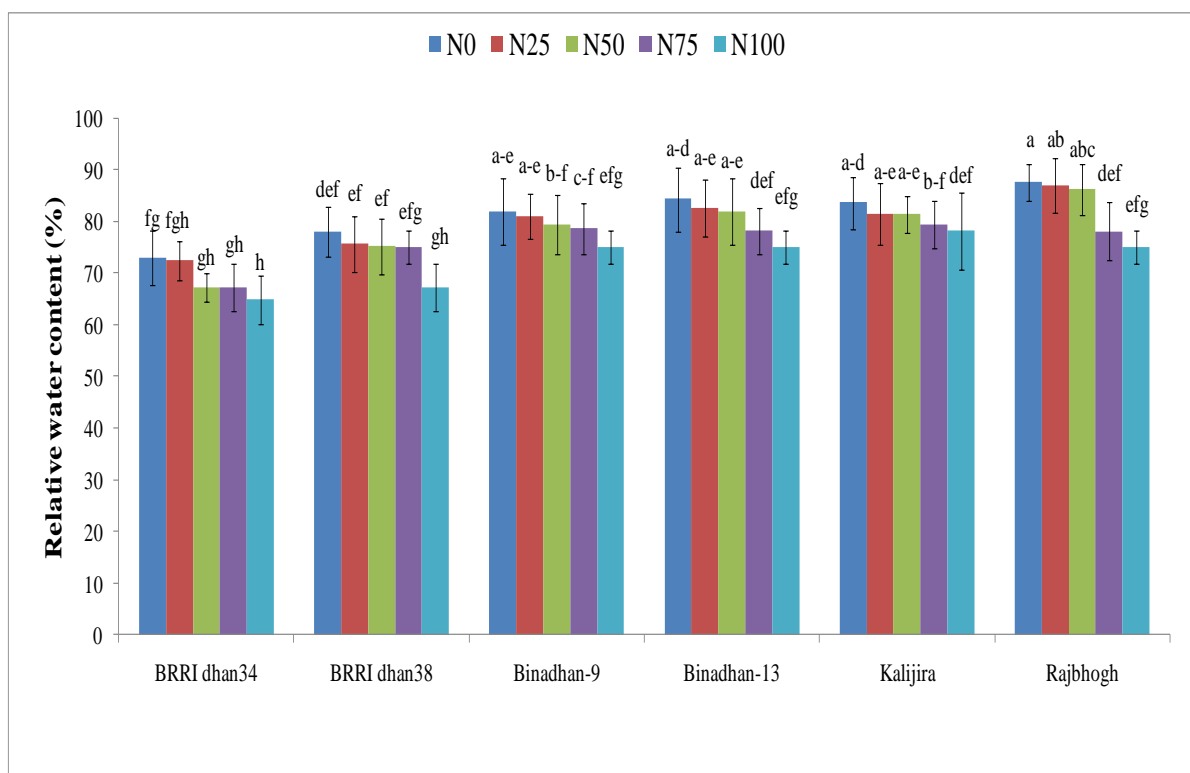


Figure 3. The combined effects of salinity and Aman rice varieties on Relative water content

Bars with different letters are significantly different at $p \leq 0.05$ applying LSD

4.2.2 Chlorophyll content

4.2.2.1 Effect of variety

The effect of varieties on chlorophyll accumulation was statistically significant (Fig 4). The chlorophyll content was found to be decreased with the increasing salinity. The highest chlorophyll ($0.4910 \text{ mg cm}^{-2}$) was in case of BRRi dhan34. The lowest chlorophyll (0.44 mg cm^{-2}) was in BRRi dhan38.

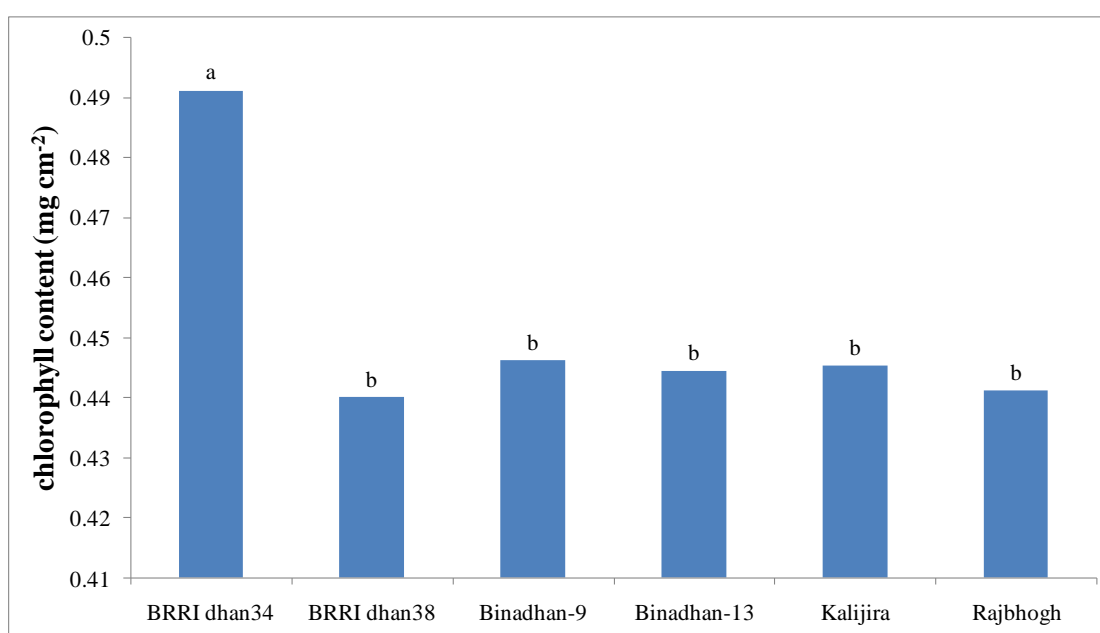


Figure 4. Effect of variety on chlorophyll content of different rice varieties.

Bars with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.2.2.2 Effect of salinity level

Different salinity levels affected on the chlorophyll production significantly through the growing period. Salinity treatment reduced total chlorophyll content compared to control (Fig5). The decline rates were 4.24% at 25 mM, 6.19% at 50 mM, 8.17% at 75 mM and 12.04% at 100 mM salinity. However,

the lowest chlorophyll content (0.440 mg cm^{-2}) found in 100 mM saline condition.

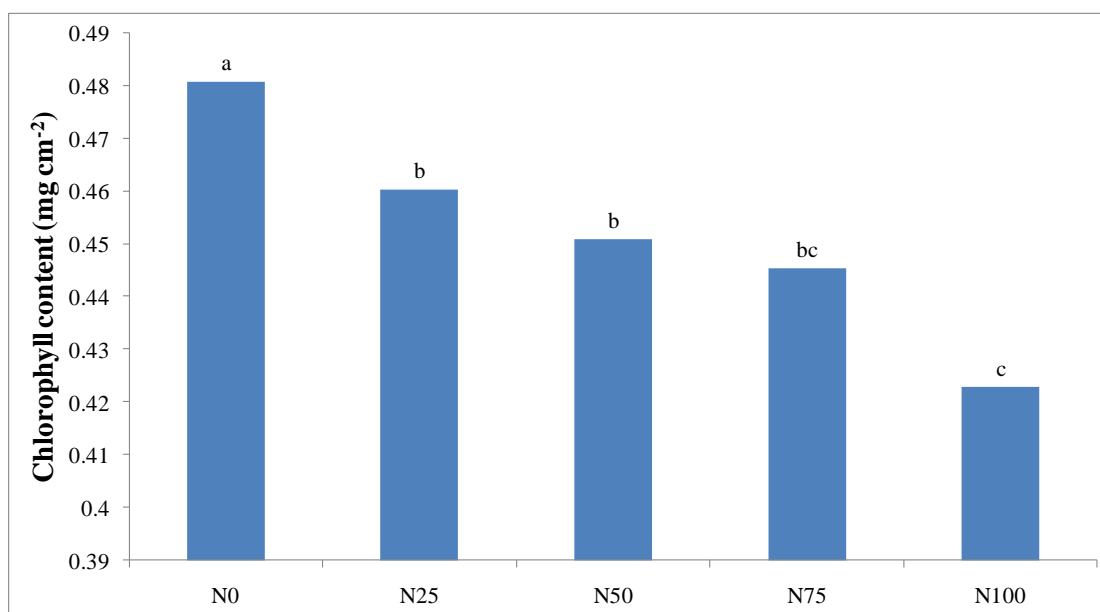


Figure 5. Effect of treatment on chlorophyll content (mg cm^{-2}) of different rice varieties.

Bars with different letters are significantly different at $p \leq 0.05$ applying LSD

4.2.2.3 Interaction effect of variety and salinity level

Interaction between varieties and salinity on the basis of total chlorophyll content was statistically significant (Fig 6). Decrease in chlorophyll content due to salt stress might be associated with the decrease in soluble protein and amino acid levels as reported by Saha and Gupta (1993).

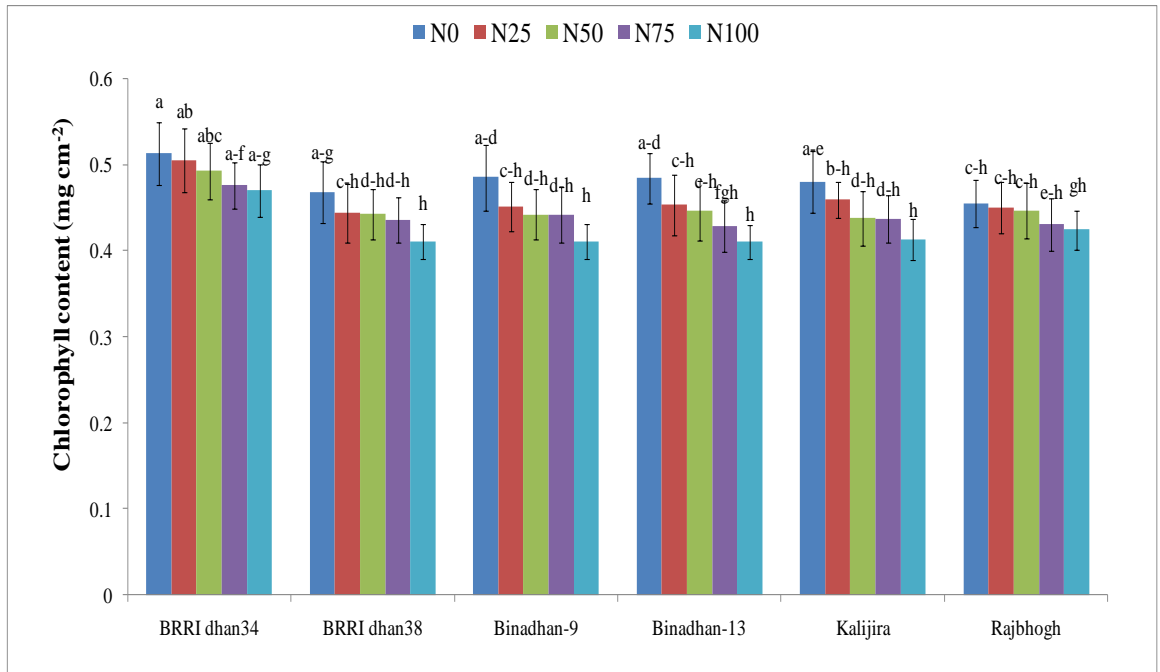


Figure 6. Chlorophyll content of different rice leaves induced by saline and their combination.

Bars with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.3 Yield contributing characters

4.3.1 Effective tillers hill⁻¹

4.3.1.1 Effect of variety

The effective tiller varied significantly due to varietal diversity (Fig 7). It was observed that Binadhan-9 found the highest (15.26) number of effective tillers hill⁻¹. On the other hand the lowest (7.55) number of effective tillers hill⁻¹ was found in Binadhan-9 which is statistically similar with Binadhan-13 (7.76).

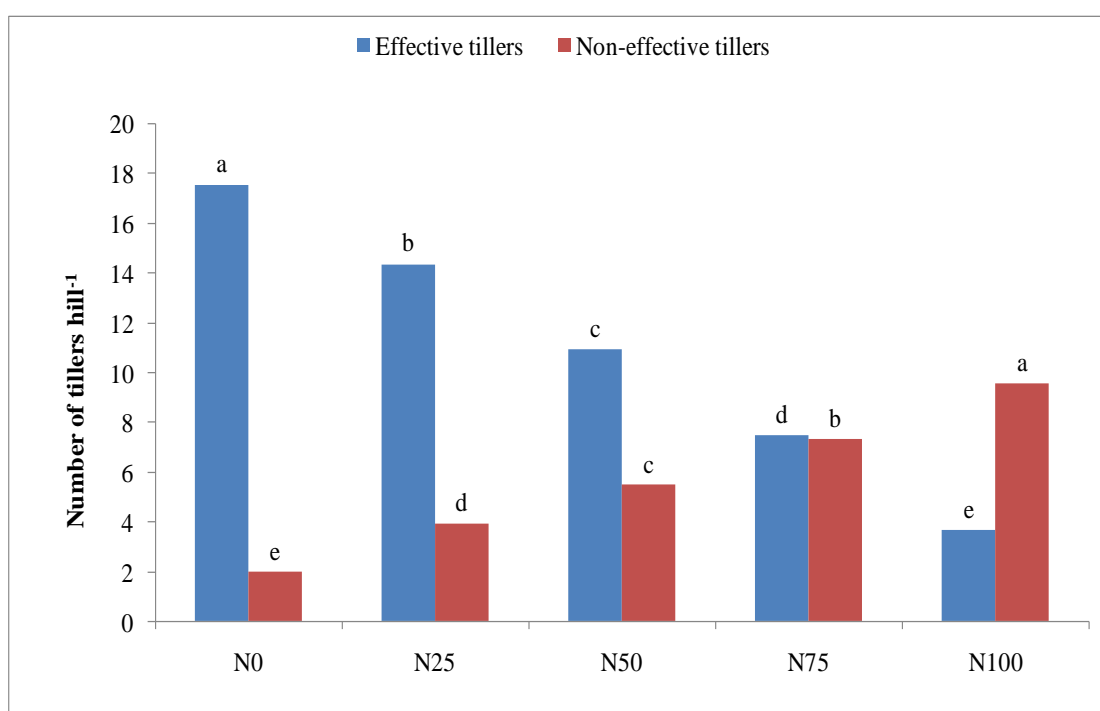


Figure 7. Effect of variety on total number of tillers hill⁻¹ of different rice varieties.

Bars with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.3.1.2 Effect of salinity level

Salinity caused a significant reduction of effective tillers compared to control (Fig 8). The highest effective tillers hill⁻¹ was found in control. After that with the increasing the salinity levels number of effective tillers hill⁻¹ was decreased. The decline rates were 18.17% at 25 mM, 37.72% at 50 mM, 57.41% at 75 mM and 79.18% at 100 mM salt stress. The lowest number of effective tillers hill⁻¹ was found in 100 mM saline condition.

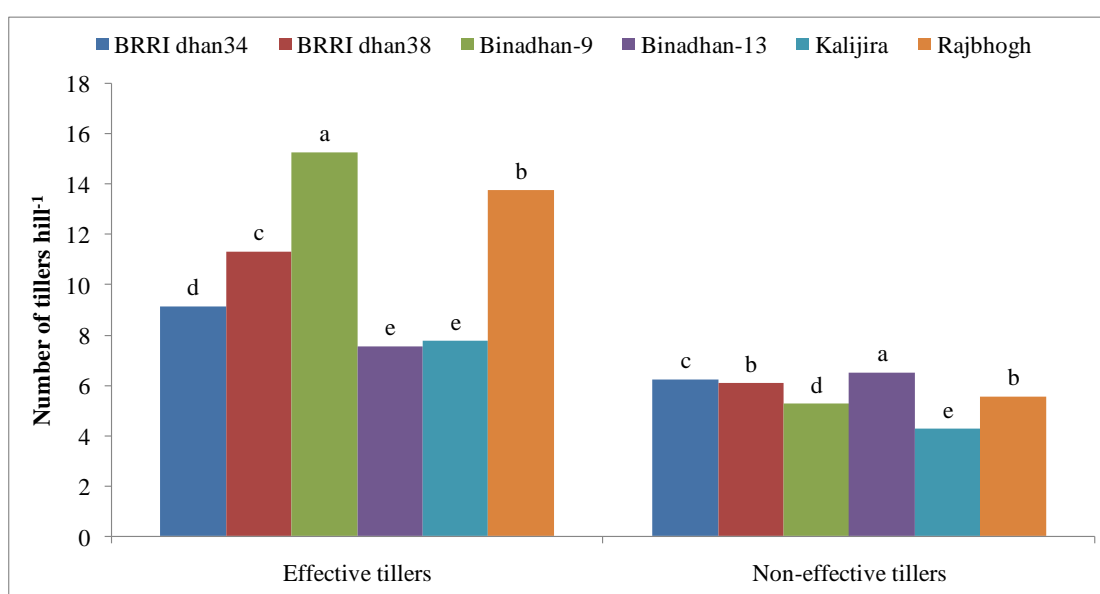


Figure 8. Effect of treatments on number total number of tiller hill⁻¹ of different rice varieties.

Bars with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.3.1.3 Interaction effect of variety and salinity level

Rice grain yields are highly dependent upon the number of panicle-bearing tillers produced per plant. There were significant differences among the varieties x salinity interaction. It was showed (Fig 9) that effective tillers hill⁻¹ decreased with increasing salinity levels. The highest number of effective

tillers was recorded in Binadhan-9 (21.88 at N₀ and 20.26 at N₂₅) and Rajbhog (20.95 at N₀) which was statistically similar with Rajbhog (18.14 at N₂₅). The lowest effective tiller was found in Kalijira (1.78) at 100 mM saline stress. Khatun *et al.* (1995); Lutts *et al.* (1995). reported that Salinity's effect on rice resulted in a decrease in the number of effective tillers and fertile florets per panicle and a reduction in individual grain mass.

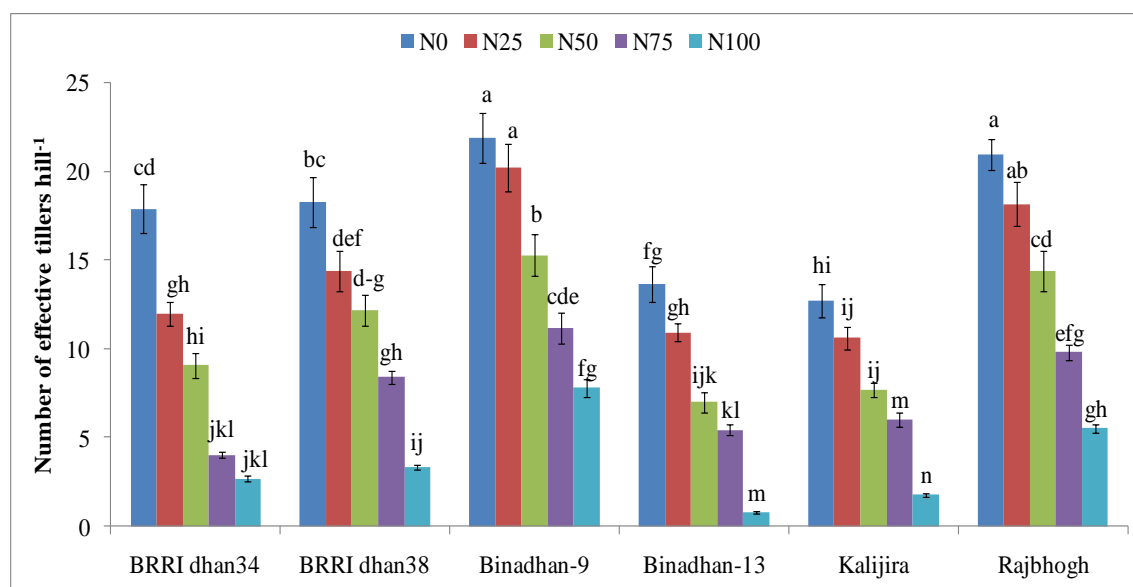


Figure 9. Number of effective tillers hill⁻¹ in rice cultivars induced by saline and their combination.

Bars with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.3.2 Non-effective tiller hill⁻¹

4.3.2.1 Effect of variety

Significant variation was observed in non-effective tillers due to the effect of variety shown in Fig 7. Binadhan-13 produced higher non-effective tillers (6.52) compared to other aman rice cultivars. The lowest number non-effective tillers found in Kalijira (4.26).

4.3.2.2 Effect of salinity level

Upon exposure to salt stress, non-effective tillers significantly decreased compared to other salinity stress (Fig 8). The number of non-effective tillers (9.54) was found in 100 mM saline condition. On the other hand, the lowest number of non-effective tillers (1.99) found in during control.

4.3.2.3 Interaction effect of variety and salinity level

Salinity stress caused increased number of non-effective tillers hill⁻¹ of different aman rice varieties. The highest non-effective tillers (11.04) were found in 100 salt stress condition of BRRI dhan38. The lowest number of non-effective tillers found in BRRI dhan34 (1.09) and Binadhan-9 (1.14) during control condition. So BRRI dhan34 showed the best result among all varieties. There were no productive tillers for all the varieties at higher salinity level (6 ds/m). The result can be supported by Zeng (2000). It was also reported that the number of non-effective tillers decreased progressively with increase in salinity levels (Desai *et al.*, 1975 and Sexena and Pandey, 1981). Young *et al.* (2003) also stated that the number of non-effective tillers hill⁻¹ decreased with increasing salinity levels in rice. The decrease in number of tillers might be due to the toxic effect of salt on plant growth.

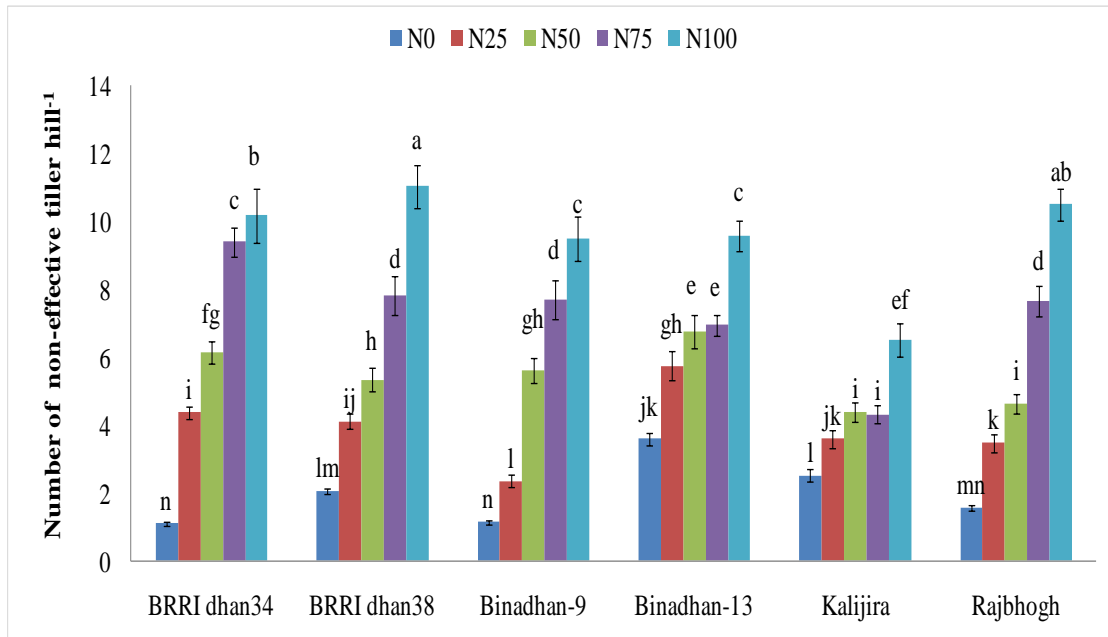


Figure 10. Effect of saline on number of non-effective tillers hill⁻¹ of different rice varieties.

Bars with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.3.3 Panicle length

4.3.3.1 Effect of variety

The panicle length was varied with the varietal diversity as shown in Fig 11. It was found that Binadhan-9 produced highest panicle length (24.03). However, the lowest panicle length found in Kalijira (21.66) which was statistically similar with BRRRI dhan38 (22.43) and Binadhan-13 (22.03).

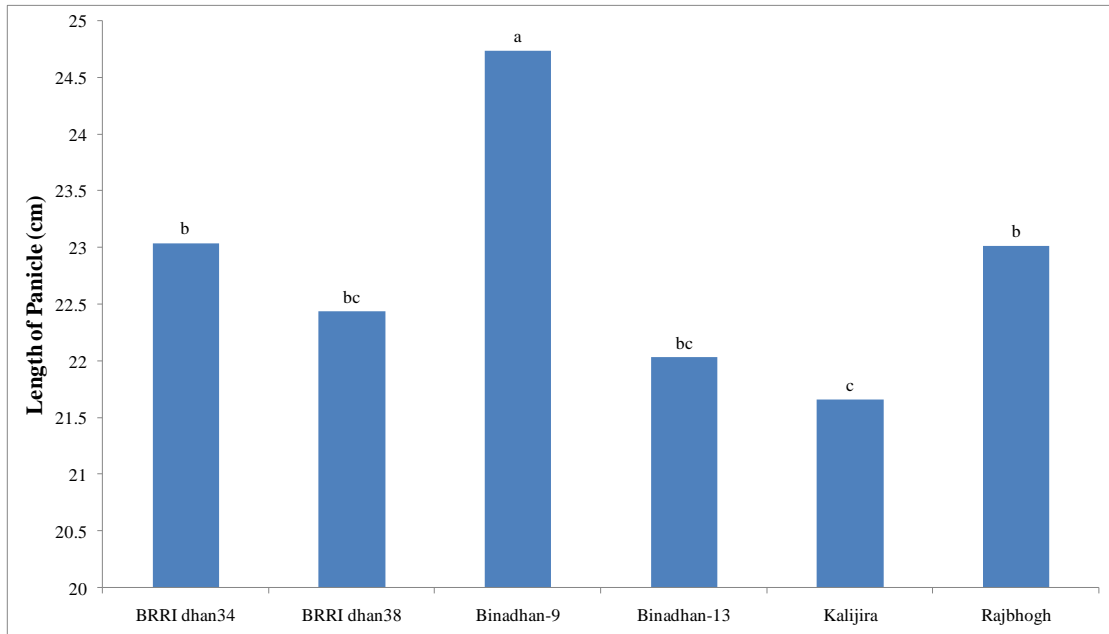


Figure 11. Effect of variety on panicle length of different rice cultivars.

Bars with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.3.3.2 Effect of salinity level

There was significant variation on panicle length due to salinity stress (Fig. 12). Saline treatment reduced the length of panicle compared to its control. The decline rates from its control were 6.66%, 10.15%, 16.88% and 24.91% due to application of 25, 50, 75 and 100 mM saline respectively. However, the highest panicle length found in N_0 condition and the lowest panicle length recorded in N_{100} condition.

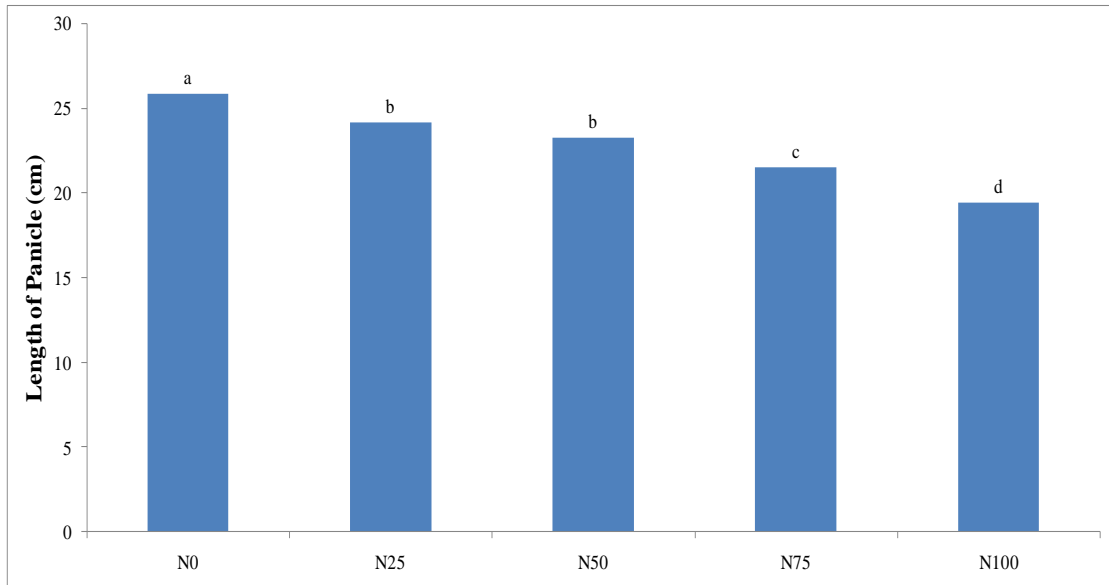


Figure 12. Effect of saline on panicle length in different rice varieties.

Bars with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.3.3.3 Interaction effect of variety and salinity level

Different growth stages showed different sensitivity to salinity considering on panicle length. Interaction between varieties and salinity levels in relation to panicle length was significant. The highest (27.95 cm) panicle length was found in Binadhan-9 at control condition which was statistically similar with the panicle length at 25 mM salinity (26.69 cm) of same variety and in BRRI dhan38 (26.80 cm) at control condition. The lowest panicle (17.45 cm) length was recorded in Kalijira at 100 mM saline condition which was statistically similar with BRRI dhan38, Binadhan-13 and Rajbhog at 150 mM salinity. Similar results were reported by Marassi *et al.* (1989) in rice. The panicle length was different among the genotypes under salinity stress. Islam *et al.* (2007) stated that panicle length of rice decreased with increase level of salinity,

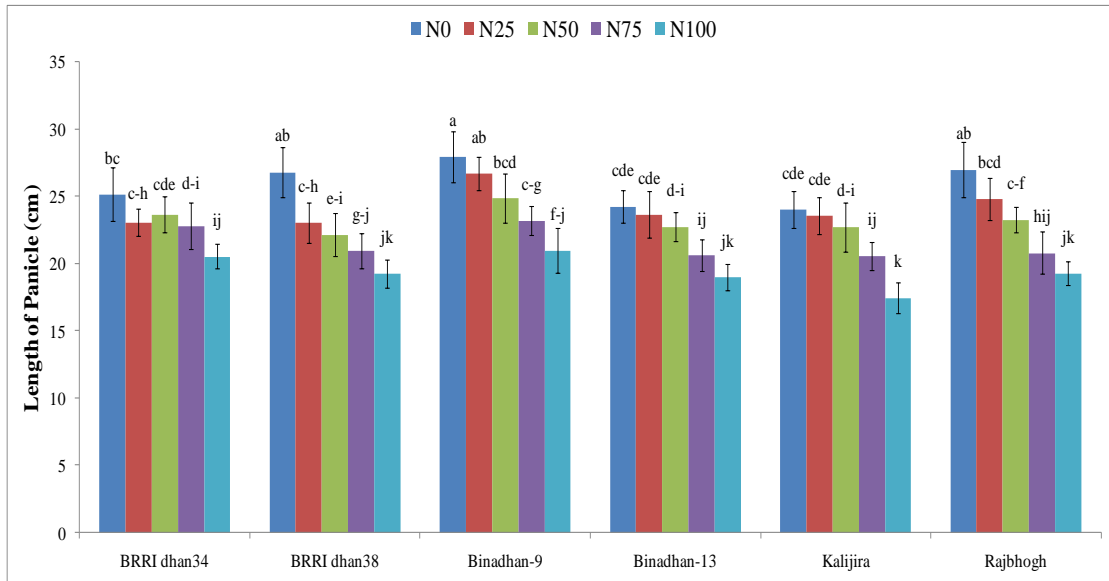


Figure 13. Effect of saline and different rice cultivars on panicle length.

Bars with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.3.4 Number of filled grain panicle⁻¹

4.3.4.1 Effect of variety

Different physiological and yield components of rice had different sensitivity to salinity. The number of filled grain panicle⁻¹ was significantly reduced due the varietal diversity (Fig 14). The highest number of filled grain (102.36) was recorded in Binadhan-9 compared to other varieties. On the other hand, lowest number (36.57) of filled grain was found in Kalijira.

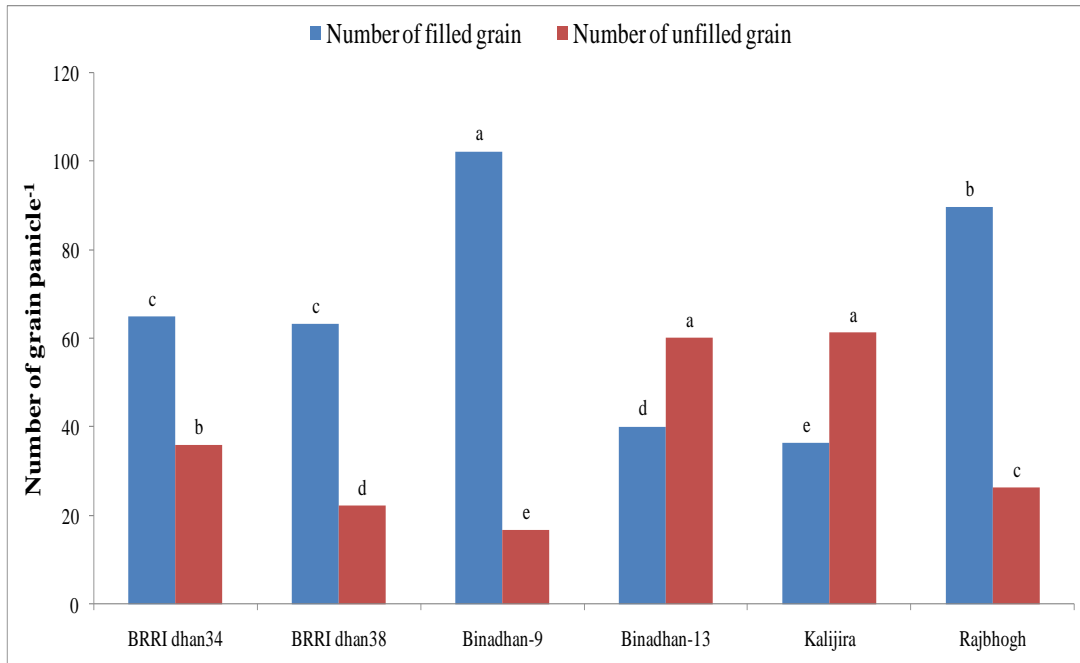


Figure 14. Effect of varieties on number of grains panicle⁻¹ in different rice cultivars.

Bars with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.3.4.2 Effect of salinity level

The effect of salinity on number of filled grains panicle⁻¹ was significantly different (Fig 15). Number of filled grain panicle⁻¹ decreased with the increasing salt stress. So, the highest number of filled grain panicle⁻¹ (80.19) was recorded in N_0 where salt stress was not used. But when salt stress was increased to 25, 50, 75 and 100 mM the decline rates were 11.35%, 17.45%, 27.35% and 31.25% respectively. The lowest number of filled grain panicle⁻¹ (55.13) found during 100 mM saline condition.

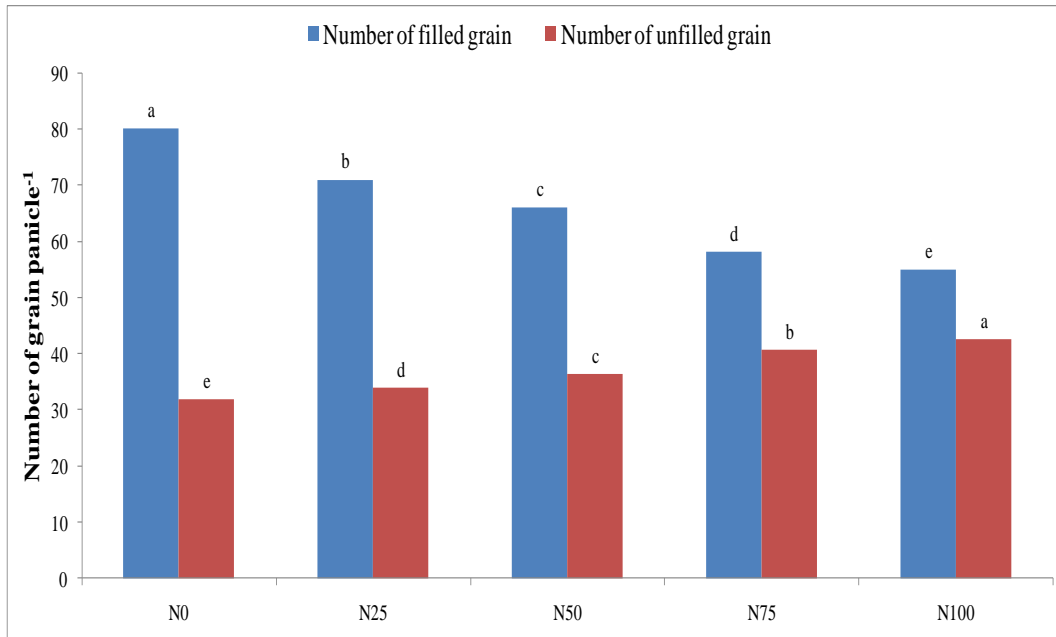


Figure 15. Effect of saline on number of grain panicle⁻¹ in rice

Bars with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.3.4.3 Interaction effect of variety and salinity level

The combined effect of salinity and varieties on number of filled grain panicle⁻¹ was significant (Fig 16). The control treatment had the most amount filled grains and after that was treatment at 25 mM in Binadhan-9. The least number of filled grains panicle⁻¹ was at 100 mM which showed 71.48% decrease compared with the control treatment of Kalijira. Therefore, increasing salinity caused decrease in number of filled grains panicle⁻¹ of rice. The decreasing rate was less in Binadhan-9 compared to other five varieties. Filled grain number per panicle had high effect on yield. Salinity decreased yield through decreasing number of filled grains per panicle. Mahmood *et al.*⁵⁰ (2009) studied the effect of salinity on rice and stated that increasing salinity significantly reduced grain filling capacity. Increased number of incompletely filled grains might be a result of assimilate shortage during grain filling,

brought about by early leaf senescence (Sheehy *et al.*, 2001; Murchie *et al.*, 2002) which in this case was caused by salinity (Shannon, 1998; Zeng, 2000).

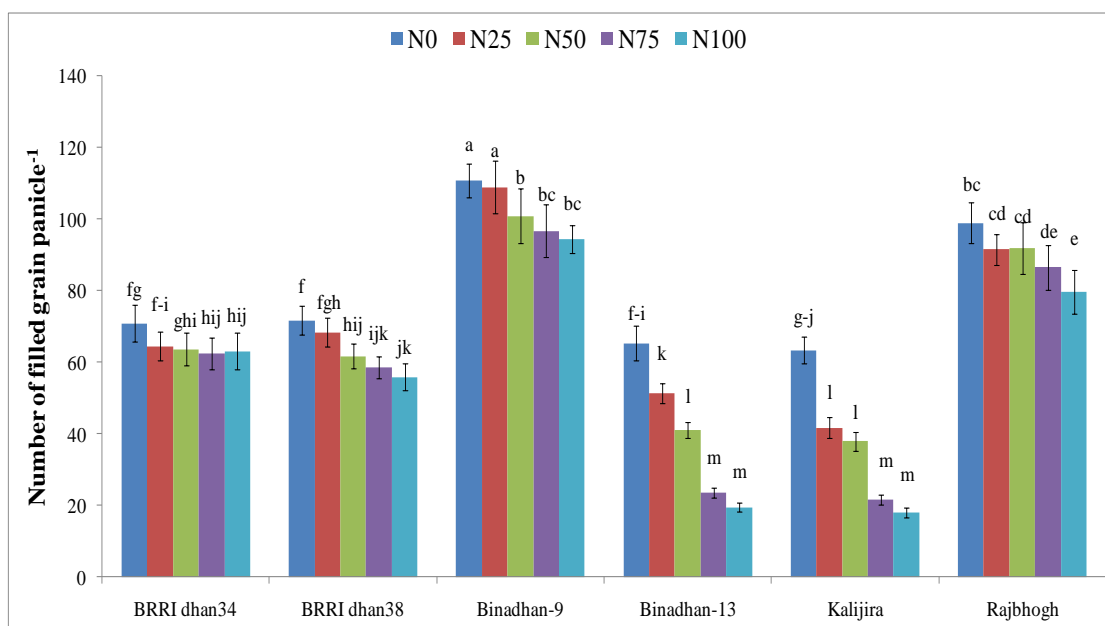


Figure 16. Number of filled grain panicle⁻¹ in rice cultivars induced by saline and their combination.

Bars with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.3.5 Number of unfilled grains panicle⁻¹

4.3.5.1 Effect of variety

As shown in Fig 14, a significant variation was observed in number of unfilled grains panicle⁻¹ in different rice varieties. Binadhan-13 (60.12) and Kalijira (61.38) had highest number of unfilled grains panicle⁻¹ compared to other aman rice cultivars. The lowest number of unfilled grain panicle⁻¹ (16.85) was recorded in Binadhan-9 compared to other aman rice varieties.

4.3.5.2 Effect of salinity level

Number of unfilled grains panicle⁻¹ was affected significantly at different levels of salt stress (Fig 15). It was found that number of unfilled grains increased with the increased levels of salinity. Increased unfilled grain negatively affects on production of rice. The highest number of unfilled grains panicle⁻¹ (42.67) was found in 100 mM treatment. But after increasing salt stress to 25 mM, 50 mM, 75mM and 100 mM the increasing rates were 6.45%, 12.03% 27.12% and 33.28% respectively. The lowest number (32.02) of unfilled grains panicle⁻¹ revealed in control treatment.

4.3.5.3 Interaction effect of variety and salinity level

The data of combined effect of salinity and varieties on number of unfilled grains panicle⁻¹ was statistically significant (Fig 17). Plants of control treatment of six rice varieties had always attained the lowest values over the salinity treatment. The lowest number of unfilled grains panicle⁻¹ was recorded in Binadhan-9 in control treatment over all the varieties. On the other hand, the highest number of unfilled grain was found in Kalijira and Binadhan-13 compared to other rice varieties. High effectiveness of salinity on number of empty grains has been reported by many researchers. Increased number of incompletely filled grains might be a result of assimilate shortage during grain filling, brought about by early leaf senescence caused in this case by salinity (Fabre *et al.*, 2005; Murchie *et al.*, 2002; Sheehy *et al.*, 2001; Zaibunnisa *et al.*, 2002; Zeng & Shannon 2000). Frequently, many spikelets on the lower primary branches do not produce a mature grain, and this loss of potential grains may adversely affect the grain number and yield. This failure in spikelet development has been attributed to a limitation in carbohydrate supply to the developing panicle (Abdullah *et al.*, 2001).

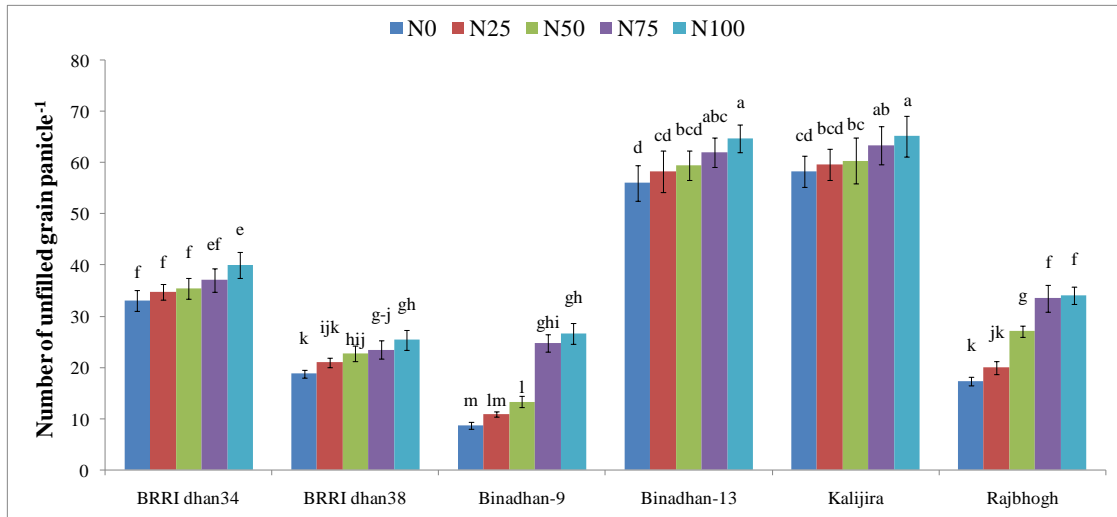


Figure 17. Number of unfilled grain panicle⁻¹ in rice cultivars induced by saline and their combination.

Bars with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.3.6 1000-grain weight

4.3.6.1 Effect of variety

Thousand grain weights were different among the varieties (Fig 18). The maximum 1000-grain weight (19.17 g) was noticed in Binadhan-38. The minimum 1000-grain weight (8.23 g) was observed in Kalijira. From this finding it may be concluded that there was varietal difference in size of the grain.

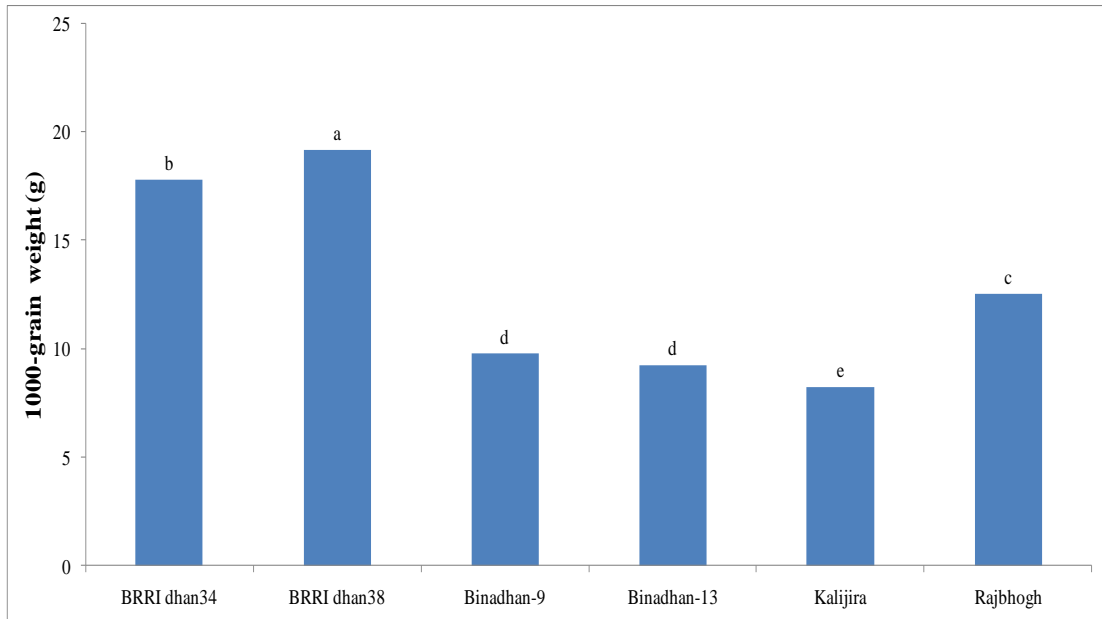


Figure18. Effect of variety on 1000-grain weight of different rice cultivars.

Bars with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.3.6.2 Effect of salinity level

The effect of salinity stress on 1000-grain weight was significant (Fig 19). 1000-grain weight represents grain size of variety. The highest 1000-grain weight (15.87 g) was found at control condition. After that with the increasing the salinity 1000-grain weight was decreased. The decline rates were 9.24% at 25 mM, 22.74% at 50mM, 29.64% at 75 mM and 35.72% at 100 mM saline stress. The lowest (10.20 g) 1000-grain weight was recorded in 100 mM saline condition.

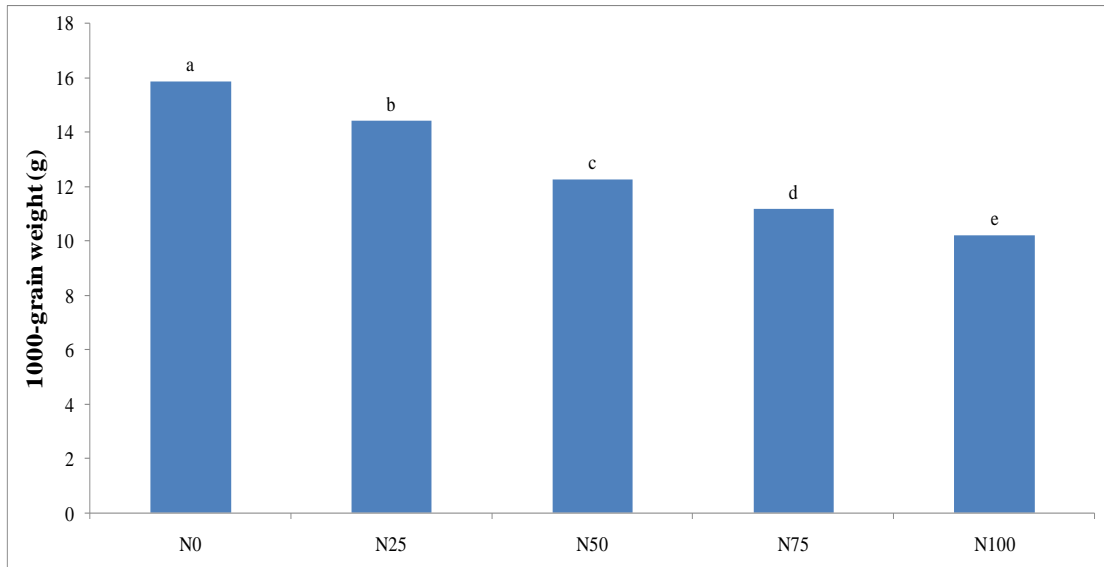


Figure 19. Effect of salinity on 1000-grain weight in different rice cultivars

Bars with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.3.6.3 Interaction effect of variety and salinity level

The interaction effect of salinity stresses to varieties on 1000-grain weight was significant (Fig 20). With the increased salinity levels the 1000-grain weight was gradually decreased. The highest 1000-grain weight (21.91 g) was observed in BRRI dhan38 at control treatment. The lowest 1000-grain weight (5.67 g) was recorded in Kalijira. Zaman *et al.* (1997)) and Aoki and Ishikawa (1971) reported that 1000-grain weight decreased with increasing the levels of salinity.

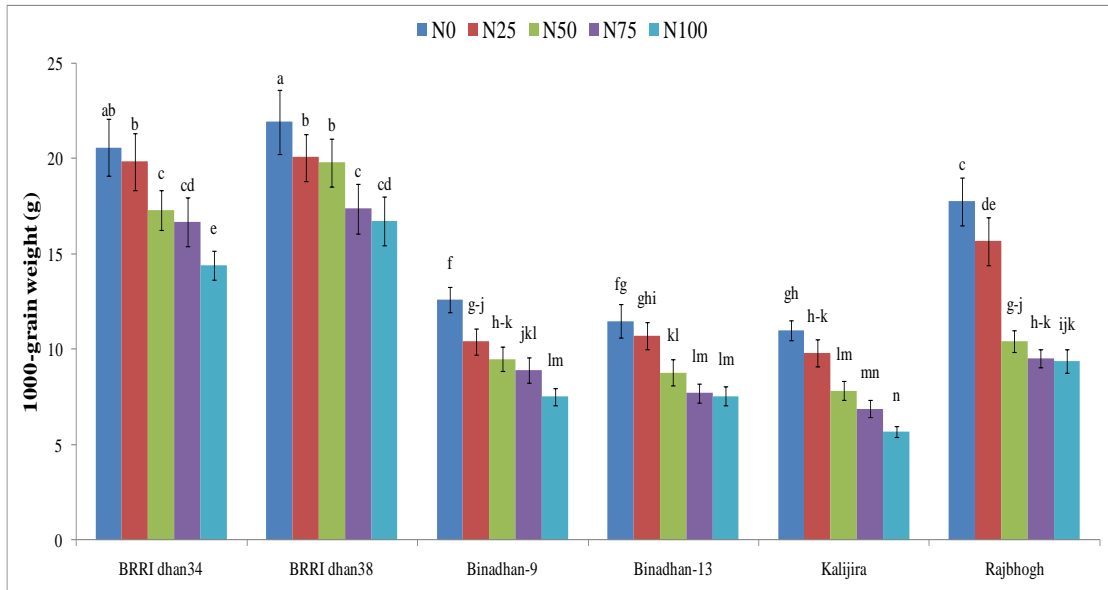


Figure 20. Interaction effect of saline and varieties on 1000-grain weight.

Bars with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.3.7 Grain yield pot^{-1}

4.3.7.1 Effect of variety

Grain yield varied significantly for varietal diversity as shown in Fig 21. The highest grain yield pot^{-1} (65.76 g) was recorded on Binadhan-9. The lowest grain yield pot^{-1} (33.52 g) was found in Kalijira.

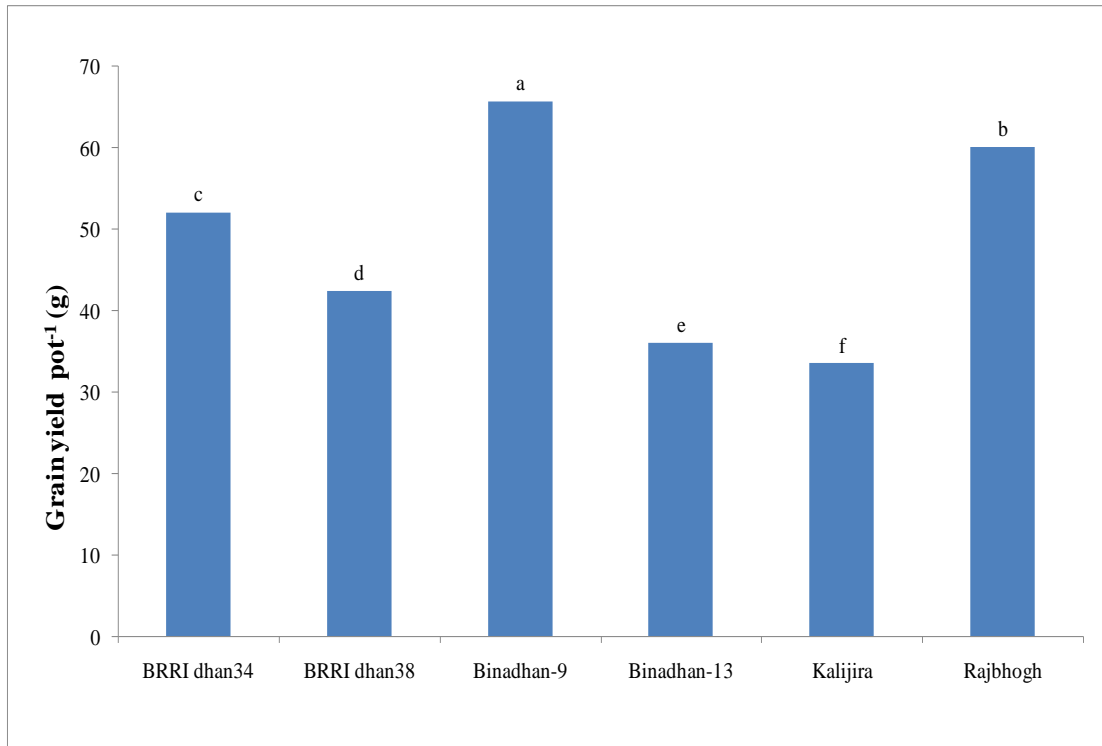


Figure 21. Effect of variety on grain yield pot⁻¹ in different rice varieties.

Bars with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.3.7.2 Effect of salinity level

Significant variation was observed in grain yield pot⁻¹ due to different salinity treatments (Fig 22). Grain yield became reduced due to saline treatment. The maximum grain yield pot⁻¹ (65.48 g) was recorded in control condition. After increase in saline grain yield was decreased. The decreased rates were 26.39% at 25 mM, 31.64% at 50mM, 33.89% at 75 mM and 39.07% at 100 mM saline treatments. The lowest grain yield (39.89 g) was noticed in 100 mM salt stress.

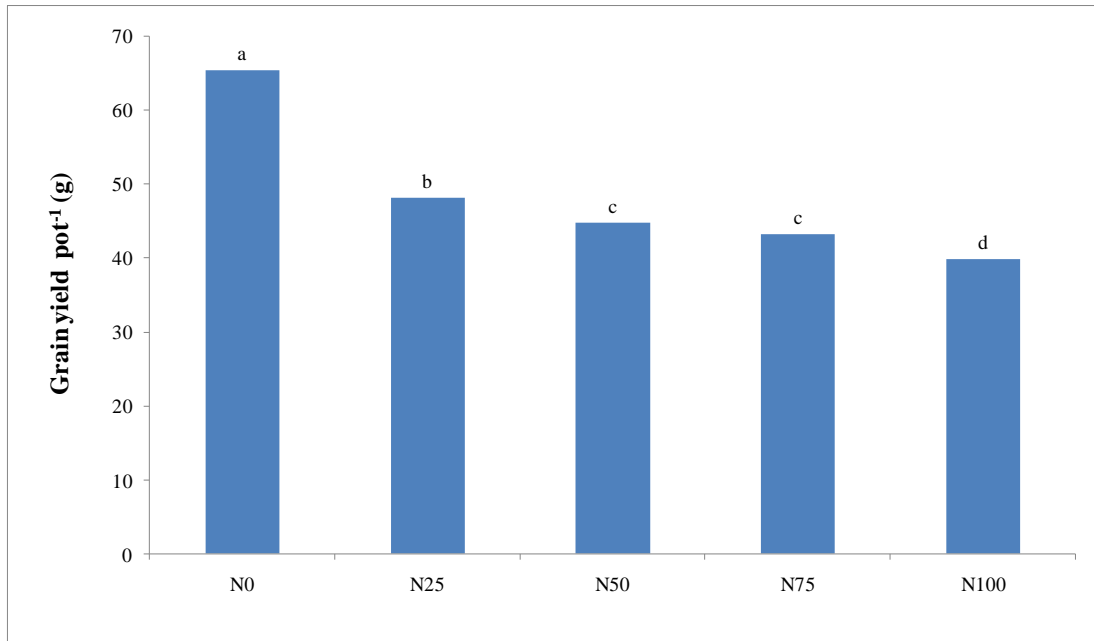


Figure 22. Effect of saline on grain yield pot⁻¹ in different rice cultivars.

Bars with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.3.7.3 Interaction effect of variety and salinity level

The interaction effect of salinity and varieties in relation to grain yield pot⁻¹ was found significant (Fig 23). The highest grain yield (87.48 g) was in control in Binadhan-9. The lowest grain yield (26.31 g) was noticed in Kalijira at 100 mM saline treatment. In all rice varieties there were decreasing trend in respect of grain yield of rice with increasing salinity level. WeonYoung *et al.* (2003) reported that grain yield decreased with raising salinity.

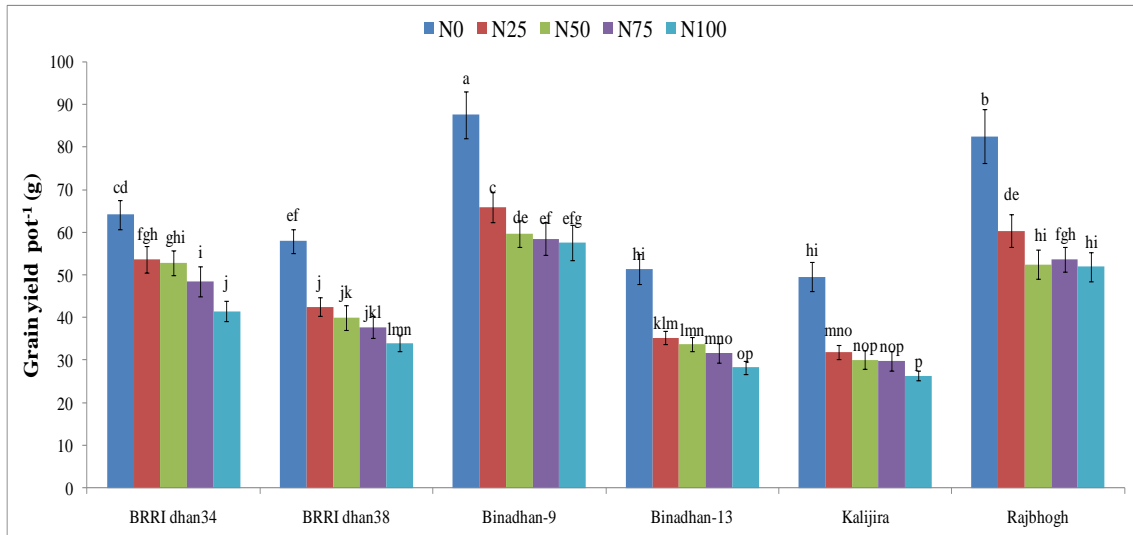


Figure 23. Grain yield pot^{-1} of different rice varieties induced by saline.

Bars with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.3.8 Straw yield pot^{-1}

4.3.8.1 Effect of variety

There was a significant variation observed in straw yield pot^{-1} due to varietal diversity (Fig 24). The highest straw yield (182.35 g) was recorded in Rajbhogh and lowest straw yield pot^{-1} (125.96 g) was found in kalijira compared to other rice cultivars.

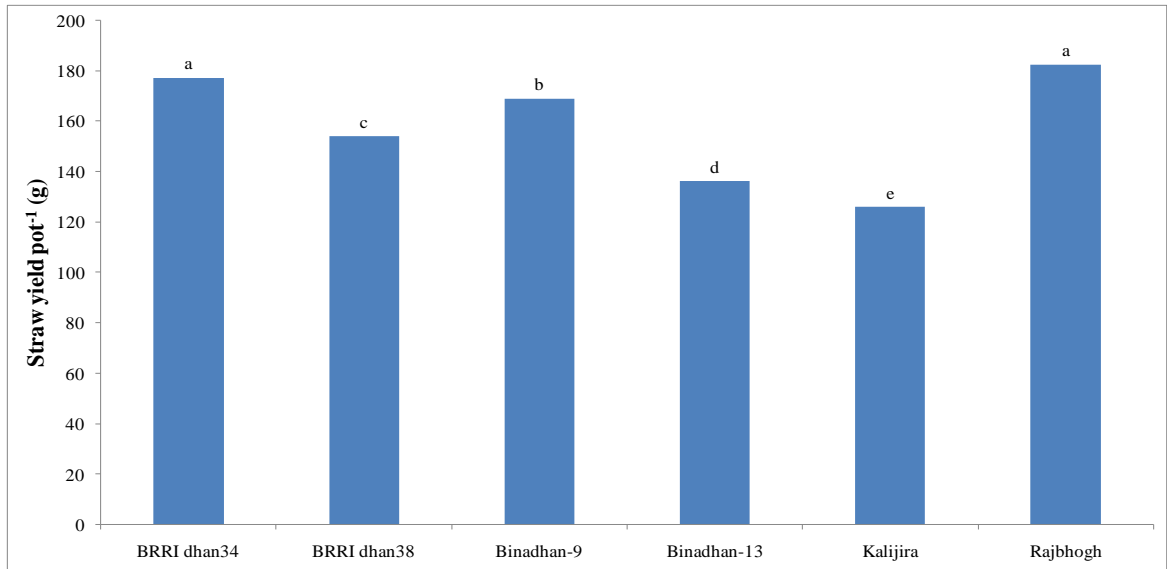


Figure 24. Effect of different aman rice varieties on straw yield pot⁻¹

Bars with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.3.8.2 Effect of salinity level

Sharp decreases in straw yield pot⁻¹ were observed due to salinity increase (Fig 25). The maximum straw yield pot⁻¹ (207.29 g) was recorded in control condition. However, when the salt stress increased straw yield pot⁻¹ was decreased. The decline rates were 17.61% at 25 mM, 22.95% at 50 mM, 36.67% at 75 mM and 42.96% at 100 mM salinity. The lowest straw yield (118.23 g) was recorded in 100 mM salinity.

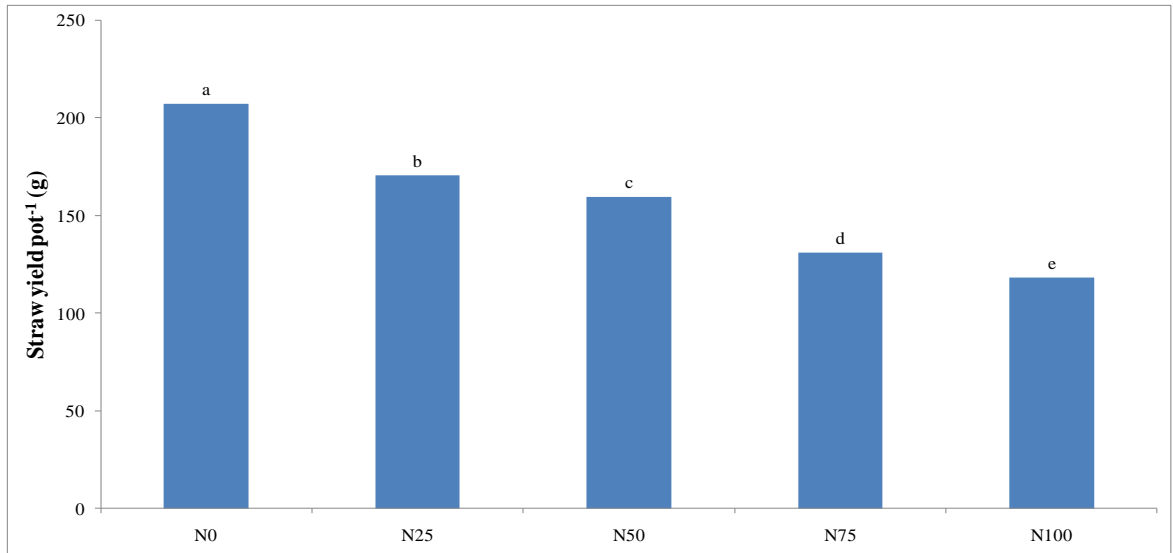


Figure 26. Effect of salinity on straw yield pot⁻¹ on different rice cultivars.

Bars with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.3.8.3 Interaction effect of variety and salinity level

The interaction between salinity and varieties was statistically significant (Fig 27). Straw yield pot⁻¹ at all cultivars gradually decreased with the increasing salinity levels compared to that of the respective control. The highest straw yield pot⁻¹ was recorded in Binadhan-9 (238.76 g), BRRi dhan38 (228 g) and Rajbhog (236.98 g). On the other hand, the lowest straw yield pot⁻¹ was found Kalijira (94.44 g), Binadhan-13 (99.51 g) and BRRi dhan38 (96.16 g).

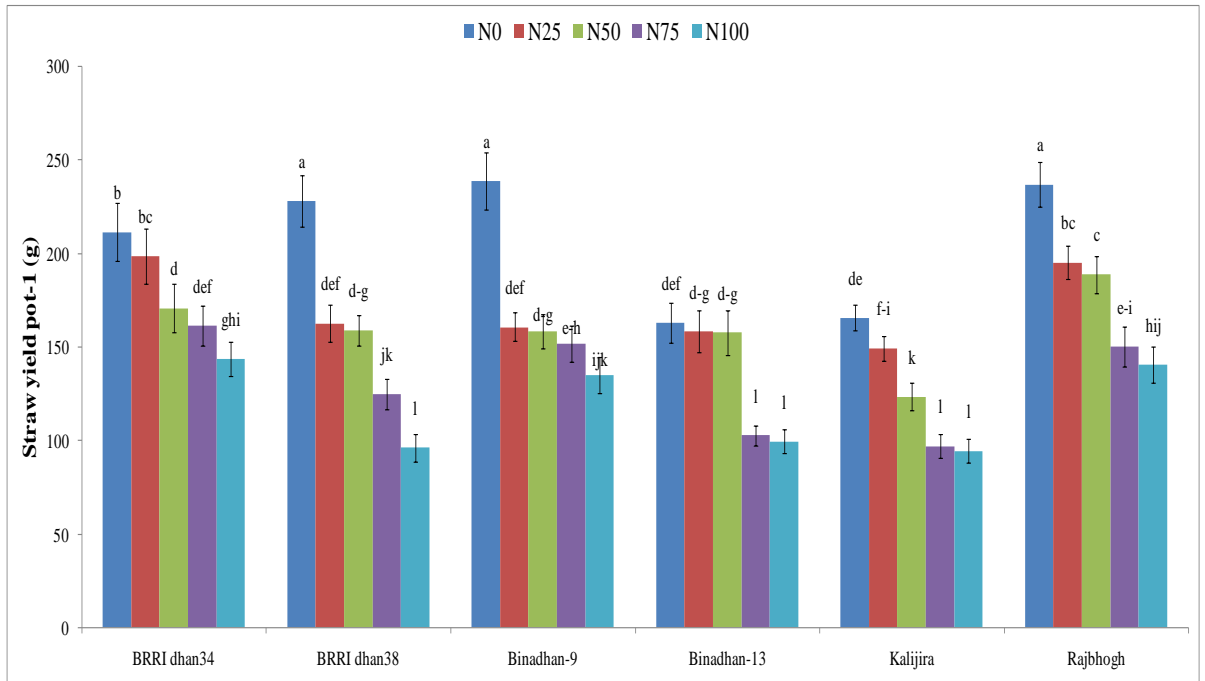


Figure 27. Interaction effect of straw yield pot⁻¹ on different rice varieties.

Bars with different letters are significantly different at $p \leq 0.05$ applying LSD.

Chapter 6

SUMMARY AND CONCLUSION

A pot experiment was undertaken to investigate the salt tolerance varieties and the effect of salinity on some growth, physiological and yield contributing characters in some aromatic rice varieties. Different levels of salinity were control (N_0), 25 mM (N_{25}), 50 mM (N_{50}), 75 mM (N_{75}) and 100 mM (N_{100}). The experiment was carried out in the Experimental shed of the Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka during the period of June, 2014 to October, 2014. Seeds of BRRI dhan34 and BRRI dhan38 were collected from Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur. Binadhan-9 and Binadhan-13 were collected from Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. Seeds of kalijira and Rajbhogh were collected from Mymensingh region.

The experiment was laid out in Randomized Completed Block Design (RCBD) with three replications. The experimental unit was earthen pot and 90 pots under study.

Data of morphological, physiological and yield and yield contributing characters were collected at 30 DAT, 45 DAT, 60 DAT, 75 DAT, 90 DAT and harvest. The recorded data were analysed using XLSTAT 2014 package. It was observed that morphological characters e.g. plant height, number of tillers hill⁻¹, number of leaves hill⁻¹; physiological characters e.g. relative water content and chlorophyll content and yield contributing characters like number of effective tillers, number of filled grain, panicle length, 1000-grain weight, straw yield and grain yield were decreased with the increasing soil salinity levels compared to those of control of plants. Number of non-effective tillers and number of unfilled grain increased with increasing salinity levels.

Different salinity with had significant effect on crop growth parameters viz. plant height, tillers hill⁻¹ and number of leaves hill⁻¹ at different DAT. The highest plant height was observed in BRRI dhan38 with the increasing level of salinity. The highest tillers hill⁻¹ was observed in Binadhan-9 in control and different saline treatments. The highest number of leaves hill⁻¹ was recorded in BRRI dhan38 in control and different salt stress.

Salt treatments had significant effect on the physiological parameters like relative water content was highest in Rajbhogh in control condition (87.62%). The chlorophyll content was highest in BRRI dhan34 (0.4910 mg cm⁻²) at control treatments.

Salinity level had significant effect on the yield and yield contributing characters viz. plant height, effective tillers hill⁻¹, length of panicle, number of filled grain, 1000-grain weight, straw yield and grain yield were highest in Binadhan-9 where non-effective tillers and unfilled grain were highest in Kalijira at control treatment.

Overall results indicated that salinity levels decreased growth and yield attributes of aromatic rice varieties. Based on result of the present experiment, together with results found in the available literature, we therefore conclude that Binadhan-9 and BRRI dhan38 performed better under saline condition where local variety Kalijira did not perform well under saline condition in different aromatic rice.

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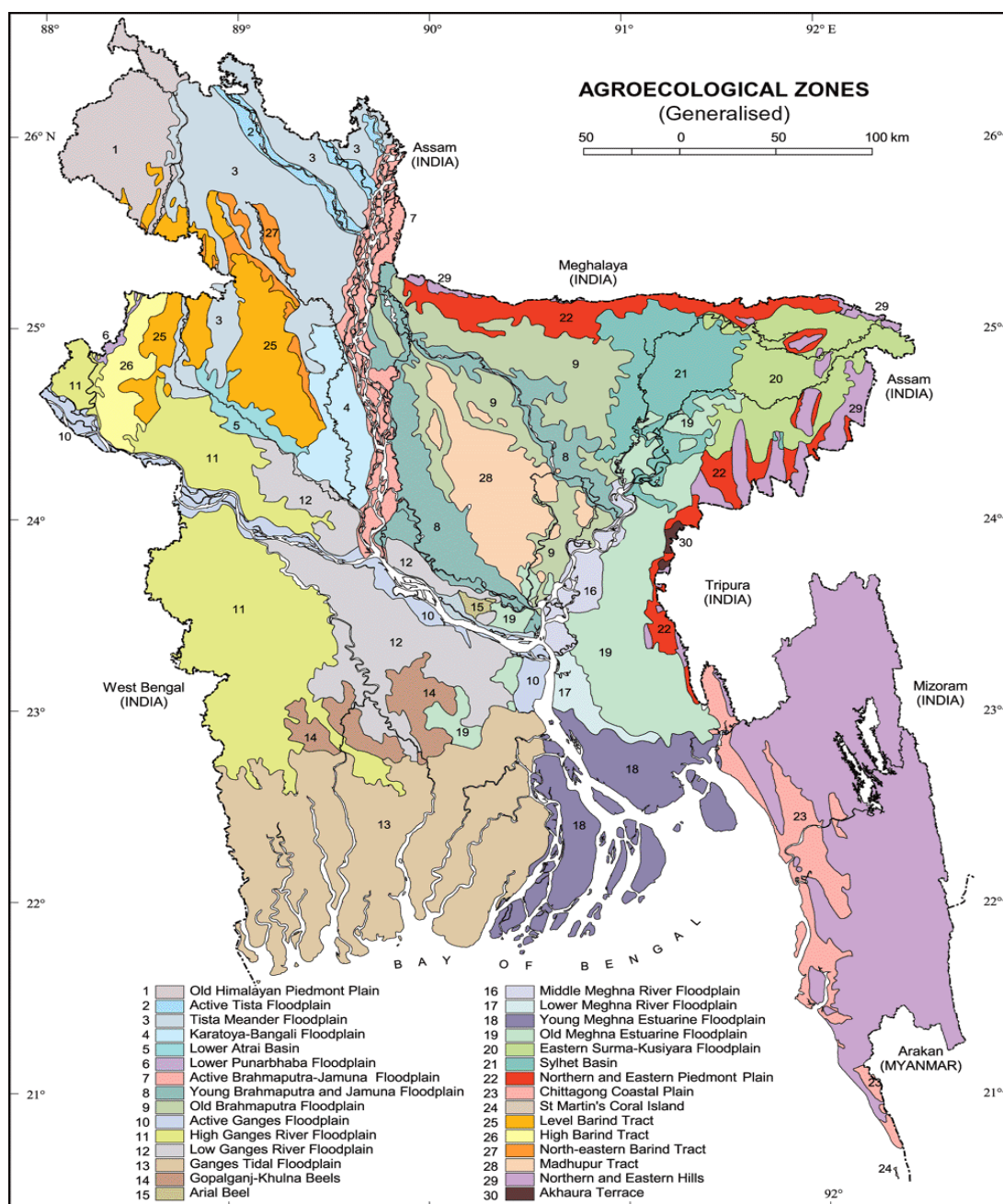
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APPENDICES

Appendix I Experimental location on the map of Agro-ecological Zones of Bangladesh



Appendix II Physical and chemical properties of experimental soil analyzed at Soil Resources Development Institute (SRDI), Farmgate, Dhaka.

Characteristics	Value
Particle size analysis	
% Sand	27
% Silt	43
% Clay	30
Textural class	Silty-clay
pH	5.6
Organic carbon (%)	0.45
Organic matter (%)	0.78
Total N (%)	0.03
Available P (ppm)	20.00
Exchangeable K (me/100 g soil)	0.10
Available S (ppm)	45

Source: SRDI (Soil Resources Development Institute), Farmgate, Dhaka

Appendix III Monthly average air temperature, rainfall and relative humidity of the experimental site during the period from June-December, 2014.

Months	Air temperature (°C)		Relative humidity (%)	Total rainfall (mm)
	Maximum	Minimum		
June, 2014	30.30	21.80	71.08	289
July, 2014	33.45	25.50	65.43	455
August, 2014	35	24.20	58	263
September, 2014	24.30	19.12	53.07	2.34
Octobeber, 2014	28.10	6.88	58.18	1.56
November, 2014	28.10	6.88	58.18	1.56
December, 2014	25.36	5.21	54.3	0.63

Source: SAU Meteorological Yard, Sher-e-Bangla Nagar, Dhaka-1207