MITIGATION OF SALT STRESS IN RICE BY EXOGENOUS APPLICATION OF SELENIUM

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MITIGATION OF SALT STRESS IN RICE BY EXOGENOUS APPLICATION OF SELENIUM

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CERTIFICATE

*This is to certify that thesis entitled, "***MITIGATION OF SALT STRESS IN RICE BY EXOGENOUS APPLICATION OF SELENIUM***" 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 MOHAMMAD ABU NAIM, Registration no. 09-03659 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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ABSTRACT

A pot experiment with three boro rice varieties *viz*. BRRI dhan45; BRRI dhan47 and Nipponbare was conducted at the experimental shed of the Department of Agronomy, Sher-e Bangla Agricultural University, Dhaka during winter season (2013-2014) to investigate the role of exogenous Selenium (Se) in growth, yield and antioxidant defense systems of rice under different salt stress condition. The experiment was carried out in seven salt stress treatments *viz*. control (without salt), S50 (50 mM salt stress), S50+Se (50 mM salt stress with 0.5 µM Se), S100 (100 mM salt stress), S100+Se (100 mM salt stress with 0.5 µM Se), S150 (150 mM salt stress) and S150+Se (150 mM salt stress with 0.5 µM Se). Salt stresses significantly reduced the plant height and tillers hill^{-1} of three 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 tillers hill^{-1}, number of filled grains panicle^{-1}, 1000 grain weight, grain yield and straw yield for three varieties. On the other hand, exogenous application of Selenium (Se) improved the plant height, effective tillers hill^{-1}, panicle length, filled grains panicle^{-1}, 1000-grain weight, straw yield and grain yield. The BRRI dhan47 can be recognized as naturally salinity tolerant rice variety. The BRRI dhan47 was observed better in response to exogenous application of Se under the stress condition. But Se application could not improve crop growth parameters, physiological parameters and yield at extreme level of salt stress (150 mM) significantly.

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LIST OF ABBREVIATIONS

AO	Ascorbate oxidase
APX	Ascorbate peroxidase
AsA	Ascorbic acid (ascorbate)
BRRI	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 *S*-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.) belongs to the Poaceae family and it is dominant over all other crops in respect of economic and social significance in Bangladesh. It is also largest cereal crop in Bangladesh. In worldwide, 474.86 milion metric tons of rice was produced from 159.64 million hectares of land with an average yield of 4.43 Mt ha^{-1} during the year of 2014-15 (USDA, 2015). USDA estimates Bangladesh to produce around 34.8 million tons of rice in MY 2014-15 (May - April), 1% from an estimated 34.59 million tons in MY 2013-14. During the year 2013-14, 19.00 million metric tons of Boro rice was produced from 4.79 million hectares of land with an average yield of 3.97 Mt ha^{-1} in Bangladesh (BBS, 2013-14). Rice is a staple crop in Bangladesh and its production has to be enhanced to meet the food requirement of an over populated country where the size of the population is still going fast.

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^-) (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).

Selenium (Se) is a widely studied trace element in humans and animals due to its role in the antioxidant defense system, which is needed for the maintenance of health and hormone balance. For the past few decades, the beneficial role of Se in plants has been investigated by several groups of researchers but the question is still unresolved whether Se is an *essential* micronutrient for plants (Terry *et al.,* 2000). Although Se has not been defined as essential, its role as a beneficial element in plants has been revealed in many plant studies (Xue *et al.,* 2001; Hasanuzzaman *et al.,* 2010). In many plant species, Se exerts a positive effect on plant growth and productivity at low concentrations (Terry *et al.,* 2000; Xue *et al.,* 2001; Turakainen *et al.,* 2004; Hasanuzzaman *et al.,* 2010). However, the precise mechanisms underlying the beneficial role of Se in plants have not been clearly elucidated yet. Some plant species supplemented with Se have shown enhanced resistance to certain abiotic stresses including salinity (Hasanuzzaman *et al.*, 2010, 2011, 2012a). Different plant studies have shown that Se could help in detoxification of reactive oxygen species (ROS) and thus enhance plant tolerance to oxidative stress (Djanaguiraman *et al.,* 2005; Hasanuzzaman *et al.,* 2010; Hasanuzzaman and Fujita, 2011).

Although, Se is not yet confirmed to be required by higher plants (Terry *et al.,*, 2000), but several studies demonstrate that at low concentrations it may exert diverse beneficial effects, including growth-promoting activities (Hartikainen and Xue, 1999; Terry *et al.*, 2000; Xue *et al.,* 2001; Turakainen *et al.*, 2004; Djanaguiraman *et al.,* 2005). Moreover, some plant species grown in Se-enriched media have shown enhanced resistance to certain abiotic stresses, e.g. drought (Kuznetsov *et al.,* 2003; Germ *et al.,* 2007b; Yao *et al.,* 2009), salinity (Kong, *et al*, 2005; Djanaguiraman *et al.,* 2005; Hawrylak, 2009;

Hasanuzzaman *et al.*, 2010), chilling (Chu *et al.*, 2010; Hawrylak *et al.*, 2010), heavy metals (Fargašová *et al.*, 2006; Hawrylak *et al.*, 2007; Pedrero *et al.*, 2008; Filek *et al.*, 2008; Srivastava *et al.*, 2009; Cartes *et al.*, 2010) and UV-irradiation (Valkama *et al.*, 2003; Hartikainen and Xue, 1999; Yao *et al.*, 2010) stress. Se exerts beneficial effects on growth and stress tolerance of plants by enhancing their antioxidative capacity (Hartikainen and Xue, 1999; Hartikainen *et al.*, 2000; Xue *et al.*, 2001; Djanaguiraman *et al.*, 2005; Kong *et al.*, 2005; Ríos *et al.*, 2009). Se increases plant resistance against oxidative stress caused by free oxygen radicals. However, agricultural crop plants are sensitive to high Se concentrations which vary among plant species (Hartikainen *et al.*, 2000; Hartikainen *et al.*, 2001; Rani *et al.*, 2005; Lyons *et al.*, 2005).

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 performance of rice varieties
ii. To investigate the oxidative stress incurred by salinity
iii. To investigate the roles an exogenous Se in mitigate salt stress-induced damages in rice.

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-spices *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 (FAOSTAT, 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 and Yabuno, 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, 2002 and Blumwald *et al.*, 2000). 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.*, 2012a, b).

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 3-9^{0}C 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 inter connected, 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 (H_2O_2), superoxide radical (O^{2-}), 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 god 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.*, 2012a).

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, Khodarahmpour *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.*, 2012b). 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, BRRI dhan41, BRRI dhan44 and BRRI 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 Abiotic stress-induced oxidative stress

The chloroplast is the main source of ROS in plants. Insufficient energy dissipation during photosynthesis can lead to the formation of a Chl triplet state that can transfer its excitation energy onto O_2 to make $^1O_2$ (Logan, 2005). O_2^{-} is produced by the photosynthetic electron transport chain (ETC) via the reduction of O_2 (Apel and Hirt, 2004), which is subsequently converted to H_2O_2 by SOD (Noctor *et al.*, 2002). Under stress conditions CO_2 fixation impaired in the chroloplast, the oxygenase activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) increases and glycolate that is produced moves from chloroplasts to peroxisomes (Takahashi and Murata, 2008). In peroxisomes, the generation of H_2O_2 involves glycolate oxidation catalyzed by glycolate oxidase (GO), the β-oxidation of fatty acids and catabolism of lipids (Halliwell, 2006). On the other hand, the generation of $O_2^{\cdot -}$ involves both the reaction of xanthine oxidase (XO) in the organelle matrix and a small electron transport chain is also an important source of ROS production in plant cells and consists of several dehydrogenase complexes that reduce a common pool of ubiquinone (Q). ROS production is likely to occur mainly in complex I (NADH dehydrogenase) and the Q zone (Møller, 2001; Blokhina *et al.*, 2003). Although mitochondrial ROS production is much lower compared to chloroplasts, mitochondrial ROS are important regulators of a number of cellular processes, including stress adaptation and PCD (Robson and Vanlerberghe, 2002). In glyoxysomes, acyl-CoA oxidase is the primary enzyme responsible for the generation of H_2O_2. Plasmamembrane-bound NADPH oxidases (NADPHox) as well as cell-wall associated peroxidases (POX) are the main sources of $O_2^{\cdot -}$ and H_2O_2 producing apoplastic enzymes activated by various forms of stress (Mittler, 2002; Mhamdi *et al.*, 2010). Additional sources of ROS in plant cells include the detoxifying reactions catalyzed by cytochromes in both cytoplasm and the endoplasmic reticulum (Urban *et al.*, 1989).

At the metabolic level abiotic stress induced signal transduction triggers the generation of reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide radicle ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicle ($OH^{\cdot}$), which consequently indirectly promotes oxidative stress by diminished antioxidant cell capacity, leading to oxidative damage, which could be at least partially responsible for stress induced damages (Yadav, 2010; Hasanuzzaman *et al.*, 2012a). Certain environmental stresses or genetic defects cause the production of ROS to exceed the Environmental stresses such as salinity, drought, extreme temperatures, metal toxicity lead to enhanced generation of ROS in plants due to disruption of cellular homeostasis and are extremely harmful to organisms at high concentrations (Hasanuzzaman *et al.*, 2012a, b). When the level of ROS exceeds the defense mechanisms, a cell is said to be in a state of “oxidative stress”. The enhanced production of ROS during environmental stresses can pose a threat to cells by causing peroxidation of lipids, oxidation of proteins, damage to nucleic acids, enzyme inhibition, activation of programmed cell death (PCD) pathway and ultimately leading to death of the cells (Mishra *et al.*, 2011).

As per the report of Tanou *et al.* (2009), it is not possible to determine the concentration of all sources to the generation of ROS under salt stress. Enhanced ROS production under salt stress induces phytotoxic reactions such as lipid peroxidation, protein degradation, and DNA mutations. Several reports showed the overproduction of ROS in plants under saline conditions and ROS-induced membrane damage is a major cause of cellular toxicity by salinity (Mittova *et al.*, 2004; Hasanuzzaman *et al.*, 2011; Hossain *et al.*, 2011).

According to Vinocur and Altman (2005), Reactive oxygen species produced in response to oxidative stress can cause permanent damage to the cellular apparatus. Reactive oxygen intermediates (ROI) typically result from the excitation of O_2 to form singlet oxygen ($^1O_2$) or the transfer of one, two, or three electrons to O_2 to form superoxide radical ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), or a hydroxyl radical ($OH^{\cdot}$), respectively. The enhanced production of ROIs during stresses can pose a threat to plants because they are unable to detoxify effectively by the ROI scavenging machinery. The unquenched ROIs react spontaneously with organic molecules and cause membrane lipid peroxidation, protein oxidation, enzyme inhibition, and DNA and RNA damage.

Shalata and Tal (1998) reported that an unfortunate consequence of salinity stress in plants is the excessive generation of ROS. The excess production of ROS under salinity stress resulted from impaired electron transport processes in chloroplast and mitochondria as well as from pathways such photorespiration causing membrane damage and chlorophyll degradation and responsible for the development of leaf chlorosis and necrosis (Choi *et al.,* 2002).

According to Asada and Takahashi (1987), ROS are a group of free radicles, reactive molecules, and ions that are derived from O_2. It has been estimated that about 1% of O_2 consumed by plants is diverted to produce ROS in various sub cellular loci such as chloroplasts, mitochondria, depending on their concentration in plants.

2.5 Antioxidant defense system

In general, plant cells are adequately equipped to keep ROS within the limits that are generated as a consequence of normal cellular metabolic activities. Under different stress conditions, however, ROS generation often exceeds the overall cellular antioxidative potential leading to stress-induced adverse effects on plant growth and physiology. A steady state balanced is required to protect plant cells from oxidative damage (Hasanuzzaman *et al.,* 2011). Plants possess an efficient non-enzymatic (AsA, GSH, α-tocopherol, phenolic compounds, alkaloids and non-protein amino acids) and enzymatic (SOD, CAT, APX, MDHAR, DHAR, GR, GPX, GST and POD) antioxidant defense systems which work in concert to control the cascades of uncontrolled oxidation and protect plant cells from oxidative damage by scavenging ROS (Gill and Tuteja, 2010b).

These antioxidant defense systems are found in almost all cellular compartments, demonstrating the importance of ROS detoxification for cellular survival (Gill and Tuteja, 2010a).

Ascorbate is an important antioxidant in plant tissues which is synthesized in the cytosol of higher plants primarily from the conversion of D-glucose to AsA. It reacts with a range of ROS such as H_2O_2, $O_2^{\cdot-}$, $^1O_2$ and $OH^{\cdot}$ at diffusion-controlled rates (Smirnoff, 2005). AsA is also responsible for keeping prosthetic metal ions in a reduced form, thereby maintaining the activity of various antioxidant enzymes (De Tullio, 2004). AsA plays an important role in plant stress tolerance (Hossain *et al.*, 2010, 2011; Hasanuzzaman *et al.*, 2011). Exogenous application of AsA influences the activity of many enzymes and minimizes the damage caused by oxidative processes through synergic function with other antioxidants (Shalata and Neumann, 2001).

Glutathione acts as an antioxidant and is involved directly in the reduction of most ROS (Noctor and Foyer, 1998). Additionally, GSH plays a key role in the antioxidative defense system by regenerating other potential water-soluble antioxidants like AsA via the AsA-GSH cycle (Foyer and Halliwell, 1976). GSH is a substrate for GPX and GST, which are also involved in the removal of ROS (Noctor *et al.*, 2002). Other functions for GSH include the formation of phytochelatins (PCs), which have an affinity to HM and are transported as complexes into the vacuole, thus allowing plants to have some level of resistance to HM (Sharma and Dietz, 2006). GSH also takes part in the detoxification of xenobiotics and acts as a storage and transport form of reduced sulfur (Srivalli and Khanna-Chopra, 2008). The role of GSH in the antioxidant defense system provides a strong basis for its use as a stress marker. The change in the ratio of its reduced (GSH) to oxidized (GSSG) form during the degradation of H_2O_2 is important in certain redox signaling pathways (Li and Jin, 2007). GSH acts as a redox sensor of environmental cues, and an increase in GSH provides resistance to plants against oxidative stress. Recent reports suggest that an increase in GSH content enhances protection to various abiotic stresses (Hossain *et al.*, 2010, 2011; Hasanuzzaman *et al.*, 2011).

Antioxidant enzymes are located in different sites of plant cells and work together to detoxify ROS. The major antioxidant enzymes are SOD, CAT, GPX, GST and AsA-GSH cycle enzymes. The AsA-GSH cycle involves 4 enzymes (APX, MDHAR, DHAR and GR) as well as AsA, GSH and NADPH which work together to detoxify H_2O_2 in a series of cyclic reactions and further regenerate AsA and GSH (Hasanuzzaman *et al.*, 2012a).

Catalases (CATs) are tetrameric heme-containing enzymes that use H_2O_2 as a substrate and convert it to H_2O and O_2, thus preventing cells from oxidative damage (Sanchez-Casas and Klesseg, 1994). CATs are present in peroxisomes, glyoxysomes, and related organelles where H_2O_2-generating enzymes are located (Agarwal *et al.*, 2009). CAT has one of the highest turnover rates of all enzymes: one molecule of CAT can convert around six million molecules of H_2O_2 to H_2O and O_2 per minute. Thus, CAT is important in removing H_2O_2, which is generated in peroxisomes by oxidases involved in β-oxidation of fatty acids, photorespiration, and purine catabolism (Gill and Tuteja, 2010). It has also been reported that apart from its reaction with H_2O_2, CAT also reacts with some hydroperoxides (Ali and Alqurainy, 2006). CAT activity shows variable trends under different abiotic stresses (Singh *et al.*, 2008; Hasanuzzaman *et al.*, 2011; Hasanuzzaman and Fujita, 2011).

APX are heme-containing enzymes involved in scavenging H_2O_2 in water-water and AsA-GSH cycles using AsA as the substrate, catalyzing the transfer of electrons from AsA to H_2O_2, producing DHA and water (Pang and Wang, 2010). The APX family consists of at least five different isoforms including mitochondrial (mAPX), thylakoid (tAPX) and glyoxisome membrane forms (gmAPX), as well as chloroplast stromal soluble form (sAPX), cytosolic form (cAPX) (Noctor and Foyer, 1998). APX activity is enhanced in plants in response to during different abiotic stress conditions (Singh *et al.*, 2008; Hasanuzzzaman and Fujita, 2011).

The univalent oxidation of AsA leads to the formation of MDHA. If MDHA is not reduced again to AsA by MDHAR, it will spontaneously disproportionate into AsA and DHA. DHA is then reduced to AsA by DHAR in a reaction requiring GSH (Chen *et al.*, 2003). Rapid regeneration is necessary in order to maintain the antioxidative capacity of AsA. The regeneration of AsA could be regulated in this cycle mainly by NADPH-dependent MDHAR activity (Mittova *et al.*, 2000) and thus it is crucial for AsA regeneration and essential for maintaining a reduced pool of AsA (Martínez and Araya, 2010). Although there are also a few reports about MDHAR activity in other physiological processes those are related to oxidative stress, research on different crops under environmental stresses revealed the regulatory role of MDHAR during oxidative stress tolerance and acclimation (Hasanuzzaman *et al.*, 2011a, b). MDHAR and DHAR are equally important in regulating the level of AsA and its redox state under oxidative stress (Eltayeb *et al.*, 2006, 2007). DHAR is also a key component of the AsA recycling system (Martínez and Araya, 2010) which regenerates AsA from the oxidized state (DHA) and regulates the cellular AsA redox state. It is thus crucial for tolerance to various abiotic stresses leading to the production of ROS. Increased DHAR activity was reported in response to various ROS-inducing stresses (Lee *et al.*, 2007; Hossain *et al.*, 2010; Hasanuzzaman *et al.*, 2011 and Hasanuzzaman *et al.*, 2014).

Glutathione reductase (GR) is a potential enzyme of the AsA-GSH cycle which catalyzes the reduction of GSH, involved in many metabolic regulatory and antioxidative processes in plants where GR catalysis the NADPH-dependent reduction of disulphide bond of GSSG and is thus important for maintaining the GSH pool (Chalapathi Rao and Reddy, 2008). Pang and Wang (2010) reported that GR also maintains a high ratio of GSH/GSSG in plant cells, also necessary for accelerating the H_2O_2 scavenging pathway, particularly under stress conditions. GR plays a crucial role in determining the tolerance of a plant under various stresses by maintaining the antioxidant machinery of the cell, conferring stress tolerance (Hasanuzzaman *et al.*, 2011).

Plant GSTs are a superfamily of multifunctional enzymes which catalyse the conjugation of electrophilic xenobiotic substrates with GSH (Dixon *et al.*, 2010). Among the enzymes related to GSH metabolism, GST isoenzymes account for approximately 1% of a plants total soluble protein (Marrs, 1996). GSTs catalyse the binding of various xenobiotics (including numerous pesticides) and their electrophilic metabolites with GSH to produce less toxic and more water-soluble conjugates (Edwards *et al.*, 2000). Besides catalyzing the conjugation of electrophilic compounds to GSH, GST isoenzymes also exhibit POX activity (Gullner and Kömives, 2001). Various abiotic stresses are powerful inducers of GST activity in plants (Dixon *et al.*, 2010). Plant GSTs are also associated with responses to various forms of abiotic stress (Hossain *et al.*, 2006; Dixon *et al.*, 2010; Hasanuzzaman *et al.*, 2011) and confer stress tolerance in plants.

The activity of ROS-0scavanging enzymes is highly correlated with antioxidant stress defense and abiotic stress tolerance. However, the activities vary with plant cultivar, stress duration and dose.

The generation of ROS and increased activity of many antioxidant enzymes during abiotic stress have been reported in different plant studies with several reports indicating that the activity of antioxidant enzymes of tolerant genotypes increased in response to abiotic stress whereas the sensitive species failed to do so (Hasanuzzaman *et al.*, 2012a).

El-Bastawisy (2010) concluded that salt tolerance was related to the endogenous levels of the enzymatic and the non-enzymatic antioxidants in wheat seedlings. Among the three wheat cultivars (H 168, Gimmeza 7 and Beni swif 1) under observation, the activities of SOD, CAT, APX and GR as well as the non-enzymatic antioxidants (AsA and GSH) increased mostly in H 168, but declined in Gimmeza 7 and particularly in Beni swif 1. H 168 had a superior antioxidant defense system and was more tolerant to NaCl than the other two cultivars due to the higher enzymatic and non-enzymatic antioxidants.

2.6 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, 2002a).

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, 1990 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 valuolar 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 (2000a) 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.* (1997) 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 and Tabita, 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 CO_2 at higher salinity. Identical findings were also noted for the vapor pressure deficit in leaves (VPDL). MR219 showed more slat 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 Mehta (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 (2011) 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.

BRRI dhan47 and Bina dhan10 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 Bina dhan10 at 0 dSm^{-1}. The highest Na content (1.69 %) in shoot was found in BRRI dhan47 at 12 dSm^{-1}. Between these two varieties BINA dhan10 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, BRRI dhan41, BRRI dhan44 and BRRI dhan46). Among the varieties BRRI 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).

2.7 Selenium and crop productivity

Selenium (Se) is a widely studied trace element in human and animal due to its role in antioxidant defense system which is needed for the maintenance of health and hormone balance. During last two decades the physiological role of Se in plants has been explored by researchers. Plant roots take up Se from soil water in either the selenate or the selenite ionic forms. In higher plants metabolism of Se is closely related to that of sulfur due to their chemical similarity. Although, Se is not yet confirmed to be required by higher plants, but several studies demonstrate that at low concentrations it may exert diverse beneficial effects, including growth-promoting activities. Moreover, some plant species grown in Se-enriched media have shown enhanced resistance to certain abiotic stresses, e.g. drought, salinity, extreme temperature, metal toxicity and UV-irradiation. Se exerts its beneficial effects on growth and stress tolerance of plants by enhancing their antioxidative capacity. It enhances plants' resistance against oxidative stress caused by reactive oxygen species (ROS) (Hasanuzzaman and Fujita, 2012)

The growth and biomass of seedlings increased at high Se foliar concentrations and decreased at low and high Se fertigation levels. The seedlings exhibited the highest values for plant height stress tolerance index (PHSI), root length stress tolerance index (RLSI), dry matter stress tolerance index (DMSI), and fresh matter stress tolerance indices (FMSI) at Se fertigation level of 7.35 μM, whereas Se foliar treatment of 7.06 μM resulted in maximum values for these indices. The seedlings foliarly sprayed with Se maintained higher DMSI and FMSI than those fertigated with Se which suggests that Se foliar spray is more effective than Se fertigation for improving drought tolerance (Nawaz *et al.*, 2014)

An application of selenium was favorable for biomass accumulation of barley plants under well-watered conditions. However, it did not significantly affect dry matter accumulation under drought stress, but Se-supplemented water-deficit plants exhibited better protection from oxidative damage because of higher CAT and GSH-Px activities and lower level of lipid peroxidation. These results suggest that selenium application can improve antioxidant defense system under drought stress conditions, and it may be recommended for arid and semiarid regions (Habibi, 2013).

Hajiboland *et al.* (2014) indicated that Se application improves some physiological parameters such as photosynthesis, accumulation of osmolyes and water use efficiency but did not change significantly plants biomass or water relation parameters.

Łabanowska *et al.* (2010) revealed that the supplementation with selenium improved the photosynthesis processes reflecting in the increase of the primary donor P^+ signal intensity in the spectrum of wheat leaves. It is suggested that starch, which is accumulated in greater amounts in plants subjected to cadmium supplementation, may act as traps for free electrons produced under stress conditions.

Ahmed (2010) revealed that increasing selenium levels decreased the fresh weights for the four plants tested statistically significant differences in appetence, plant production, and fresh weights of the plant produced were studied. Next, the amount of selenium retained in the edible plants, no edible plant, and soil for each was analyzed by acid digestion followed by hybride generation atomic absorption analysis. Finally, inhibition effects on the seeds of the addition with an inorganic form of a maximum of 95% retained in the edible portion of lettuce plants.

The addition of inorganic Se forms significantly increased Se content in lupin sprouts in a dose-dependent manner. The highest Se content in lupin sprouts was observed when germination was carried out with selenate solutions at 20 °C (11 μg/g of DW) or 25°C (14 μg/g of DW). The Se-enriched sprouts presented an improvement in antioxidant activity (up to 117.8 and 103.5 μmol of Trolox/g of DW) as well as in essential amino acid content, and no cytotoxicity was observed on HL-60 human leukemic cells. Lupin seeds germinated with 8 mg/L selenate solutions for 5 days at 20 °C exhibited a higher germination rate (>90%) and a higher concentration of some essential amino acids than those obtained in selenite solutions in the same germination conditions. Therefore, the employment of selenate solutions at a concentration of 8 mg/L and germination for 5 days at 20 °C may be suggested for the production of Se-enriched lupin sprouts (Frias *et al.*, 2009).

Se treatment was associated with a 43% increase in seed production. The Se-treated Brassica plants had higher total respiratory activity in leaves and flowers, which may have contributed to higher seed production (Lyons *et al.*, 2009).

Different components of the antioxidant defense system played a pivotal role in overcoming oxidative damage by Se in the macroalga, and explained the lack of morphological and ultra structural alterations in *Ulva* sp. exposed to selenate (Schiavon *et al.*, 2012).

Se enhanced the salt tolerance of seedlings by protecting the cell membrane against lipid peroxidation. The growth-promoting effect of low Se concentrations (5 and 10 μM) under saline conditions could be due to the antioxidative activity of Se, increase in proline accumulation and/or decrease in content of chloride ions in the shoots tissues. Thus, optimal Se supplementation presents a promising potential for use in conditions of relatively high levels of NaCl in the medium (Hawrylak, 2009).

Sajedi *et al.* (2011) indicated that the activity of superoxide dismutase and malondialdehyde content under water deficit stress increased, but grain yield was reduced. The highest grain yield was obtained from optimum irrigation, while in the case of with water deficit stress at V8 stage it was non significant. Selenium spray increased activity of superoxide dismutase enzyme, malondialdehyde content of leaves in V8, R2 and R4 stages and also grain yield. Application of microelements increased the leaves superoxide dismutase enzyme activity and malondialdehyde content. Selenium and microelements spray under water deficit stress conditions during vegetative growth and dough stage increased grain yield in comparison to not spraying elements under water stress conditions.

Treatments with 1.0 and 2.0 mg Se kg^{-1} promoted biomass accumulation of wheat seedlings. Treatments at 1.0, 2.0, and 3.0 mg Se kg^{-1} significantly increased root activity, proline content, peroxidase (POD), and catalase (CAT) activities, carotenoids (Car) content, chlorophyll content, and reduced malondialdehyde (MDA) content of wheat seedlings. Lower Se treatment did not significantly effect on chlorophyll content and MDA content, although it also increased some antioxidant index (proline and Car content, POD and CAT activities) in wheat seedlings. These results suggest that optimal Se supply is favorable for growth of wheat seedlings during drought condition (Yao *et al.*, 2009).

Selenium due to its physiological and toxicological importance has become an element of interest to many plant scientists. There are reports in literature of selenium beneficial role in plants. It enhances growth of plants (Hartikainen and Xue, 1999), reduces 20 damage caused by UV-induced oxidative stress (Hartikainen and Xue, 1999; Hartikainen *et al.,* 2000; Valkama *et al.,* 2003), enhances chlorophyll contents under light stress (Seppanen *et al.,* 2003), stimulates senescing plants to produce antioxidants, and improves plant tolerance to drought stress by regulating water status (Kuznetsov *et al.,* 2003; Djanaguiraman *et al.,* 2005; Ekelund and Danilov, 2001). Selenium is known to increase superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities thus activating the protective mechanisms against oxidative stress. It is also reported to exert an antioxidant effect directed towards a decreased concentration of intracellular active oxygen species by inducing the biosynthesis of proline and peroxidase production (Kuznetsov *et al.,* 2003).

In plants, selenium uptake and translocation takes place through sulphate transporters (STs) and phosphate transporters (PiTs). Once absorbed by the plants, the inorganic Se is converted into more bioavailable organic forms like selenomethionine (SeMet). It is presumed that STs located in root cell membranes are involved in the uptake of selenate while PiTs are generally accepted to be involved in selenite uptake (Hopper and Parker, 1999). A large number of enzymes such as APS reductase (APSR), ATP sulphurylase (ATPS), sulphite reductase (SiR) etc. take part in sulphate assimilatory pathway to incorporate selenate or selenite into selenocysteine (SeCys) and selenomethionine (SeMet) (Rotte and Leustek, 2000). Selenium uptake may be influenced by genetic factors and cultivars may differ in their Se content (Eurola *et al.,* 2004). Rate and method of Se application are reported to affect grain Se levels while other factors like the yield of the crop may also affect grain Se concentration through dilution effects, i.e., a low yield crop of dry areas may have higher grain Se than a high yielding crop grown on an irrigated site (Curtin *et al.,* 2006).

The soil and/or foliar application of Se fertilizers (agronomic biofortification) are often used to improve the Se concentration of food. Moreover, plants act as an effective buffer that can protect humans from toxic Se intakes that may take place with direct Se supplementation (Hartikainen, 2005). The application of selenate fertilizers (soil and/or foliar application) has proved to be more effective to increase plant Se concentrations than selenite fertilization (Gissel-Nielsen *et al.*, 1984; Singh *et al.*, 1980) and hence selenate is the predominant form of Se in Se fertilization of plants (Broadley *et al.,* 2007). The chemical form of Se, soil characteristics, time of foliar and method of basal application affects the relative 21 effectiveness of soil or foliar applied Se fertilizers (Lyons *et al.,* 2003). Stephen *et al.* (1989) reported an increase of 32% in Se concentrations of wheat plants by sodium selenate fertilization on silt loam soil. They supplied sodium selenate at varying rates of 5, 10, 15 and 20 g Se ha^{-1} as prills drilled with the wheat seed, Se seed coating or a foliar spray at mid tillering and/or ear emergence and found foliar application at ear emergence stage to be more effective than other methods of Se application. In Finland, Se is added at a rate of 10 mg kg^{-1} as a soil amendment in NPK fertilizer (Eurola and Hietaniemi, 2000) and has proved to be a safe, easy, effective and cost efficient approach to increase Se levels in a human population. Cartes *et al.,* (2005) found that treatment of soil with selenite and selenate (0-10 mg kg^{-1}) increased the Se concentration in ryegrass seedlings and recorded a significant positive correlation between shoot Se concentration and glutathione peroxidase (GSH-Px) activity.

The higher shoot Se concentration in selenate treated plants suggested that activity of this enzyme was related with the chemical form of applied Se rather than with the concentration of Se in plant tissues. More recently, Ducsay *et al.* (2009) reported that soil Se application significantly increased the Se content in the dry matter of roots, straw and grains of wheat. The application of 0.2 mg Se kg^{-1} of soil gave highest Se content in grain (0.732 mg kg^{-1}), straw (0.227 mg kg^{-1}) and roots (1.375 mg kg^{-1}) dry matter whereas the lowest dose of Se (0.05 mg Se kg^{-1} of soil) gave 0.155 mg Se kg^{-1} in grain. The results of the study showed that Se concentration was highest in grain and lowest in the straw dry matter.

The foliar application of Se on wheat significantly increased the levels of plasma Se (53% increase after 6 weeks) in Serbia. It caused an increase in the activity of glutathione peroxidase in blood and decreased oxidative stress parameters (Djujic *et al.* 2000a). An increase in the levels of copper, iron and zinc by Se-enriched wheat was observed in erythrocytes, compared to consumption of low-Se wheat (Djujic *et al.* 2000b). Germ *et al.* (2007a) found that foliar application of 1 mg sodium selenate per liter doubled the concentration of Se (43-46 ng Se g^{-1} DM) compared to the control (21-24 ng Se g^{-1} DM) and it also increased the respiratory potential in young plants without any visible toxic effects.

Xu and Hu (2004) reported in their study on Se-enriched rice that Se concentration was significantly enhanced dose dependent in rice. They reported an increase in the antioxidant activity of Se enriched rice by Se foliar application. 22 The low rate of selenate (10 g ha^{-1}) was found to be equally effective in both basal and foliar application while foliar gave better results at 50 g ha^{-1} and no significant difference was observed at the high rate of 500 g ha^{-1} (Ylaranta, 1983). In the follow-up trials, foliar applied selenate at the 3-4 leaf stage was found to be more effective than basal application in variety of soils. The wheat grain Se level increased from 16 to 168 μg kg^{-1} by the foliar application of selenate at the rate of 10 g ha^{-1} while basal application of 9g raised it to ust 77 μg kg^{-1}. Thus it was concluded that foliar application was the more effective method with the exception from low rainfall areas (Ylaranta, 1984).

The variation among cereal crop cultivars for nutrients like zinc and iron (Graham *et al.*, 2001) suggest the possibility of such high variation for Se. In an experiment conducted in controlled field by Lyons *et al.* (2005), it was observed that significant genetic variation does not exist among modern wheat genotypes however, larger variation and higher grain Se concentration was observed in diploid wheat (*Aegilops taushii*) and rye. The soil physical and chemical properties significantly affect variation in Se accumulation in wheat and even within few meters of field; large variation in grain Se concentration might occur (Lyons *et al.*, 2005). Therefore the homogeneity of the field site for available soil Se is very important for the assessment of genotypic variation in grain Se concentration and content.

The hydroponic conditions provide a better medium to study the uptake efficiency for Se. It not only removes the limitations of heterogonous soil conditions but also provide more reliable information about Se accumulation by different genotypes (Cary and Allaway, 1969). The experiments carried out in water cultures on lettuce revealed that sodium selenite ($Na_2SeO_3.5H_2O$) applied at the rate of 5 μM in nickel (Ni) contaminated medium prevented the toxic effects of Ni and stimulated plant growth by increasing the concentration of assimilation pigments (Hawrylak *et al.*, 2007).

2.8 Effects of selenium on rice under salt stressed condition

Three Se levels were developed by the addition of 0, 20 and 200 mg L^{-1} Na_2SeO_3 and rice seedlings were harvested in week 4. Se uptake in roots of rice showed significant differences among all varieties and Se uptake significantly increased with increased in Se levels. Other root parameters (length, average diameter, surface area, volume and number of root tips) did not show any significant differences at different Se treatments (Alifar *et al.*, 2014).

Liu *et al.* (2004) reported that a suitable supply of selenium could promote rice growth and excessive selenium could injure rice plant, causing lower biomass, especially in the roots. The supply of selenite could enhance the selenium contents of rice shoots and roots in solution culture and in soil culture. The selenium concentrations in roots were much higher than those in shoots supplied with the same rates of selenium and phosphorus.

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 December-May, 2013-14. 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-calcarious 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

Three rice genotypes BRRI dhan45, BRRI dhan47 and Nipponbare were used in the experiment. The features of two varieties are presented below:

BRRI dhan45: BRRI dhan45 variety is grown in Boro season. It released by Bangladesh Rice Research Institute (BRRI) in 2005. It is a salt sensitive variety. It completes its life cycle in 140-145 days. Its life cycle is same as BRRI dhan29 but yield is high. It attains a plant height 90-100 cm. 1000-seed weight is 26 g, grain is medium and white in color. Protein content is 7.2% and yield is 6.0-6.5 t ha-1.

BRRI dhan47: BRRI dhan47 is a salt tolerant variety grown in Boro season released by BRRI. It completes its life cycle in 152 days. It can tolerate salinity 12-14 dS/m during seedlings stage and 6 dS/m during maturity stage. Grain is medium and there is a white spot in the grain. Its plant height is 105 cm and yield is 6.0 t ha^{-1}.

Nipponbare: Nipponbare is a Japanese Boro rice variety. It completes its life cycle in 140 days. Plant height is about 90 cm. Grain is medium size and white in color. It can give yield 6.5 0 t ha^{-1}.

3.4.2 Earthen pot

63 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 32 DAT, 47 DAT, 59 DAT and 75 DAT. There were four 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), S50 (50mM), S100 (100mM) and S150 (150mM). When no salt added it termed as control (C) while 175.5g salts in S50, 351g salts in S100, and 526.5g salts in S150 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 Protectant treatments

Selenium (Se) was used as a protectants. The concentration of Se was 0.5mM and applied as a solution 0.56675g powder form of Se was mixed with 2 ml distilled water and then in 60 L water to prepare solution.

3.7 Treatments

The experiment consisted of two factors as mentioned below:

a) Factor A: varieties

- i. BRRI dhan45
- ii. BRRI dhan47
- iii. Nipponbare

b) Factor B: Salinity level

- i. Control (C)
- ii. 50 mM NaCl (S50)
- iii. 50 mM NaCl+ 0.5 mM S (S50 + Se)
- iv. 100 mM NaCl (S100)
- v. 100 mM NaCl+ 0.5 mM S (S100 + Se)
- vi. 150 mM NaCl (S150)
- vii. 150 mM NaCl+ 0.5 mM Se (S150+Se)

3.8 Design and layout

The experiment was laid out in Randomized Completely Block Design (RCBD) with three replications. There were 63 pots all together replication with the given factors. Two separate sets of pots were palced for growth and yield analysis.

Conduction of the experiment

3.9 Seed collection

Seeds of BRRI dhan 45 and BRRI dhan 47 were collected from Bangladesh Rice Research Institute, Joydebpur, Gazipur. Seeds of Nipponbare were collected from Faculty of Agriculture, Kagawa University, Japan.

3.10 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.

3.11 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).

3.12 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.

3.13 Uprooting and transplanting of seedlings

One Seedling of thirty days old was uprooted carefully from the seedbed and transplanted in the respective pots at the rate of single seedling hill^{-1} on January 22, 2014.

3.14 Intercultural operations

3.14.1 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.

3.14.2 Plant protection measures

Before heading green leafhopper infestations were observed in the crop and they were successfully controlled by applying Durshban two times on 55 DAT and 62 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.

3.15 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

3.16 Detecting maximum tillering and panicle initiation stages

Maximum tillering and panicle initiation stages were detected through field observations. When the number of tillers 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.

3.17 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 at 15 days interval up to harvest
- Number of tillers plant^{-1} at 15 days interval up to harvest

3. Physiological parameters:

- Chlorophyll content
- Relative water content (RWC)

4. Yield contributing and other parameters:

- Panicle length

- Number of grains panicles^{-1},
- Number of filled grains panicle^{-1}
- number of unfilled grains panicle^{-1}
- 1000-grain weight^{-1}
- Number of effective tillers plant^{-1}
- Number of non-effective tillers plant^{-1}

5. Yields:

- Grain yield pot^{-1}
- Straw yield pot^{-1}

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

3.18.1 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.

3.18.2 Number of tillers 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 tillers plant^{-1}.

3.19 Procedure of sampling phenological parameters

3.19.1 Chlorophyll content

Three leaves were randomly selected from each pot. The top and bottom of each leaves 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.

3.19.2 Relative water content (RWC)

Three leaves 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 80∘C for 48 h. Then calculation was done using the following formula:

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

3.20 Procedure of sampling yield contributing parameter

3.20.1 Plant height

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

3.20.2 Effective tillers plant^{-1}

The total number of tillers plant^{-1} was counted from pots and were grouped in effective and non-effective tillers plant^{-1}.

3.20.3 Panicle length

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

3.20.4 Number of total grains panicle^{-1}

Grains of 5 randomly selected panicle of each replication were counted and then the average number of grains for each panicle was determined.

3.20.5 Grain yield pot^{-1}

The grains were separated by threshing plant^{-1} and then sun dried and weighed.

3.20.6 Straw yield pot^{-1}

The straw were separated by threshing plant^{-1} and weighed.

3.20.7 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.

3.21 Statistical analysis

The data obtained for different parameters were 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 dhan47 at all growth duration (27.96 cm at 30 DAT, 45.47 cm at 45 DAT, 66.27 cm at 60 DAT, 87.74 cm at 75 DAT, 95.72 cm at 90 DAT and 89.57cm at harvest) compared to BRRI dhan45 and Nipponbare. Sultana *et al*, (2014) found the similar result that BRRI dhan47 gave the higher plant height than BINA dhan10. Lee *et al.* (2003) observed that tolerance level of *indica* was higher than that of *japonica*.

4.1.1.2 Effect of salinity level

There was a significant variation observed in plant height due to different salinity treatments. Salinity reduced the plant height compared to its respective control in all growth duration. However, Se increased plant height up to 150 mM salt stress for all stage (Table 1). But in case of Se treated with 150mM salt stress plant height statistically simillar with 150mM salt stress at all stages except 60 DAT. The highest result found in control and only Se treated plant (30.30 and 28.91cm at 30 DAT, 46.04 and 44.58cm at 45 DAT, 67.33 and 64.70cm at 60 DAT and 94.80 and 91.54cm at 75 DAT, 100.80 and 96.81 cm at 90 DAT, 92.08 and 89.15cm at harvest respectively). Choi *et al.* (2003) observed that the plant height decreased in the 0.5% saline water in the soil. Similar opinion was also postulated by Saleque *et al.* (2005). During the vegetative period, the most common salinity effect was stunting of plant growth, whereas leaf withering was less apparent (Alam *et al.*, 2001).

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	At Harvest
BRRI dhan 45	23.44b	35.95c	47.71c	78.93b	81.80c	73.61c
BRRI dhan 47	27.96a	45.48a	66.27a	87.73a	95.72a	89.56a
Nipponbare	26.34c	42.12b	58.58b	82.13b	87.28	79.43b
LSD $_{(0.05)}$	**1.14**	**1.70**	**2.74**	**3.58**	**4.13**	**3.87**
Salinity level						
C	30.30a	46.04a	67.33a	94.80a	100.80a	92.08a
S50	26.60b	41.63c	61.86b	89.00bc	94.13bc	84.70bc
S50+Se	28.91a	44.58ab	64.70ab	91.54ab	96.81ab	89.15ab
S100	23.77c	40.56c	53.61c	79.84d	84.13d	78.21d
S100+Se	26.52b	42.72bc	60.66b	85.48c	90.17cd	82.10cd
S150	21.86d	35.76d	44.26d	68.32e	74.71e	68.17e
S150+Se	23.42cd	36.96d	50.22c	71.55e	77.17e	71.67e
LSD $_{(0.05)}$	**1.75**	**2.60**	**4.19**	**5.47**	**6.3227**	**5.91**
CV (%)	**7.09**	**6.64**	**7.65**	**6.93**	**7.52**	**7.68**

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

Sharp decreases in plant height was observed in response to salt stress, compared to the untreated control at 30, 45, 60, 75, 90 DAT and at harvest for three rice varieties (Table 2). However, Se supplementation with salt treatment increased plant height for three varieties. But in case of 150 mM saline treatment, plant height became statistically similar with Se treated with salt treatment at 30, 60, 75, 90 DAT and at harvest for BRRI dhan45, at 30, 45, 75, 90 DAT and at harvest for BRRI dhan47 and at 30, 45, 60, 75 and at harvest for Nipponbare. Nawaz *et al.* (2014) found the similar result that application of Se either foliar spray or fertigation improves the plant height under abiotic stress condition.

Table 2. Plant height of three Boro rice varieties at different growth duration induced by saline, Se and their combination.

Variety	Salinity treatment	Plant height (cm)					
		30 DAT	45 DAT	60 DAT	75 DAT	90 DAT	At Harvest
BRRI dhan 45	C	27.63c-f	42.26d-g	61.23d-g	94.93ab	96.53a-d	86.53b-e
	S50	23.03hij	35.50ghi	54.26ghi	87.70a-d	90.26cde	76.60e-h
	S50+Se	25.23fgh	39.33e-i	55.60f-i	90.70a-d	93.36b-e	83.66c-g
	S100	21.23ij	32.93j	40.50j	75.50ef	75.90fg	71.13hij
	S100+Se	24.16ghi	37.56hi	51.40hi	84.06cde	85.73def	75.10f-i
	S150	20.96j	31.30k	31.63k	56.40g	64.13h	60.66k
	S150+Se	21.83ij	32.73j	39.36jk	63.23g	66.70gh	61.56jk
BRRI dhan 47	C	32.63a	49.70a	75.76a	96.36a	105.73a	102.00a
	S50	28.83b-e	46.73abc	67.46bcd	92.46abc	99.56abc	96.16ab
	S50+Se	31.73ab	48.66ab	74.20ab	95.10ab	103.23ab	98.56a
	S100	26.06efg	46.56a-d	62.30def	88.90a-d	91.76cde	84.96c-f
	S100+Se	28.13c-f	45.96a-d	69.67abc	89.50a-d	99.43abc	88.03bc
	S150	22.96hij	39.86fgh	52.23hi	75.16ef	84.46ef	77.63d-h
	S150+Se	25.36fgh	40.83efg	62.26def	76.66ef	85.86def	79.66c-h
Nipponbare	C	30.63abc	46.16a-d	65.00cde	93.10abc	100.13abc	87.73bcd
	S50	27.93c-f	42.66c-f	63.86cde	86.83bcd	92.56b-e	81.33c-h
	S50+Se	29.76a-d	45.76a-d	64.30cde	88.83a-d	93.83b-e	85.23c-f
	S100	24.03ghi	42.20def	58.03e-h	75.13ef	84.73ef	78.53c-h
	S100+Se	27.26def	44.63a-d	60.93d-g	82.90de	85.33ef	83.16c-g
	S150	21.66ij	36.13hij	48.93i	73.40f	75.53fg	66.23ijk
	S150+Se	23.06g-j	37.33ghi	49.03i	74.76ef	78.86f	73.80ghi
CV (%)		**7.09**	**6.64**	**7.65**	**6.93**	**7.52**	**7.68**
LSD (0.05)		3.03	4.51	7.26	9.47	10.95	10.25

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

Salinity stresses greatly affected the development and viability of tillers in this experiment. All the rice varieties in this experiment were significantly influenced by salinity levels in terms of tiller production Varietal variation had significant effect on tillers hill^{-1} over time (Table 3). The highest tillers hill^{-1} was found in BRRI dhan47 compared to BRRI dhan45 and Nipponbare (9.04 at 30 DAT, 15.50 at 45 DAT, 19.92 at 60 DAT, 22.91 at 75 DAT and 22.28 at 90 DAT throughout the growing period. This result is supported by Zeng and Shannon (2000b).

Table 3. Effect of variety and salinity level on number of total tiller 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 dhan45	5.61c	10.07c	15.98c	19.32b	20.24b	19.67b
BRRI dhan47	9.04a	15.50a	19.92a	22.91a	22.28a	22.24a
Nipponbare	6.06b	12.27c	18.76b	19.80b	19.62b	18.72b
LSD $_{(0.05)}$	**0.34**	**0.67**	**0.87**	**1.19**	**0.98**	**1.07**
Salinity level						
C	9.90a	16.20a	23.46a	26.64a	26.07a	25.18a
S50	7.76b	13.42b	19.77b	22.29b	22.18b	22.94b
S50+Se	9.41a	15.67a	22.40a	25.13a	25.11a	23.41b
S100	6.28d	11.83c	17.77c	19.75c	20.46c	20.34c
S100+Se	6.83c	12.95b	18.22c	20.92bc	22.12b	20.03c
S150	3.98e	8.93d	12.83d	14.88d	14.58d	15.24d
S150+Se	4.45e	9.31d	13.09d	15.12d	14.46d	14.33d
LSD $_{(0.05)}$	**0.53**	**1.02**	**1.34**	**1.82**	**1.50**	**1.64**
CV (%)	**8.11**	**8.56**	**7.74**	**9.29**	**7.62**	**8.56**

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

Different salinity treatments affected tiller production significantly throughout the growing period. Salinity treatment reduced tiller number compared to control (Table 3). On the contrary, Se with saline treatments increased tiller number (21%, 9%, and 12% at 30 DAT; 17%, 9% and 4% at 45 DAT; 13%, 3% and 2% at 60 DAT; 13%, 6%, 2% at 75DAT; 13%, 8% at 90 DAT and 2% at harvest, respectively) where higher level of salinity treatment did not affect by Se application (150 Mm salinity stress at 90 DAT and harvest). Sometimes medium level of salinity treatment gave similar result with Se treated salinity treatment. 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 (Zeng and Shannon, 2000b). Similarly, 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 data (Table 4) showed that salinity also reduced the tiller number hill^{-1} in Boro rice cultuvars. On the other hand, the magnitude of decrease was less in BRRI dhan47 as compared to BRRI dhan45 and Nipponbare. The Se treated salt-stressed seedlings had significantly higher tiller number hill^{-1} (18%, 6% at 30 DAT; 37%, 5% at 45 DAT; 25%, 2% at 60 DAT; 18%, 5% at 75 DAT, 14%, 13% at 90 DAT and 3%, 8% at harvest in BRRI dhan45; 27%, 10%, 16% at 30 DAT; 4%, 6%, 6% at 45 DAT; 7%, 4%, 6% at 60 DAT; 13%, 8%, 5% at 75 DAT; 15%, 4%, 3% at 90 DAT and 1%, 3% at harvest in BRRI dhan47 and 32%, 8%, 19% at 30 DAT; 17%, 17%, 14% at 45 DAT; 10%, 2%, 5% at 60 DAT; 7%, 5%, 4% at 75 DAT; 10%, 8%, 5% at 90 DAT and 2%t harvest in Nipponbare at Se treated 50, 100 and 150 mM NaCl stresses, respectively), compared to the seedlings subjected to salt stress without Se treatment. Sometimes 150 mM, Se could not give any higher result compared to without Se treatment for all varieties. As a result, tiller number was statistically similar or decreased from its control. This result indicates that Se improves the tiller production under lower salinity condition which is similar with Nawaz *et al.* (2014).

Table 4. Effect of S**e** on number of tillers hill^{-1} of Boro rice varieties under saline and non-saline conditions at different age.

Variety	**Salinity level**	**Number of tillers hill^{-1}**					
		30 DAT	45 DAT	60 DAT	75 DAT	90 DAT	At harvest
BRRI dhan 45	C	8.18cd	13.92ef	22.84ab	26.36ab	25.83ab	24.90ab
	S50	6.50ef	10.14h	17.38ghi	20.50defg	21.90def	22.06bcd
	S50+Se	7.71d	13.88ef	21.76bcd	24.27bc	25.05abc	22.76bc
	S100	5.31h	9.76hi	14.82jk	18.35g	19.44fg	19.23def
	S100+Se	5.66fgh	10.28h	15.09ijk	19.32efg	22.03def	20.73cde
	S150	3.01k	6.59jk	10.38mn	13.62h	14.40hi	14.76ghi
	S150+Se	2.88k	5.92k	9.59n	12.83h	13.03i	13.23i
BRRI dhan 47	C	12.14a	18.52a	24.31a	28.01a	27.00a	26.73a
	S50	9.27b	16.48bc	21.64bcde	24.35bc	23.45bcd	24.43ab
	S50+Se	11.76a	17.22ab	23.16ab	27.64a	27.05a	24.60ab
	S100	8.64bc	14.39def	18.71fgh	20.37defg	21.74def	23.36bc
	S100+Se	9.51b	15.27cde	19.43efg	21.92cde	22.61cde	22.70bc
	S150	5.53gh	12.90fg	15.66ij	18.54fg	16.84gh	16.70fgh
	S150+Se	6.44efg	13.72ef	16.55hij	19.55efg	17.28g	17.16fg
Nipponbare	C	9.39b	16.17bcd	23.24ab	25.55ab	25.39ab	23.93ab
	S50	6.65e	13.64ef	20.30cdef	22.02cde	21.19def	22.33bc
	S50+Se	8.77bc	15.91bcd	22.28abc	23.49bcd	23.24bcd	22.86bc
	S100	4.90h	11.33gh	19.78def	20.54defg	20.21ef	18.43ef
	S100+Se	5.32h	13.29f	20.15cdef	21.54cdef	21.73def	16.66fgh
	S150	3.40jk	7.29jk	12.45lm	12.49h	12.52i	14.26hi
	S150+Se	4.03ij	8.29ij	13.13kl	12.97h	13.09i	12.60i
CV (%)		**0.92**	**1.78**	**2.32**	**3.16**	**2.6038**	**2.85**
LSD (0.05)		**8.11**	**8.56**	**7.74**	**9.29**	**7.62**	**8.56**

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 a significant variation observed for relative water content due to varietal variation (Fig 1). BRRI dhan47 (56.51%) recorded the highest relative water content compared to BRRI dhan45 (31.11%) and Nipponbare (39.25%). In this experiment BRRI dhan47 showed the better tolerance than other two varieties.

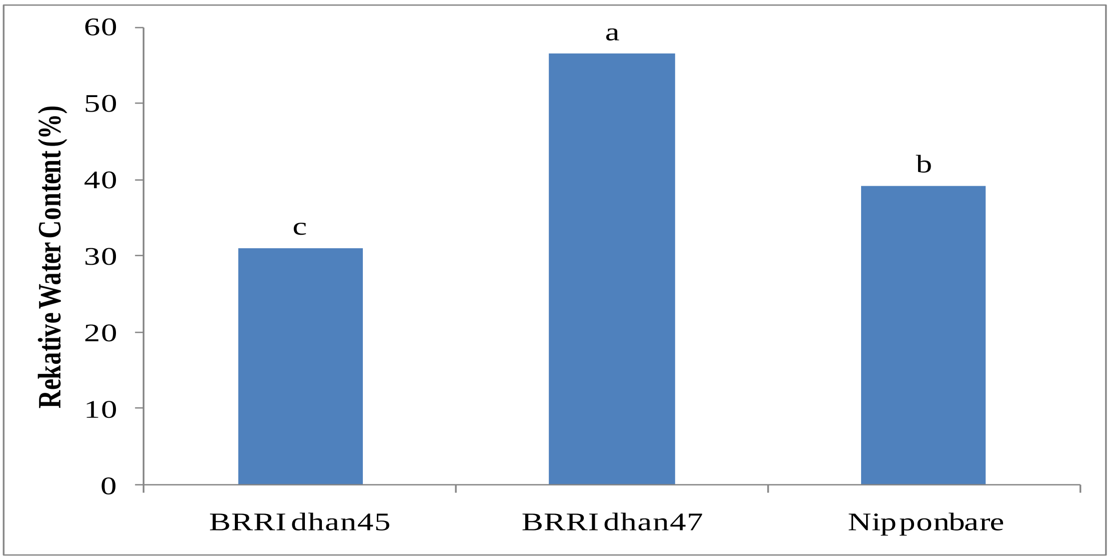

Figure 1. Effect of variety on relative water content of Boro rice (Bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

4.2.1.2 Effect of treatment

Sharp decreases in relative water content (12%, 20% and 35% at 50, 100 and 150 mM salt stressed condition) were observed in response to salt stress, compared to untreated control (Fig 2). Moreover, Se could increase relative water content under all salt stressed condition. But At 150 mM stressed condition, Se application gave statistically similar result. Similar decrease in RWC 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).

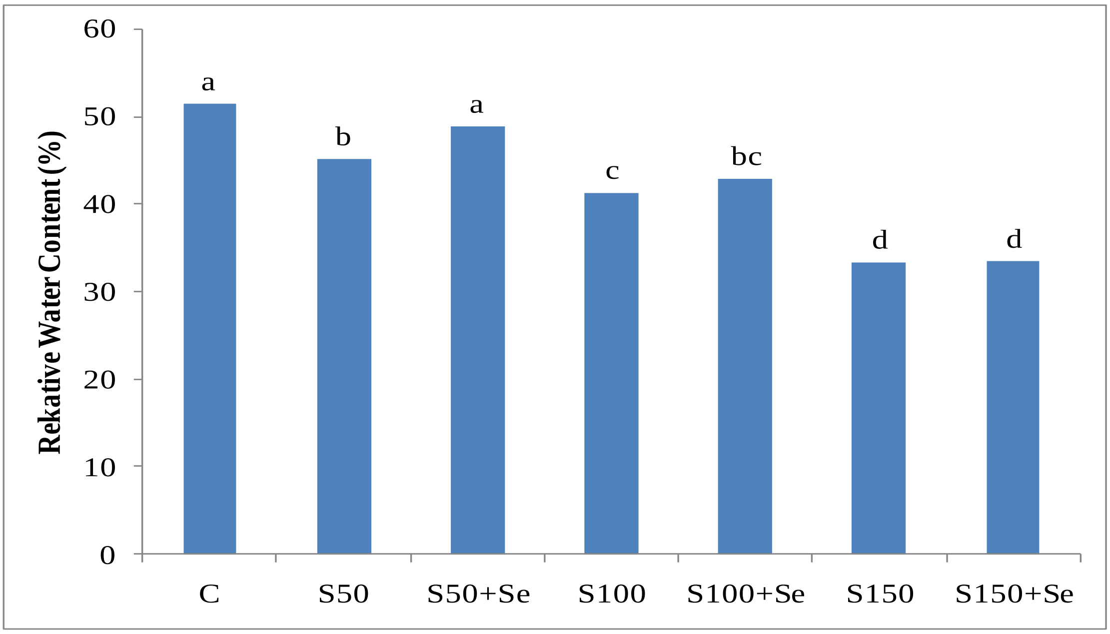

Figure 2. Effect of salinity level on relative water content of Boro rice (Bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

4.2.1.3 Interaction effect of variety and salinity level

Upon exposure to salt stress, leaf relative water content decreased significantly in all rice varieties when compared to their controls (Fig 3). However, decline in RWC was lower in BRRI dhan47 as compared to BRRI dhan45 and Nipponbare. At 100 mM of NaCl it was decreased by 20%, 19% and 20% in BRRI dhan45, BRRI dhan47 and Nipponbare respectively over control while at 150 mM the RWC decreased by 36%, 25% and 48% (Fig 3). The application of Se effectively maintained the RWC in salt stressed seedlings. In BRRI dhan 45, Se could increase RWC by 6%, 4% and 1% in seedlings exposed to 50, 100 and 150 mM NaCl respectively. In case of BRRI dhan 47, the increases were 9%, 4% and 1% at 50, 100 and 150 mM. Se increased the RWC 9% and 3% for Nipponbare at 50 and 100mM saline condition. But at 150 mM NaCl, RWC was decreased by 2% for Nipponbare (Fig 3). Decrease in LAI might have been due to decrease in leaf expansion in salinity stress condition. This result corroborates with Bal and Dutt (1984). The enhanced water content in plants due to exogenous application of Se was also observed by other researchers (Habibi, 2014).

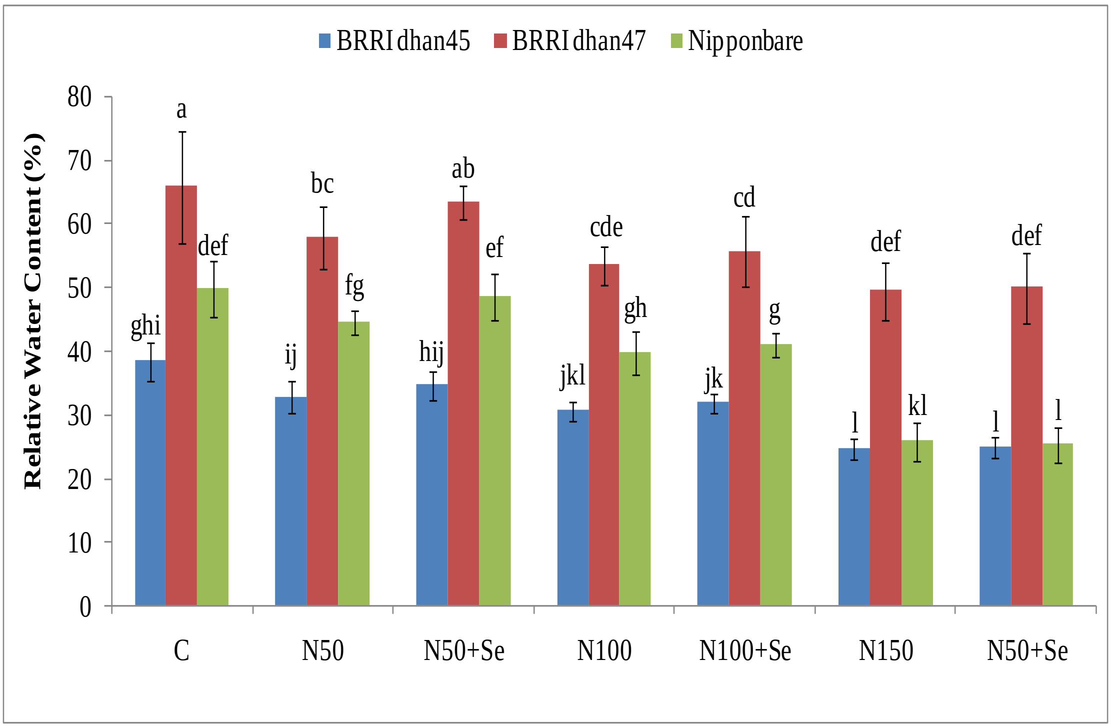

Figure 3. Relative water content of Boro rice leaves induced by saline, Se and their combination (Bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

4.2.2 Chlorophyll content

4.2.2.1 Effect of variety

Variety showed significant variation in chlorophyll content (Fig 4). Nipponbare the highest chlorophyll content (0.0450mg cm^{-2}) compared to BRRI dhan45 (0.0405 mg cm^{-2}) and BRRI dhan47 (0.0399mg cm^{-2}). The salt caused reduction in chl content, in three rice varieties. However, the reduction was higher in salt sensitive BRRI dhan45 (Fig 4).

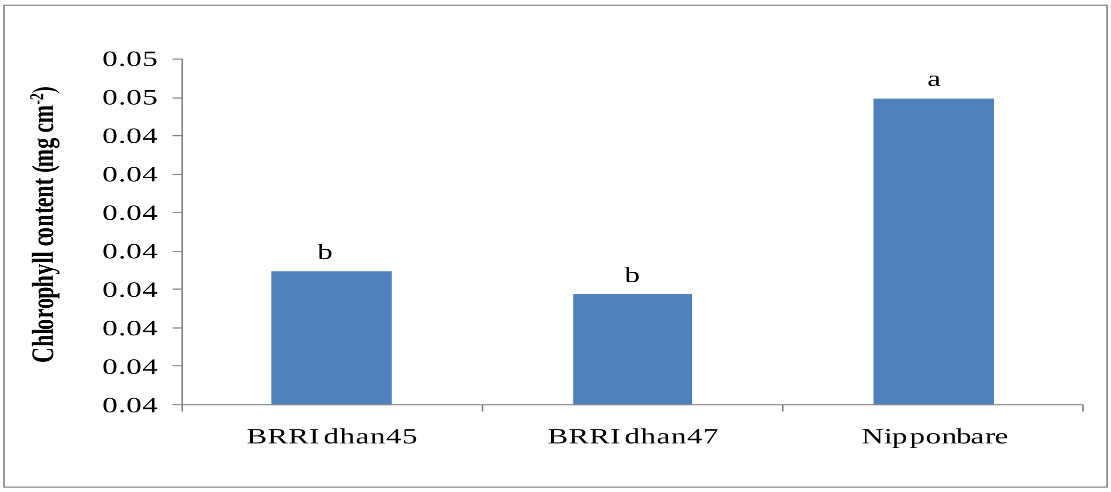

Figure 4. Effect of variety on chlorophyll content of Boro rice varieties (Bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

4.2.2.2 Effect of salinity level

Different salinity level affected chlorophyll production significantly throughout the growing period. Salinity treatment reduced total chlorophyll content compared to its respective control (Fig 5). On the contrary, Se with saline level increased chlorophyll content (1, 2 and 4% at 50, 100 and 150 mM, respectively). Salt stress often causes alteration in photosynthetic pigment biosynthesis (Maxwel and Jhonson, 2000). Similar decrease in chl content was observed by Amirjani (2011) in rice.

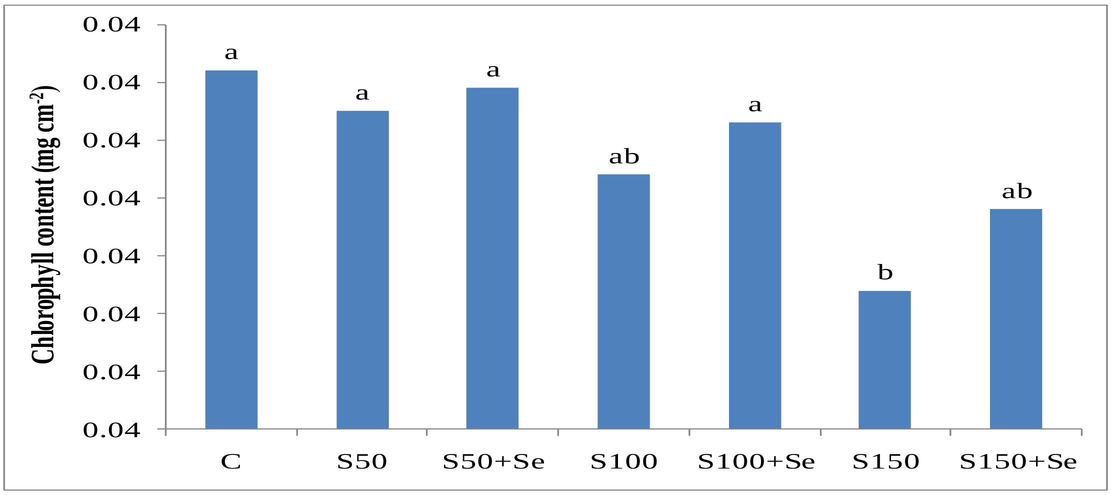

Figure 5. Effect of treatment on chlorophyll content (mg cm^{-2}) of Boro rice (Bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

4.2.2.3 Interaction effect of variety and salinity level

Chlorophyll content was also affected by salinity stress, according to Fig 6. In case of BRRI dhan45, chlorophyll content decreased 4, 6, and 16% at 50, 100 and 150 mM salinity stress, respectively (Fig 6). Reductions in chl content were 1, 4 and 4% at 50, 100 and 150 mM salinity stress in case of BRRI dhan47, respectively. Se treatment significantly increased the chl content under stress condition. The highest amount chl (0.0461 mg cm^{-2}) was found in Nipponbare under control and the lowest amount chl was 0.03688 mg cm^{2} in BRRI dhan45 under 150 mM salinity treated with Se. However, exogenous application of Se in salt treated seedlings could elevate the chl content which might be due to the higher biosynthesis of the pigment. These results are in agreement with Hasanuzzaman *et al.* (2010).

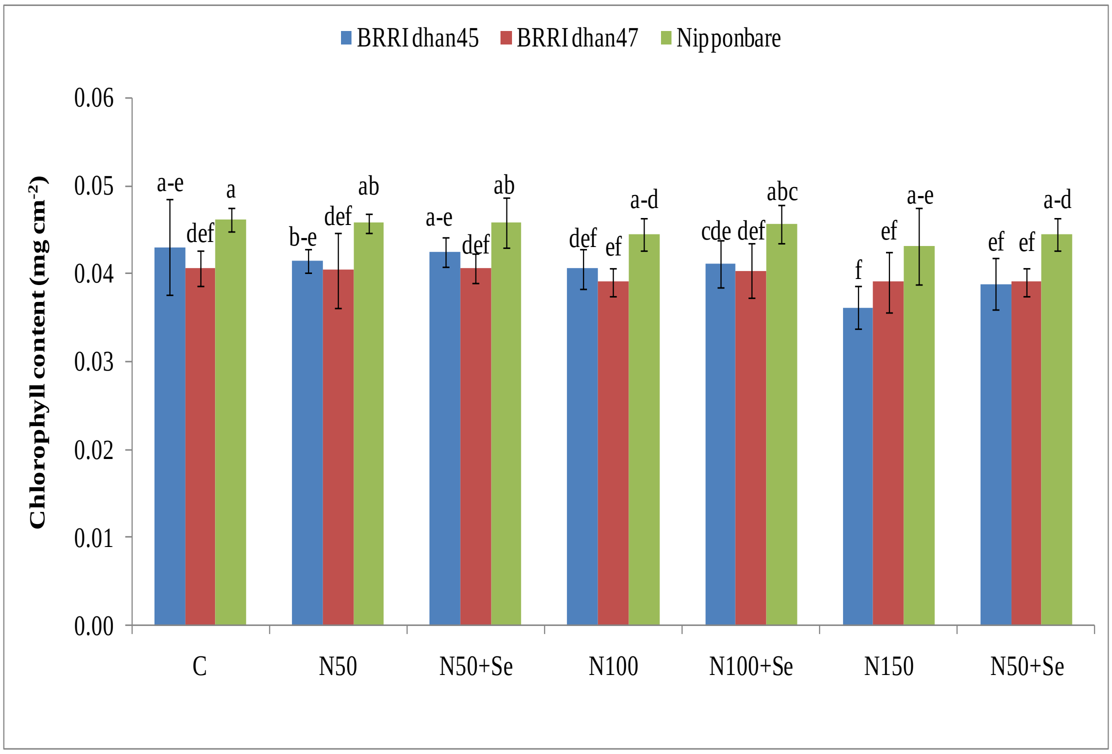

Figure 6. Chlorophyll content of Boro rice leaves induced by saline, Se and their combination (Bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

4.3 Yield contributing characters

4.3.1 Effective tiller hill^{-1}

4.3.1.1 Effect of variety

The effective tiller varied significantly due to variety shown in Fig 7. It was observed that BRRI dhan47 produced significantly the highest effective tiller (18.17) compare to BRRI dhan45 (16.40) and Nipponbare (15.07).

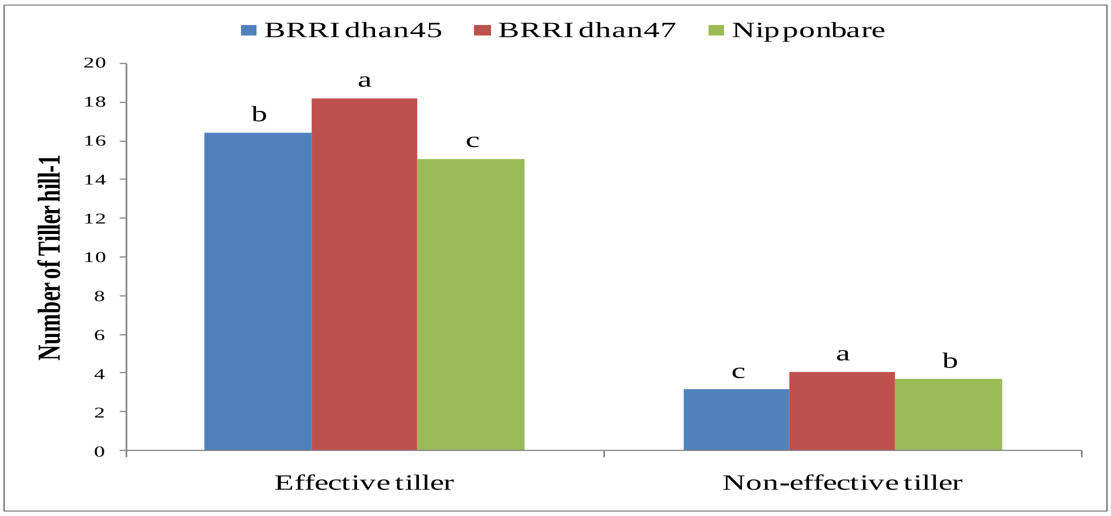

Figure 7. Effect of variety on total number of tillers hill^{-1} of Boro rice (Bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

4.3.1.2 Effect of salinity level

Salinity caused a significant reduction of effective tiller compared to control (Fig 8). The highest effective tiller was found in control. On the contrary, Se increased effective tiller number compared to its respective control but not similar with control and only Se treated plant (12 and 4% at 50 and 100 mM stressed condition, respectively). 150 mM salt stressed condition was also not affected by Se treatment (Fig 8). Khatun *et al.* (1995) found that salinity delayed flowering, reduced the number of productive tillers, the number of fertile florets per panicle. Salt tolerance indexes in terms of seed yield, seed weight/ panicle, spikelet number/panicle, and tiller number/plant were reduced with increasing salinity (Zeng *et al.*, 2002).

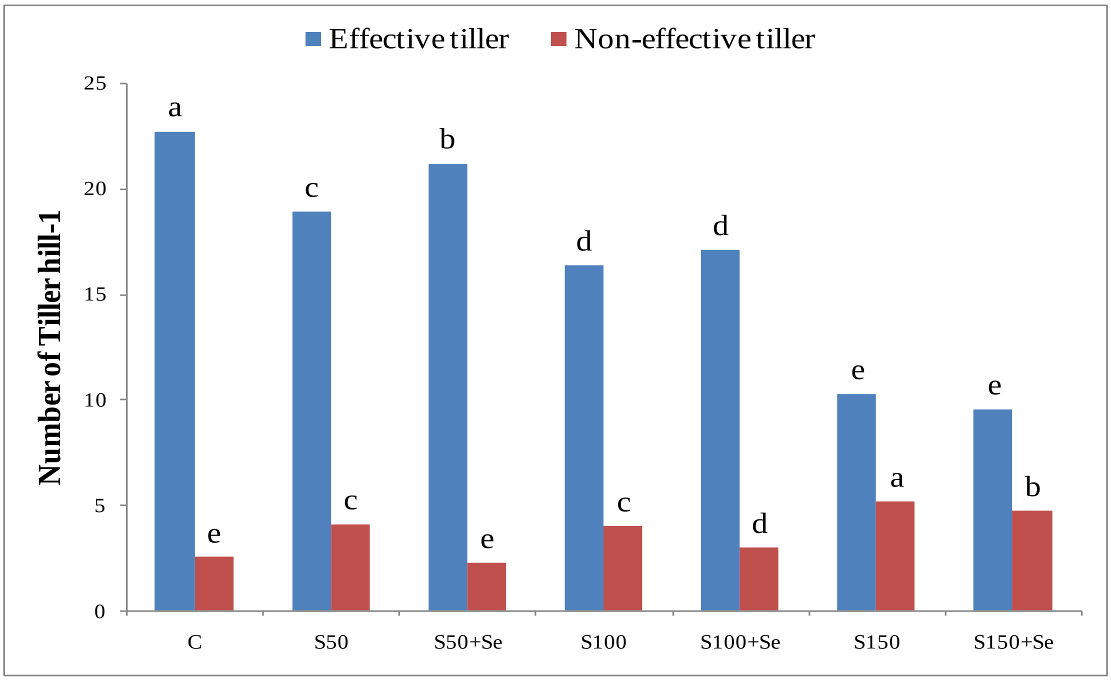

Figure 8. Effect of treatment on number tiller hill^{-1} of Boro rice (Bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

4.3.1.3 Interaction effect of variety and salinity level

Exposure to salt stress resulted in significant decreases in effective tiller number hill^{-1}: 17, 31 and 56% for BRRI dhan45; 17, 23 and 54% for BRRI dhan47 and 16, 31 and 57% for Nipponbare at 50, 100 and 150 mM salinity stressed conditions respectively when compared to unstressed control plant (Fig 9). Addition of exogenous Se combination with salinity stress significantly increased the effective tiller number up to 100 mM by 13 and 14% for BRRI dhan45; all stages by 10, 4 and 10% for BRRI dhan47 and only 50 mM by 13% for Nipponbare in Se treated 50, 100 and 150 mM stressed plant respectively (Fig 9), when compared to plants exposed to salt stress alone. Furthermore, in case of 150 mM salt stressed condition for BRRI dhan47 and 100 mM and 150 mM for Nipponbare, there had no statistically difference from each other of both varieties. Yao X. *et al.* (2009) found that optimal Se supply is favorable for growth yield of wheat seedlings during abiotic stress condition.

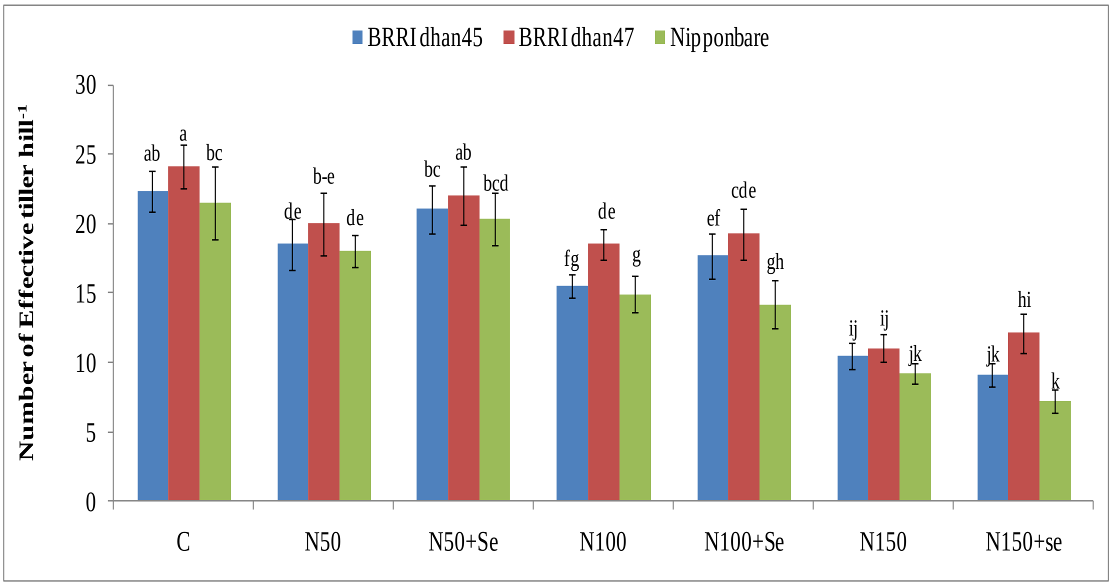

Figure 9. Number of effective tillers hill^{-1} in Boro rice cultivars induced by saline, Se and their combination (Bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

4.3.2 Non-effective tiller hill^{-1}

4.3.2.1 Effect of variety

Significant variation was observed in non-effective tiller due to the effect of variety shown in Fig 7. BRRI dhan47 produced higher non-effective tiller (4.09) compared to BRRI dhan45 (3.19) and for Nipponbare (3.71). Although BRRI dhan47 is a salt tolerant variety, BRRI dhan45 and Nipponbare gave lower number of non-effective tiller hill^{-1} than BRRI dhan47 (Fig 7).

4.3.2.2 Effect of salinity level

Upon exposure to salt stress, non-effective tiller decreased significantly compared to their controls (Fig 8). The highest non-effective tiller number was found in 150 mM salt stressed plant (5.15). Moreover, the lowest non-effective tiller was found in only Se treated plant (2.25). Number of non effective was increased with the increased of salinity level.

4.3.2.3 Interaction effect of variety and salinity level

Salinity stress caused increased number of non-effective tiller hill^{-1} of three rice varieties. The highest non-effective tiller was found in 150mM salt stressed condition of BRRI dhan47 (Fig 10). Upon salinity stress treatment the number of non-effective tiller was increased by 37, 47 and 66% for BRRI dhan45; 77, 86 and 123% for BRRI dhan47 and 75, 42 and 127% for Nipponbare at 50, 100 and 150 mM, respectively, as compared to their respective control (Fig 10). Se supplementation reduced the number of non-effective tiller in the salt stressed condition at all stages for all three varieties. But in high saline condition, the reduction of non effective tiller was statistically similar with the saline condition. However, Exogenous application of Selenium showed positive effects and increased yield contributing characters in ryegrass (Hartikainen *et al.*, 2000), lettuce (Xue *et al.*, 2001), potato (Turakainen *et al.*, 2004), and pumpkin (Germ *et al.*, 2005).

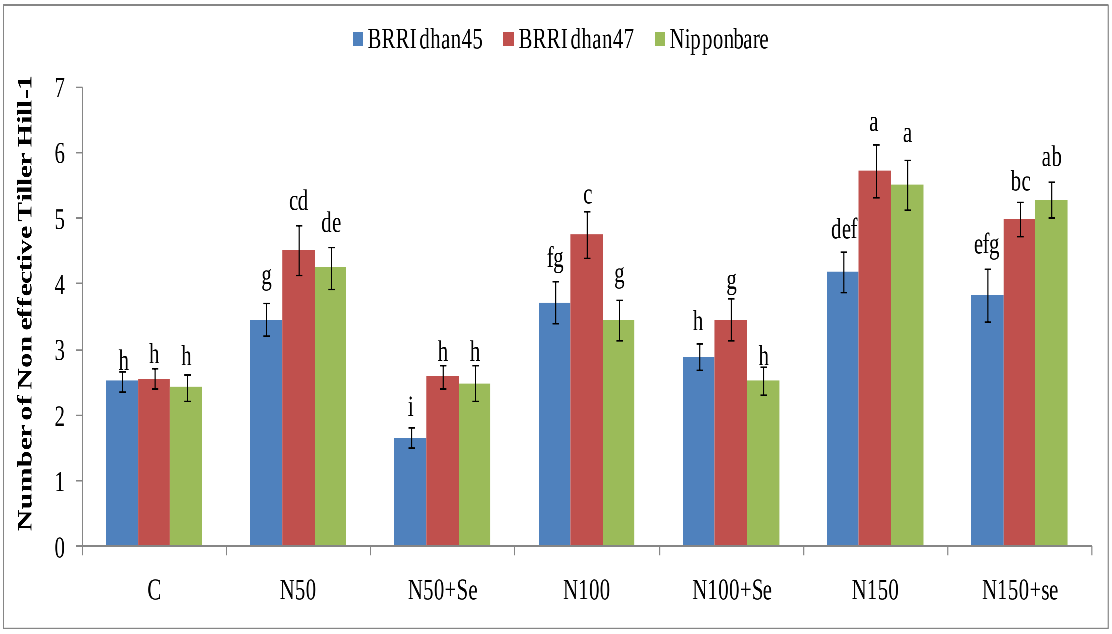

Figure 10. Effect of Se on number of non-effective tillers hill^{-1} of BRRI dhan45, BRRI dhan47 and Nipponbare under saline and control condition (Bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

4.3.3 Panicle length

4.3.3.1 Effect of variety

The panicle length was varied significantly due to variety shown in Fig 11. It was observed that BRRI dhan47 produced significantly the highest panicle length (25.46 cm) compare to BRRI dhan45 (14.43 cm) and Nipponbare (17.99 cm). This result was supported by Asch *et al.*, (1997) who worked with eight rice cultivars and found that cultivars differed in their salt uptake and tolerant cultivars had lower salt effect on yield and yield components than the susceptible ones.

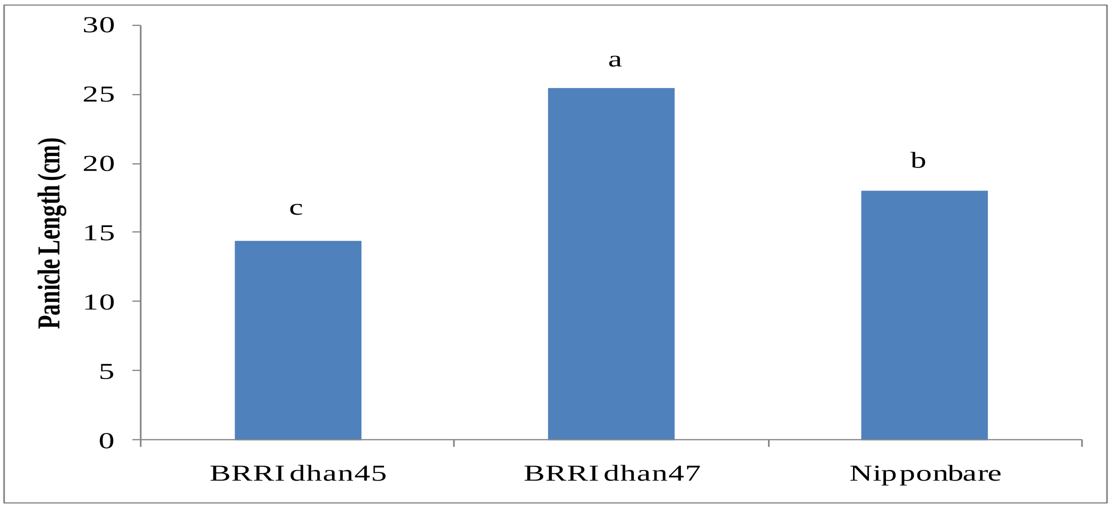

Figure 11. Effect of variety on panicle length of Boro rice varieties (Bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

4.3.3.2 Effect of salinity level

Length of panicle was also affected by salinity stress, according to according to Fig 12. Saline treatment reduced the length of panicle compared to control and Se treated plant. 15, 26 and 39% reduced due to 50, 100 and 150 mM salinity stress, respectively. Furthermore, the reduction was less in Se treated stressed plant compared to respective control (Fig 12). But Se could not affect 150 mM salt stressed condition where it gave similar result with 150 mM stressed condition. Reduction in panicle length was observed with increase of salinity level by Sajjad (1984b), Heenan *et al.* (1988) and Khatun *et al.* (1995) and Hasamuzzaman *et al.* (2009). However, exogenous application of Se increases the panicle length which is supported by the Emam, M.M. *et al.* (2014).

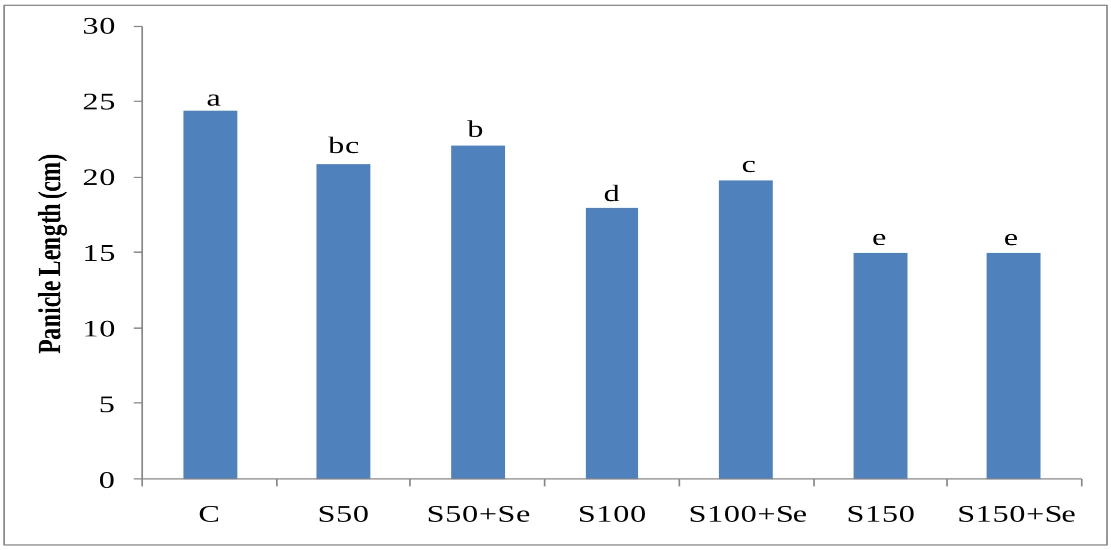

Figure 12. Effect of salinity level on panicle length of Boro rice (Bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

4.3.3.3 Interaction effect of variety and salinity level

Length of panicle of BRRI dhan45, BRRI dhan47 and Nipponbare varieties were decreased by 18%, 33%, 54%; 9%, 19, 30% and 20%, 30%, 36% in 50, 100 and 150 mM respectively, as compared to their respective control (Fig 13). In contrary, exogenous Se supplementation caused increased the length of panicle for BRRI dhan47 and Nipponbare varieties all saline condition. But in case of BRRI dhan45 panicle length increased up to 100 mM saline condition. Moreover, it had no statistically significance with each other. Maximum reduction in length of panicle due to salinity stress was observed in BRRI dhan45 compared to BRRI dhan47 and Nipponbare.

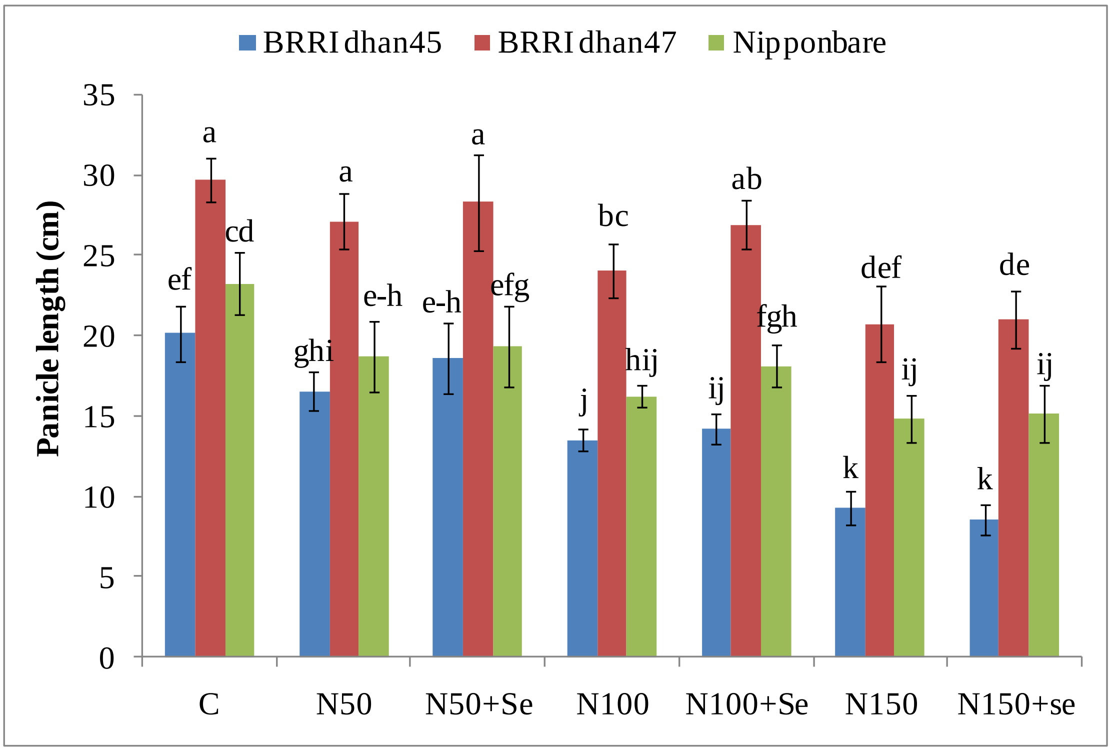

Figure 13. Effect of Se on panicle length of BRRI dhan45, BRRI dhan47 and Nipponbare under saline and control condition (Bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

4.3.4 No. of filled grain panicle^{-1}

4.3.4.1 Effect of variety

Significant variation was observed in filled grain panicle^{-1} due to the effect of variety shown in Fig 14. Productuion of filled grain panicle^{-1} was higher in BRRI dhan47 (83.70) compared to BRRI dhan45 (40.78) and for Nipponbare (61.02).

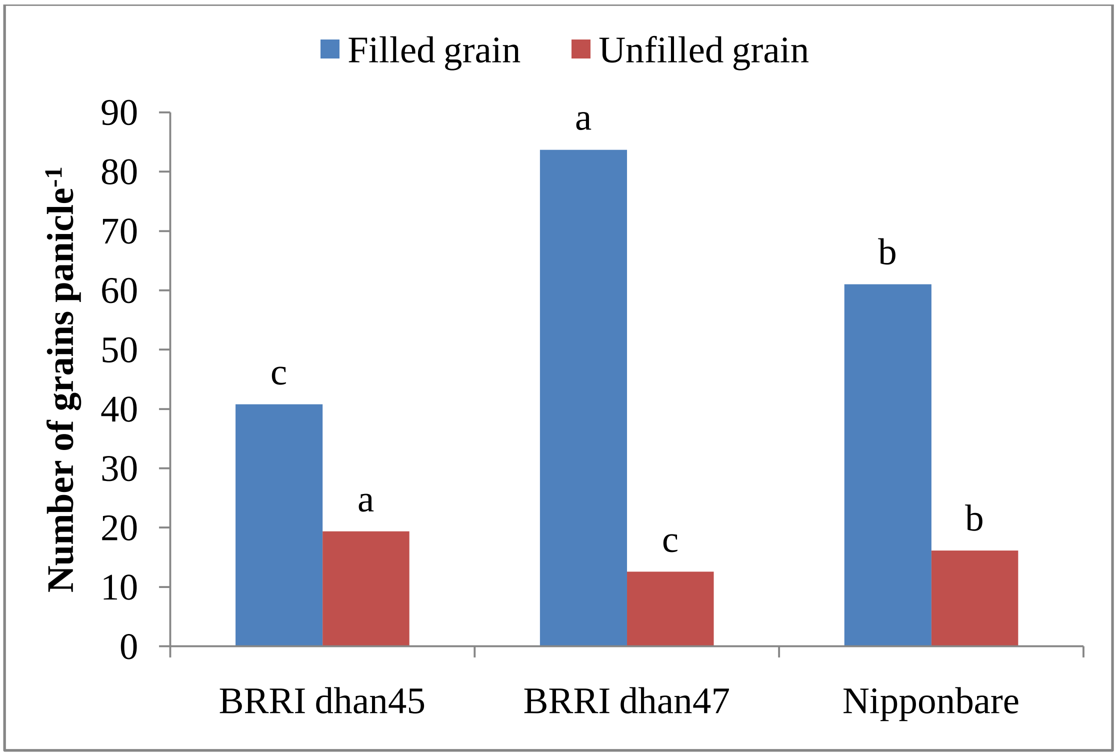

Figure14. Effect of variety on grain panicle^{-1} of Boro rice varieties (Bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

4.3.4.2 Effects of level

Different salinity level affected on the number of filled grain panicle^{-1} significantly throughout the growing period. Salinity treatment reduced total number of filled grain panicle^{-1} compared to its respective control (Fig 14). On the contrary, Se with saline level increased the number of filled grain panicle^{-1} (12, 11 and 6% at 50, 100 and 150 mM, respectively). Filled grain panicle^{-1} also decreased significantly with increase in level of salinity. The lowest filled grains panicle^{-1} was observed at 150 mM NaCl level (Hasamuzzaman *et al.,* 2009). Zaibunnisa *et al.* (2002) and Zaman *et al.* (1997) reported that filled grain per panicle decreased by salinity.

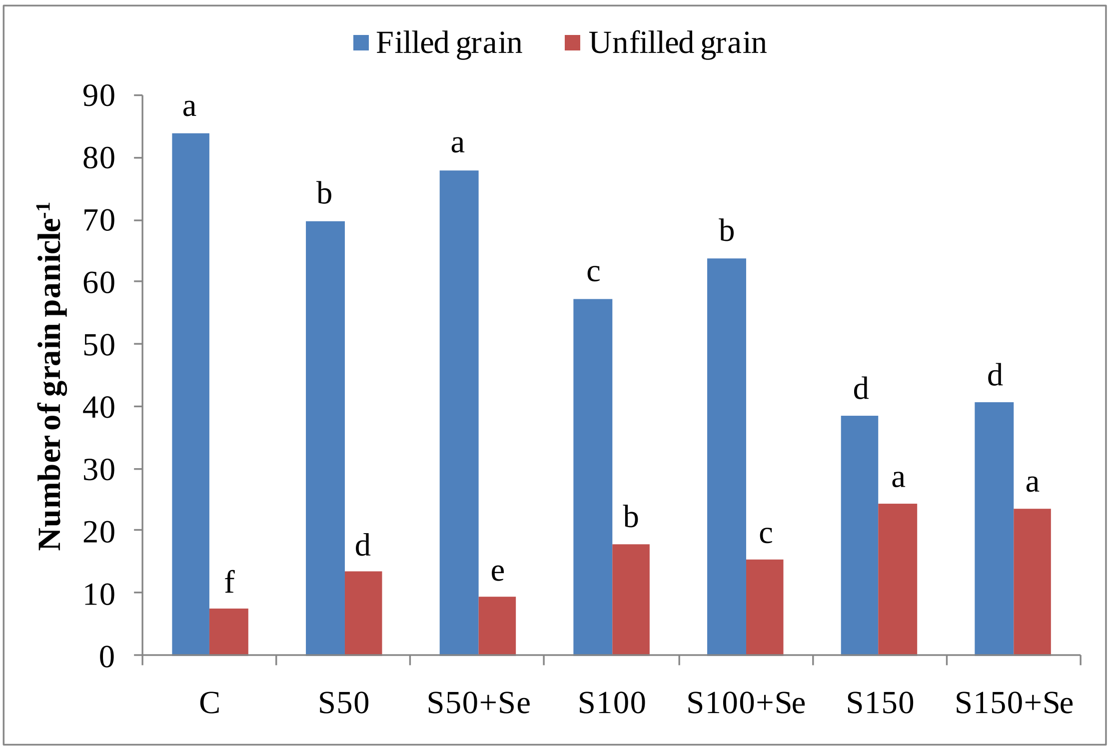

Figure 15. Effect of level on number grain panicle^{-1} of Boro rice (Bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

4.3.4.3 Interaction effect of variety and salinity level

Number of filled grain panicle^{-1} of BRRI dhan45, BRRI dhan47 and Nipponbare varieties were decreased by 27%, 40%, 71%; 10%, 26, 48% and 17%, 32%, 49% in 50, 100 and 150 mM respectively, as compared to their respective control (Fig 13). In contrary, exogenous Se supplementation caused increased the number of filled grain panicle^{-1} for BRRI dhan45 and BRRI dhan45 varieties all saline condition. But in case of Nipponbare number of filled grain panicle^{-1} increased up to 100 mM saline condition. Moreover, it had no statistically significance with each other. However in 150 mM salinity decreased the number of filled grain panicle^{-1} for Nipponbare.

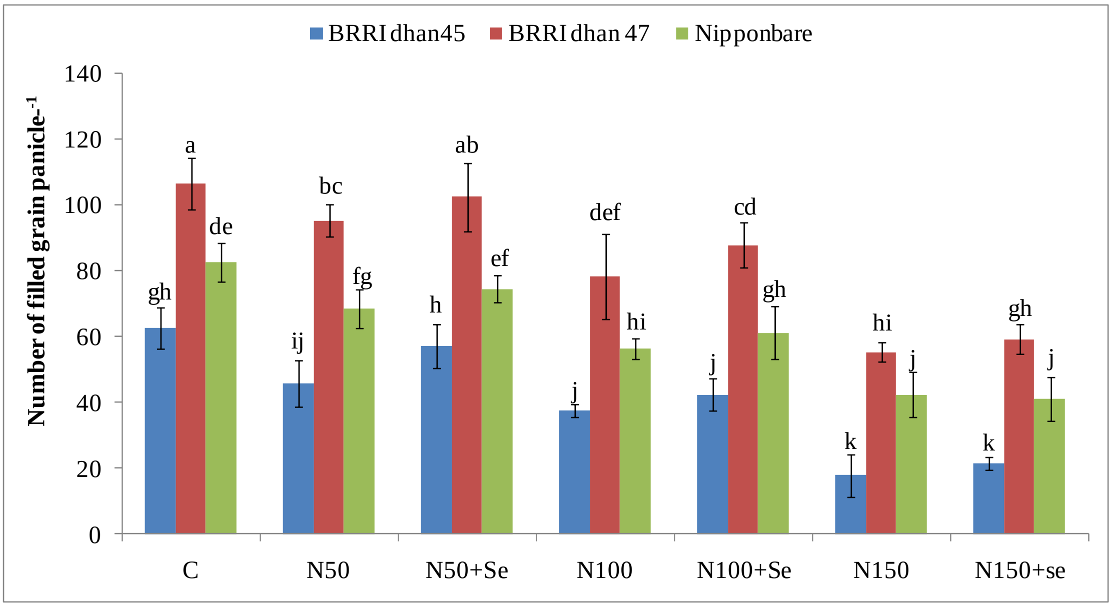

Figure 16. Effect of Se on filled grain panicle $^{-1}$ of BRRI dhan45, BRRI dhan47 and Nipponbare under saline and control condition (Bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

4.3.5 Number of unfilled grain panicle^{-1}

4.3.5.1 Effect of variety

Significant variation was observed in number of unfilled grain panicle^{-1} due to the effect of variety shown in Fig 14. BRRI dhan45 produced higher number of unfilled grain panicle^{-1} (19.38) compared to BRRI dhan47 (12.57) and for Nipponbare (16.15).

4.3.5.2 Effects of salinity level

Upon exposure to salt stress, number of unfilled grain panicle^{-1} decreased significantly compared to their controls (Fig 15). The highest number of unfilled grain panicle^{-1} r was found in 150 mM salt stressed plant (24.48). Moreover, the lowest number of unfilled grain panicle^{-1} was found in only Se treated plant (9.46).

4.3.5.3 Interaction effect of variety and salinity level

Number of unfilled grain panicle^{-1} was increased with the increase of salinity which was 88, 130and 227% for BRRI dhan45; 49, 138, 248% for BRRI dhan47 and 92, 154, 204% for Nipponbare at 50, 100 and 150 mM of salinity stress, respectively (Fig 17). Extent of reduction was higher in BRRI dhan47 than BRRI dhan45 and Nipponbare. However, exogenous application of Se decreased the number of unfilled grain panicle^{-1} for all *varieties* under saline and non-saline conditions. The Se application decreased the number of unfilled grain panicle^{-1} under saline conditions in three varieties. Significantly lowest number of unfilled gran panicle^{-1} was recorded in controlled condition and Se treated 50 mM saline condition of BRRI dhan47. However, the height number of unfilled grain panicle^{-1} was observed in 150 mM saline condition for BRRI dhan45 (Fig 17).

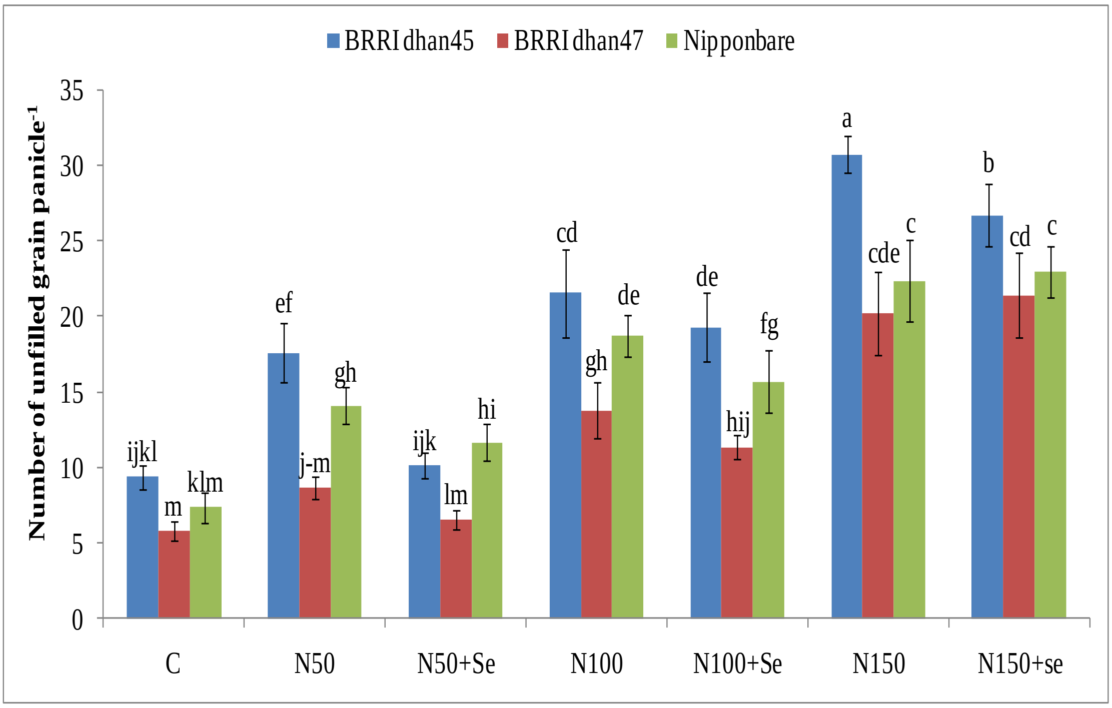

Figure 17. Effect of Se on unfilled grain panicle^{-1} of BRRI dhan45, BRRI dhan47 and Nipponbare under saline and control condition (Bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

4.3.6 1000 grain weight

4.3.6.1 Effect of variety

Weight of 1000 grains showed significant variation among the different varieties (Fig 18). BRRI dhan47 produced highest 1000 grain weight (20.42g). The lowest 1000 grain weight (18.65g) was obtained from BRRI dhan45. Thousands grain weight of Nipponbare was 19.44 g (Fig 18).

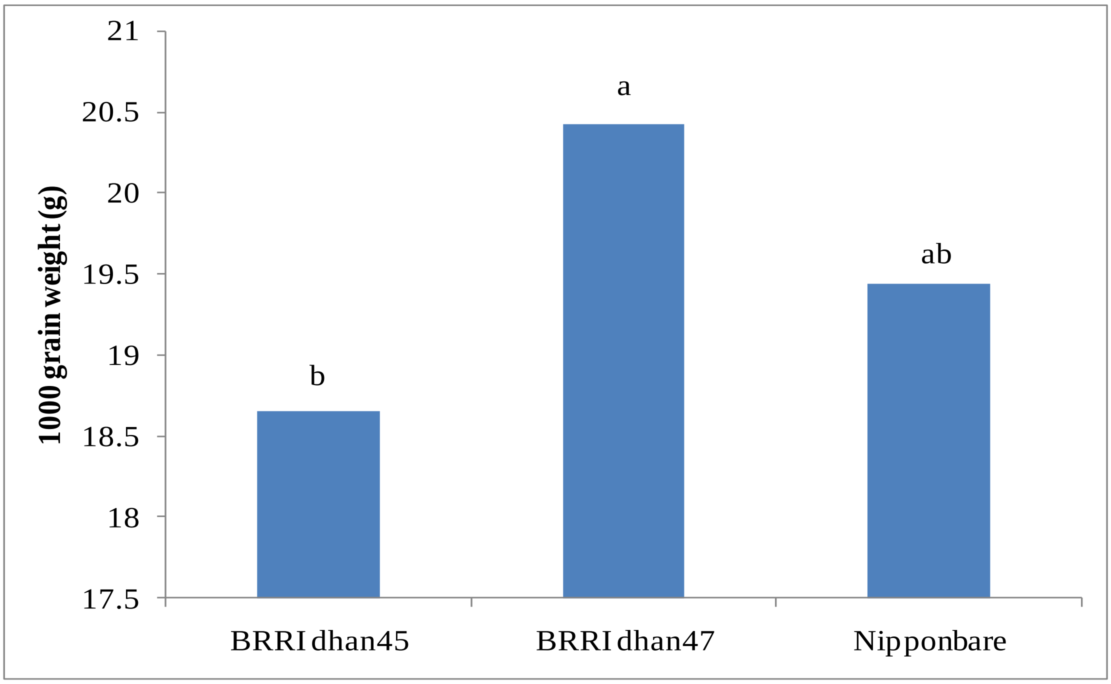

Figure 18 .Effect of variety on 1000 grain w eight of Boro rice varieties (Bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

4.3.6.2 Effect of level

Salinity reduced 1000 grain weight compared to control. Se treatment increased 1000 grain weight under stressed condition (8 and 6% at 50 and 100 mM). But in 150 mM stressed condition produced application of Se did not affect on the 1000 grain weight (Fig 19). This might be due to lower accumulation of carbohydrates and other food materials due to salt stress. Khatun and Flowers (1995) reported that 1000-grain weight decreases with increase in levels of salinity.

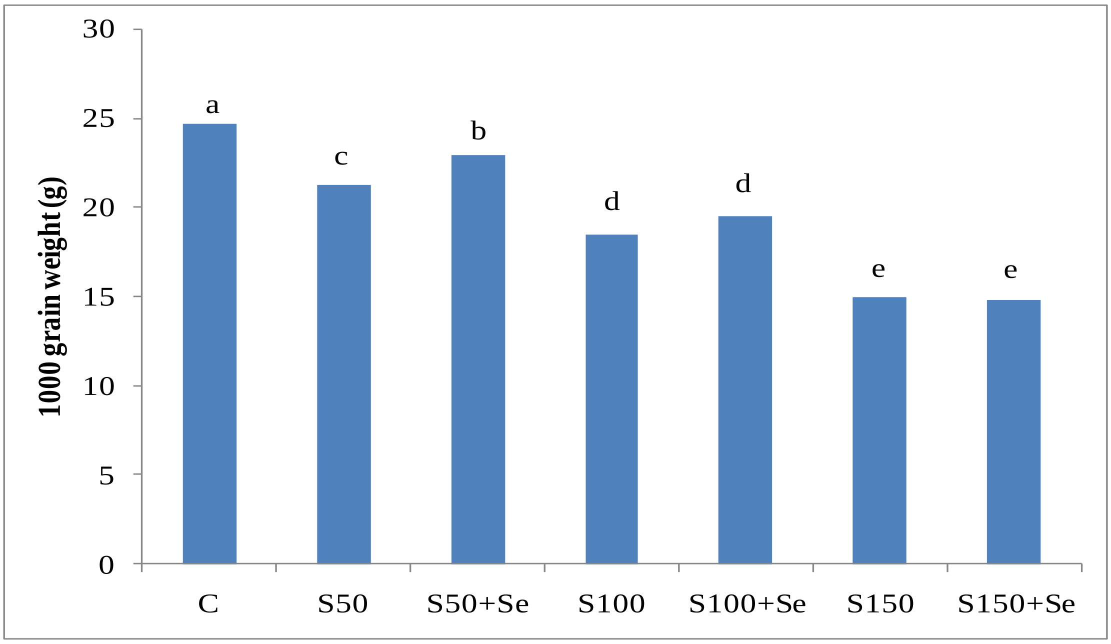

Figure 19. Effect of level on 1000 grain w eight of Boro rice varieties (Bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

4.3.6.3 Interaction effect of variety and salinity level

As shown in Fig 20, 1000 grain weight of rice plants decreased under salinity stress. Marked decreases in 1000 grain weight were observed (16, 28 and 40% in BRRI dhan45; 16, 25 and 40% in BRRI dhan47 and 10, 24 and 38% in of Nipponbare at 50, 100 and 150 mM, respectively) in response to salt stress. Anyhow, salt stressed plants treated with Se application up to 100 mM had significantly higher 1000 grain weight in all varieties compared to plants which were subjected to salt stress without Se. Even so, 1000 grain weight significantly decreased after treating with Se at 150 mM salt stress in Nipponbare variety of rice plant compared to plants which were treated with 150 mM without Se treatment (Fig 20). Highest 1000 grain weight was found in control (25.81 g) of BRRI dhan47 which was similar to Se treated 50 mM (24.147 g) stressed plant of that variety. Comparing varieties, under control conditions, BRRI dhan47 produced more 1000 grain weight in comparison to BRRI dhan45 and Nipponbare.

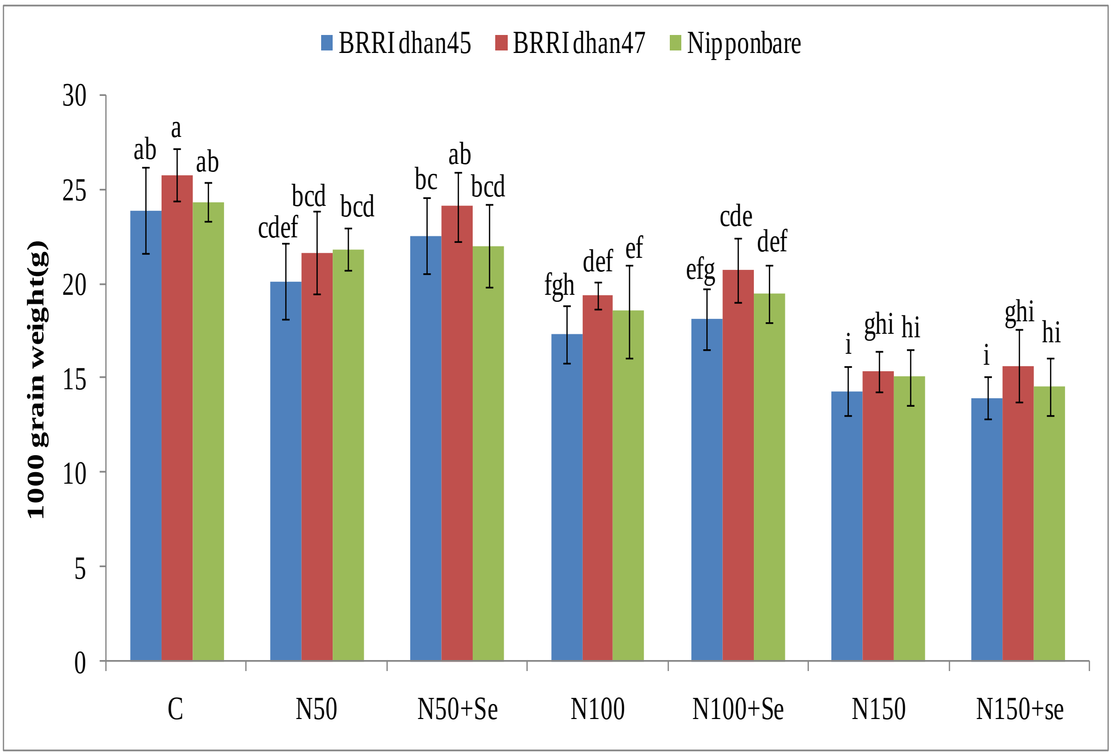

Figure 20. Effect of Se on 1000 grain w eight of BRRI dhan45, BRRI dhan47 and Nipponbare under saline and control condition (Bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

4.3.7 Grain yield pot^{-1}

4.3.7.1 Effect of variety

Grain yield varied significantly for different varieties shown in Fig 21. The highest grain yield (65.21 g) was recorded by BRRI dhan47 compared to BRRI dhan45 (56.48 g) and Nipponbare (59.43 g). The grain of BRRI dhan45 as revealed from Fig 21 was statistically similar to that of Nipponbare.

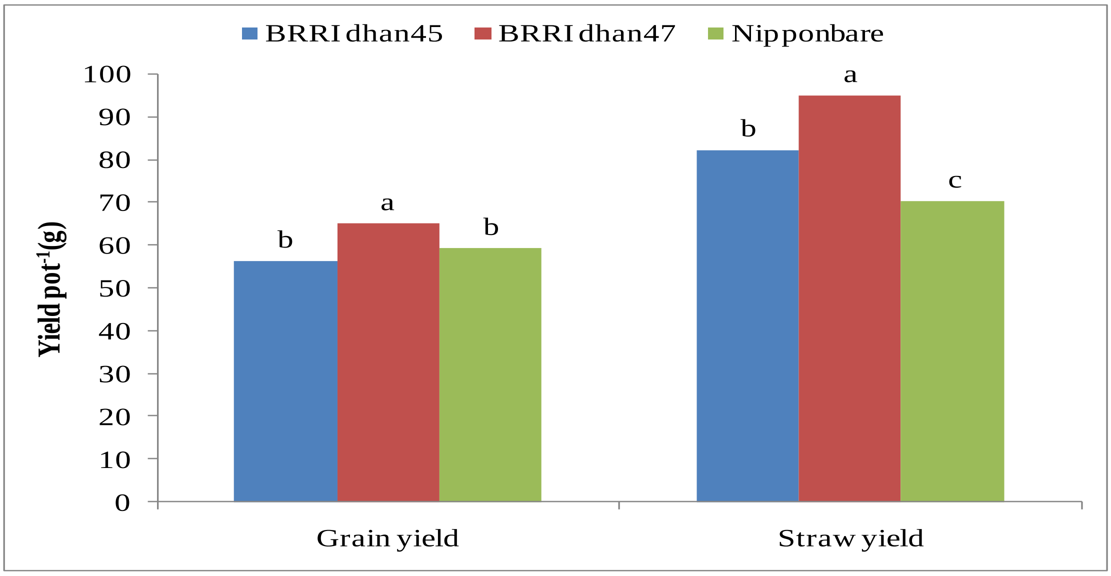

Figure 21. Effect of variety on yield pot^{-1} of rice varieties (Bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

4.3.7.2 Effect of salinity level

Significant variation was observed for grain yield due to different salinity treatments (Fig 21). Grain yield became reduced due to saline treatment. However, Se treated under salt stressed condition increased grain yield at all salt stressed condition (10, 3, and 1% at 50, 100 and 150 mM stress treatment, respectively) compared to their respective control. But increase of grain due to application of Se at 100 and 150 mM statistically similar which are treated with only salinity (Fig 21). Under salinity stress, the loss of grain yield results from a combination of reductions in plant stand, spikelet number per panicle, fertility, and harvest index. Among all these contributing components studied, the fertility of grain is found most severely affected and thus causes significant reduction in total yield of grain. In addition to fertility, panicle length and panicle numbers are two important affected characters that contribute to grain yield. The magnitude of salt induced yield losses could not be attributed to a single factor. Different physiological, and biochemical factors at different stages of rice plants might be involved. One factor may be the overall control mechanism (before flowering) of sodium uptake through root properties and its subsequent distribution in different vegetative and floral parts especially in leaves where it causes leaf mortality thereby reducing transportation of total assimilates to the growing region (Munns 2002). 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 (Murty and Murty, 1982). Also reduced viability of pollen under stress condition could result in failure of seed set (Abdullah *et al.*, 2001). Grain yield reduction of rice varieties due to salt stress is also reported by Linghe *et al.* (2000).

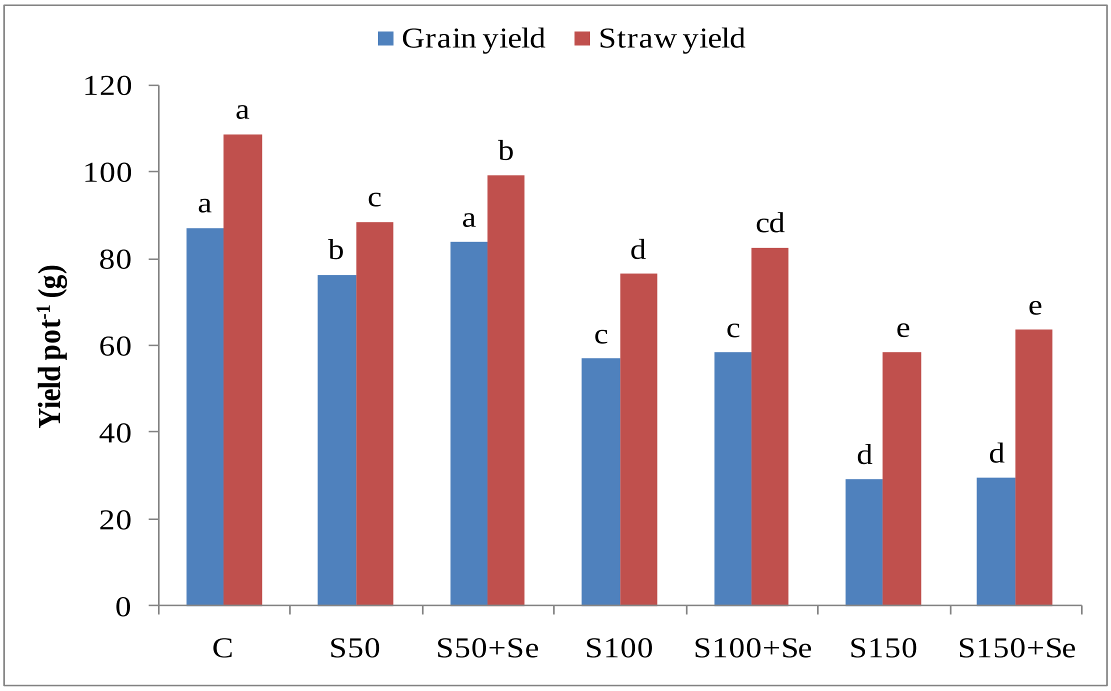

Figure 22. Effect of treatment on yield pot^{-1} of rice (Bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

4.3.7.3 Interaction effect of variety and salinity level

Salinity caused (Fig 22) a significant reduction in grain yield of rice plants of three varieties compared to those in non-saline solution and magnitude of decrease was less in BRRI dhan47 as compared to BRRI dhan45 and Nipponbare. 10, 38 and 68% grain yield decreased for BRRI dhan45; 12, 27 and 64% for BRRI dhan47 and 15, 39 and 67% for Nipponbare at 50, 100 and 150mM, respectively. The highest grain yield was found in control (90.253 g) and Se treated plant witn 50mM salinity (87.317g) of BRRI dhan47 variety (Fig 22). For all that, the lowest grain yield was found in 150 mM saline condition of three varieties which was statistically similar after treating with Se.

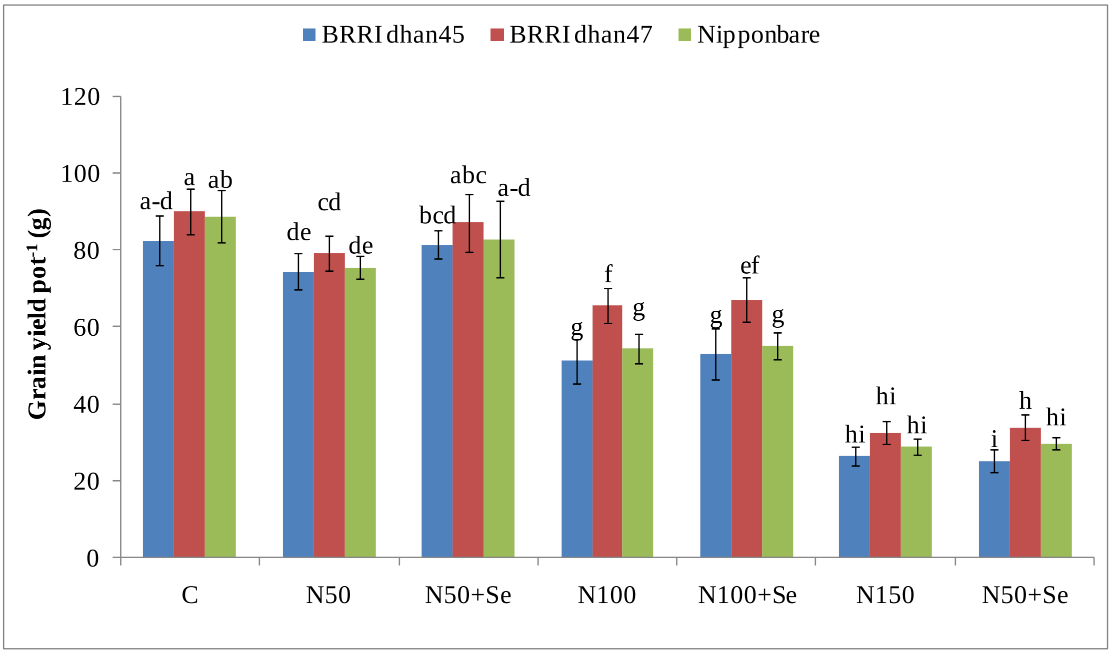

Figure 23. Grain yield pot $^{-1}$ of two rice varieties induced by saline, Se and their combination (Bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

4.3.8 Straw yield pot^{-1}

4.3.8.1 Effect of variety

Straw yield varied significantly for different varieties shown in Fig 21. The highest straw yield (95.09 g) was recorded by BRRI dhan47 compared to BRRI dhan45 (82.424 g) and Nipponbare (70.48 g).

4.3.8.2 Effect of salinity level

Sharp decreases in straw yield were observed (19, 30 and 46% at 50, 100 and 150 mM stressed condition, respectively) due to NaCl salinity stress (Fig 21). Furthermore, Se treatment increased straw yield under stress condition.

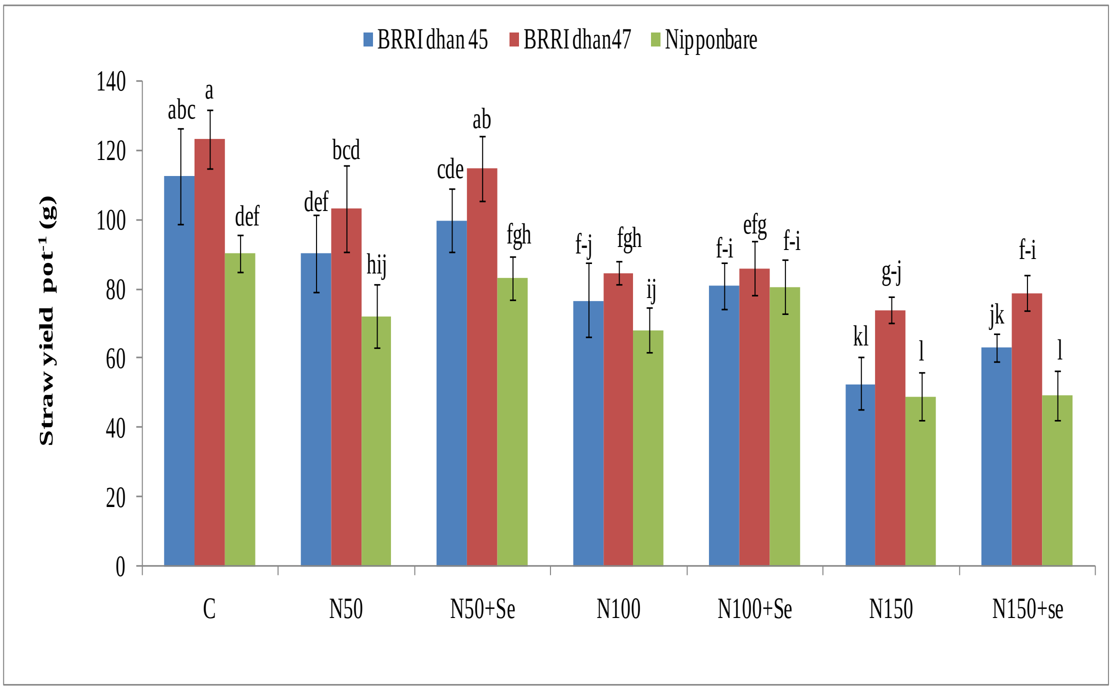

Figure 24. Straw yield pot^{-1} of two rice varieties induced by saline, Se and their combination (Bars with different letters are significantly different at $p \leq 0.05$ applying LSD)

4.3.8.3 Interaction effect of variety and salinity level

Straw yield was noticeably decreased in three varieties under salt stressed condition. Straw yield was decreased by 20, 32 and 53% for BRRI dhan45; 16, 31, 40% for BRRI dhan47 and 20, 24 and 46% for Nipponbare at 50, 100 and 150mM respectively, compared to respective control (Fig 24). The highest straw yield was observed in control (123.393 g) of BRRI dhan47 variety when grown under non-saline treatment. Se application for BRRI dhan47 at 50 mM also produced 114.883 g straw yield pot^{-1} which is statistically similar with the highest result (Fig 24). However, exogenous application with Se mitigated the salt effect at all saline condition but at 150 mM salinity yield was statistically similar for three varieties.

Chapter 6

SUMMARY AND CONCLUSION

This study was conducted to mitigate salt stress in Boro rice by exogenous application of selenium. Three varieties were used for this experiment which was done at the Experimental shed of the Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka during the period of December, 2013 to April, 2014. BRRI dhan45 and BRRI dhan47 were collected from Bangladesh Rice Research Institute (BRRI), Gazipur, Dhaka and Nipponbare was collected from Kagawa University, Japan.

The experiment was laid out in a Randomized Completely Block Design (RCBD) with three replications. There were 63 pots all together replication with the given factors. Empty earthen pots with 18 inch depth were used for the experiment. There were 21 treatment combinations. The treatments were control (C), 50 mM NaCl (S50), 50 mM NaCl with Selenium (S50+Se), 100 mM NaCl (S100), 100 mM NaCl with Selenium (S100+SA), 150 mM NaCl (S150), 150 mM NaCl with Selenium (S150+SA), for three rice varieties. The salinity treatments were applied on 32, 47, 59 and 75 DAT.

Different salinity with or without Se treatments had significant effect on crop growth parameters viz. plant height and tillers hill^{-1} at different DAT. The highest plant height was observed in BRRI dhan47 with control (32.63 cm), 50mM saline with Se (31.73 cm) at 30 DAT; control (49.70 cm), 50mM saline with Se (48.56 cm) at 45 DAT; control (75.76 cm), 50mM saline with Se (74.20 cm) at 60 DAT; control (96.36 cm), 50mM saline with Se (95.10 cm) at 75 DAT; control (105.73 cm), 50mM saline with Se (103.23 cm) and control (102 cm), 50mM saline with Se (98.96 cm) at harvest. The highest tillers hill^{-1} was observed in BRRI dhan47 with control (12.146), 50mM saline with Se (11.76) at 30 DAT; control (18.52), 50mM saline with Se (17.22) at 45 DAT; control (24.31), 50mM saline with Se (23.16) at 60 DAT; control (28.01), 50mM saline with Se (27.64) at 75 DAT; control (27.00), 50mM saline with Se (27.05) and control (26.73), 50mM saline with Se (24.60) at harvest.

Salinity treatments had significant effect on the physiological parameters viz. relative water content was highest in BRRI dhan47 with control (65.815%), S50+Se (63.31%). The chlorophyll content was highest in BRRI dhan45 with control (0.0461 mg cm^{-2}), S50+Se (0.0458 mg cm^{-2}).

Salinity level had significant effect on the yield and yield contributing characters viz. plant height, effective tillers $hill^{-1}$, length of spike, spikelet $spike^{-1}$, 1000 grain weight, grain yield, straw yield and harvest index was highest in BRRI dhan47 control and S50+Se treatment. Where, non- effective tiller was highest in BRRI dhan45 at S150.

Based on result of the present experiment, together with results found in the available literature, we therefore conclude that exogenous Se solution application is an effective way to overcome the adverse effects of osmotic stress on growth, physiology and yield components of rice. It could be partially attributed to the increase in non-enzymatic and enzymatic antioxidants. In all the cases BRRI dhan47 was a better performer under salt stress. All parameters decreased at any level of salt stress. Exceptions were non-effective tiller $hill^{-1}$, unfilled grain $panicle^{-1}$ which increased in response to salinity. BRRI dhan45 gave lowest performance compare to other two varieties because it was a saline sensitive. Nipponbare showed medium performance but it can tolerate salinity sometimes.

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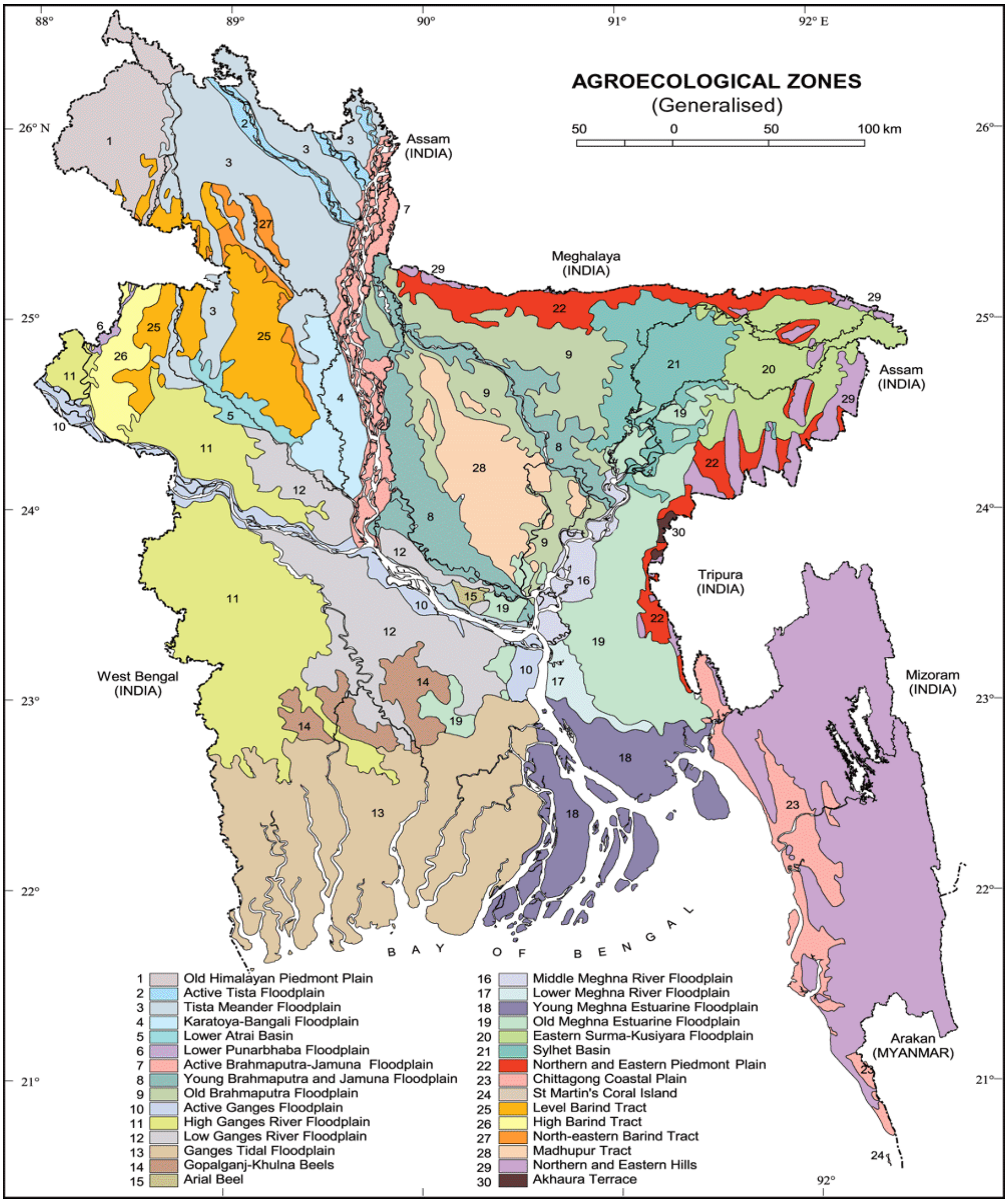
APPENDICES

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 November 2013 to March 2014

Months	Air temperature (°C)		Relative humidity (%)	Total rainfall (mm)
	Maximum	Minimum		
November, 2013	28.10	6.88	58.18	1.56
December, 2013	25.36	5.21	54.3	0.63
January, 2014	21.17	15.46	64.02	00
February, 2014	24.30	19.12	53.07	2.34
March, 2014	29.78	22.37	48.66	0.12

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