GERMINATION AND GROWTH PERFORMANCE OF THREE RICE VARIETIES UNDER SALINITY STRESS

A THESIS

BY

AHMED ABUBAKAR ABDULKADIR

Student No. 1705194
Session: 2017- 2018
Thesis Semester: January - June, 2018

Submitted to the Department of Agricultural Chemistry
Hajee Mohammad Danesh Science and Technology University, Dinajpur
in partial fulfillment of the requirements for the degree of

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

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JUNE, 2018

DEDICATED
TO MY
BELOVED PARENTS
&
RESPECTED TEACHERS

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June 2018 The Author

ABSTRACT

Salinity is major constrain to rice production in southern part of Bangladesh. Rice yield reduction is caused by salinity due to its adverse effect on many important physiological processes. The present study was therefore designed to select salt tolerant rice varieties. The research was conducted in three rice varieties (Nonabokra, Botessor and BRRI dhan28) for the coastal areas of Bangladesh. Germination of three rice varieties were studied under three salinity levels in the laboratory of HSTU dinajpur, the experiment was conducted during the period of July to December 2017. The results showed that Nonabokra were tolerant and Botessor was moderately tolerant based on germination test experiment. However, germination test, shoot and root length of Nonabokra, Botessor and BRRI dhan28 seeds under different salinity levels were also performed. The highest germination (90%, 86.67 % and 100%) was found in Nonabokra, Botessor and BRRI dhan28, respectively with the treatments of 3 dSm^{-1}, 6 dS m^{-1}, and 9 dS m^{-1}. The highest shoot and root weight of seedlings were found in 3 dS m^{-1}. It was observed that all salinity effluents could also delay germination and growth of crops such as BRRI dhan28. However, overall view suggests that the salinity was not suitable for germination of the rice varieties except Nonabokra. Subsequent studies on growth, biochemical constituents, and mineral composition of three rice varieties under different salinity levels in the pots were conducted experiment and the result revealed that the growth of rice plants were arrested instantly at 9 dS m^{-1}. The highest proline accumulation was found in Nonabokra while the lowest accumulation was in BRRI dhan28. The highest proline increment (3.19) was in Nonabokra, while the lowest (0.55) was in BRRI dhan28. The total chlorophyll content were observed from 14.54 to 0.55. The carotene content ranger from 4.30 to 0.66. The sodium concentration was ranger from 459 to 71.67. The potassium concentration of rice varieties was founded as from1180 to 248.3. The calcium concentration was from 1077 to 140.3. The magnesium concentration were from 1158 to 165. The sulfur concentration were from 801 to 133.7. The phosphors concentration were from 12.86 to 1.95. The zinc concentration were from 3.66 to 0.35. The overall result revealed that among the tested rice varieties one variety were selected of which Nonabokra appeared as salt tolerant, while Botessor was moderately tolerant, and BRRI dhan28 was more susceptible to salinity. So it can be suggested that Nonabokra is more appropriate variety to cultivate in the coastal area of Bangladesh.

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CHAPTER I

INTRODUCTION

Rice (*Oryza sativa* L.) belongs to the family Gramineae. It is the staple food crop in Bangladesh and one of the most important cereal crops throughout the world. This staple food ranked first position by production 34.7 million tons during the year 2017 among all cereals in Bangladesh (BBS, 2017). Total rice cultivated area 11.7 million hectares. Among the low and middle income countries of the world rice is the most important cereal crop. The developing countries contribute 96.24% of the total world rice production. Anonymous (2012) studied rising sea levels, salinization, erosion and human settlements lead to the loss of rice fields in an alarming speed. Maclean *et al.,* (2002) reported rice that is a salt sensitive crop species for which soil salinity is a major factor restricting yield throughout substantial areas of Africa and south and southeastern Asia. Ponnamperuma (1984) observed salinity and drought stress are among the most serious challenges to crop production in the world today, particularly in developing countries. Zhou *et al.,* (2007) and Shobbar *et al.,* (2010) reported salinity of soil or water is of increasing importance to agriculture because it causes a stress condition to crop plants. As far as rice is concerned, a species native to swamps and freshwater marshes, secondary salinization is becoming an increasingly serious production constraint. Akbar and Ponnamperuma (1980) studied several physiological pathways like photosynthesis, respiration, nitrogen fixation and carbohydrate metabolism have been observed to be affected by high salinity. Chen *et al.,* (2008) reported variations in sensitivity to salt during the life cycle increase the complexity of tolerance evaluation. Flowers (2004) studied some of the growth parameters such as root growth, seedling height, and leaf area and tiller number have been proposed as morphological markers for the screening of tolerant genotypes in rice. Singh *et al.,* (2017) reported one of such efforts is to cultivate rice with elevated level of salt tolerance on salt affected marginal lands. Over 30 percent of the net cultivable area of Bangladesh lies in the coastal zone. Out of 2.85 million hectares of coastal and offshore land, about 1.5 million hectares are affected by varying degrees of salinity. The coastal saline soils are distributed unevenly in 64 Upazila of 13 District, covering portions of eight agro-ecological zones (AEZ) of the country. Seraj and Salam (2000) reported Salinity affects plants at all stages of development, but sensitivity sometimes varies from growth stage to the next. Several studies indicated that rice is tolerant during germination, becomes very sensitive during early seedling stage (2-3 leaf stage), gains tolerance during vegetative growth stage becomes sensitive during pollination and fertilization and then become increasingly more tolerant at maturity (Pearson *et al.,* 1966; IRRI, 1967). Rice is the grain that has shaped the cultures, diets and economies of billions of Asians. It is a staple food to feed more than 3 billion people and to provide 50- 80% daily calorie intake. Khush (2005) declared People get 66% calorie from rice in Malaysia. The Malaysian rice production is lower than its consumption and thus resulting in rice importation which gave the rice self-sufficiency of about 70% for many years. Khan and Abdullah (2003) reported that the rice crop identified as salt-susceptible both in seedling and reproductive stages. Salinity is a major abiotic stress for all stages of rice due to anthropogenic contributions to global warming, the rate of sea-level rise is expected to increase and possess dramatic effect on rice production especially in coastal areas. Hakim *et al.,* (2013) reported increasing global warming increases the average temperatures which might cause the 'melting' of polar ice caps, resulting from the rise-up of the sea-water level gains (2.8 – 3.1 mm^{-1} year), and thus causing salty water intrusion into the coastal areas. Selamat and Ismail (2008) reported that fifty per cent yield is being lost of the salt-sensitive rice genotypes due to salinity. In arid and semi-arid regions, limited water and hot dry climates frequently increase salinity levels that limit or prevent crop production. Michael *et al.,* (2004) observed that the major inhibitory effect of salinity on plant growth has been attributed to: 1) osmotic effect 2) ion toxicity 3) nutritional imbalance leading to reduction in photosynthetic activities and other physiological disorders. Ali *et al.,* (2004) identified Na^+ and Cl^- derived from NaCl are well known as the toxic ions to damage the plant cells in both ionic and osmotic levels. Plant growth and development are directly inhibited, leading to low yield prior to plant death. Mansour and Salama (2004); Chinnusamy *et al.,* (2005) declared in normal conditions, the Na^+ concentration in the cytoplasm of plant cells was low in comparison to the K^+ content, frequently 10-2 versus 10-1 and even in conditions of toxicity, most of the cellular Na^+ content was confined into the vacuole (Apse *et al.,* 1999). Salt tolerance in plants is generally associated with low uptake and accumulation of Na^+, which is mediated through the control of influx and/ or by active efflux from the cytoplasm to the vacuoles and also back to the growth medium (Jacoby, 1999). Energy-dependent transport of Na^+ and Cl^- into the apoplast and vacuole can occur along the H^+ electrochemical potential gradients generated across the plasma membrane and tonoplast (Hasegawa *et al.,* 2000). Increasing of Na and Cl ion and decreasing of potassium (K), phosphorus (P), nitrogen (N), calcium (Ca), and magnesium (Mg) in different tissues of crop 875 plant with increasing NaCl concentration in growing medium (El-Sayed Emtithal *et al.,* 1996). Therefore, researchers and policy makers must invent ways for the efficient utilization of the salinity prone areas. The selection of salt tolerant rice variety might be the best approach to bring the salinity susceptible areas under rice cultivation. Shereen *et al.,* (2005); Ali *et al.,* (2004) reported although much research have been done for understanding the influences of saline habitats on seed germination, growth, reproduction and population dynamics of crop plants. Khan *et al.,* (2002) report on total dry matter accumulation, mineral nutrients and yield of coarse rice under saline environments is still scanty. Therefore, the study was undertaken to determine dry matter accumulation, nutrient concentration and yield of rice under varied salinity stress. Abiotic stress is a major factor around the world in limiting plant growth and productivity (Osakabe *et al.,* 2011 and Jamil *et al.,* 2010). Exposure of plants to a stressful environment during various developmental stages appears to induce various physiological and developmental changes. Islam *et al.,* (2008) reprted that the soil salinity, particularly due to NaCl, can be considered as the single most widespread soil toxicity problem that global rice production faces at present. Hong *et al.,* (2007) studied that the some toxic effects of salt stress include decreased germination and seedling growth. Zeng and Shannon (2000); Ashraf (2010) suppressed leaf expansion which ultimately reduces photosynthetic area and dry matter production. Mansour and Salama (2004) observed K^+ in plant tissues evidently decreases when plants are exposed to salt stress, especially rice genotypes. Basu *et al.,* (2002) absorved translocation of salt into roots and to shoots is an outcome of the transpirational flux required to maintain the water status of the plant and unregulated transpiration may cause toxic levels of ion accumulation in the shoot. Yeo (1998) studied lower transpiration rate, coupled with reduced ion uptake by the roots, or reduced xylem loading, may cause poor supply via the xylem. So it is possible that an inadequate supply of ions to the expanding region may restrict cell division and/or expansion when plants are grown at high levels of NaCl. Berstein *et al.,* (1995) observed that many reports show salt induced reduction in photosynthetic pigments in many plant species such as rice. Cha-um *et al.,* (2007) reported that the plants also show the high chlorophyll degradation symptom, chlorosis, as a common morphological and physiological characteristic in response to salt stress. Harinasut *et al.,* (2000) reported that the chlorophyll content of salt stressed rice can be described as a function of the leaf sodium content (Yeo and Flowers, 1983). The response of plants to excess NaCl is complex and involves changes in their morphology, physiology and metabolism (Hilal *et al.,* 1998) reported keeping in mind all these observations, experiments were designed to study the effect of NaCl stress on germination percentage, seedling growth, ion content (Na, K and Ca), protein content and photosynthetic pigments i.e. Chl a, Chl b and carotenoids Seed germination is affected by the increase in salinity. Pushpam and Rangasamy (2002) studied soil salinization is a serious problem in the entire world and it has grown substantially causing loss in crop productivity (FAO, 2006). It is a major constraint limiting agricultural productivity on nearly 20% of the cultivated and irrigated area worldwide. Zheng *et al.,* (2001) identified that the Salinity affects almost every aspect of the physiology and biochemistry of plants and significantly reduces yield. High exogenous salt concentrations affect seed germination, water deficit, ion imbalance of the cellular ions resulting in ion toxicity and osmotic stress (Khan and Panda, 2006). Salt stress has been reported to cause an inhibition of growth and development, reduction in photosynthesis, respiration and protein synthesis in sensitive species. Meloni *et al.,* (2003); Pal *et al.,* (2004) studied that the Yield is a very complex character which comprise of many components and these yield components are related to final grain yield which are also severely affected by salinity. Shereen *et al.,* (2005) observed the differences in yield response of rice to soil salinity can be related to climatic variations. In particular, a low relative humidity of the air during the growing season can enhance the yield losses per unit increase of salt concentration because the potential yield is higher in the dry season, as a consequence of longer and more intense solar radiation in the dry season than in the wet season (Asch *et al.,* 2000; Eynard *et al.,* 2005). Zeng and Shannon (2000) studied the effect of salinity on seedling growth and yield components of rice and stated that harvest indices were significantly reduced by salinity at 3.4 dS m^{-1}

or higher. For better understanding, it is necessary that the salinity stress is started and stopped, to enable the quantification of damage and the differences in sensitivity over the cycle, by comparisons of salinity levels with the same duration of stress in different stages of rice growing (Zeng *et al.,* 2001) studied symptoms of salt injury in rice are stunted growth, rolling of leaves, white leaf tips, white blotches in the laminae, drying of older leaves and poor root growth. Worldwide numerous reports have been published on salinity stress on rice but very little information is available on the effects of salinity on the local variety of Dinajpur district. Thus, there is a need to assess the salt tolerant levels of some local rice varieties in Bangladesh condition.

In the present study was conducted with the following objectives:

- To analyze genotypic variations of rice vareities at germination and early seedling growth stage under salinety stress.
- To evaluate the effect of salinity stress during different growth stages of rice.

CHAPTER II

LITERATURE REVIEW

2.1 Salinity: an overview

Salinity as an abiotic stress widely limit the crop production severely (Shannon MC 1998) reported that a saline soil is usually the reservoir of a number of soluble salts such as Ca^{2+}, Mg^{2+}, Na^{+} and anions SO_4^{2-}, Cl^{-}, HCO_3- with exceptional amounts of K^{+}, CO_3^{2-}, and NO_3^{-}. A soil can be termed as saline if its EC is 4 dS m^{-1} or more (USDA-ARS 2008) (equivalent to approximately 40 mM NaCl) with an osmotic pressure of approximately 0.2 MPa. Salinity is the condition when the EC is sufficient to cause yield reduction of most crops. The pH of saline soils generally ranges from 7-8.5 (Mengel 2001). However, the pH in saturated soil can vary provoking severe crop damage the arid and semi-arid zones, characterized by low precipitation and high evaporation are the most affected due to minimum lixiviation of salt from the soil profile resulting in increased salt accumulation. Salinity prone areas found in the arid and semiarid zones are usually accounted to the accumulation of salts over ages. Moreover, weathering of the parental rocks has accelerated the process a lot (Szabolcs 1989, Regasamy 2002) stated that the salinity is a well off natural phenomenon occurring near sea shores due to sea water flooding. Salinity has been a potential threat affecting almost 900 million ha of which nearly accounts for 20% of the globally cultivated area and also half of the total irrigated land of the world (Munns 2002, FAO 2007) described globally salt affected area accounts to about 1 billion ha of land (Fageria 2012) in India the scenario accounts for about 8.4 million ha land affected by salinity (Tyagi 1998) in India, irrigated land accounts for only 15% of total cultivated land, nevertheless, it has at least twice the productivity of rain-fed land and yields about one third of the world's consumption. In accordance to the above facts and keeping in view the present alarming scenario, development of salt tolerant genotypes is definitely an urgent need of the hour.

2.2 Effect of salinity stress on rice crops

Rice is a crop with great economic importance (Bajaj 2005 and Harkamal 2007) and is cultivated across 114 countries globally (FAO 2004) however, the abiotic and biotic stresses can reduce its yield. This problem will be worse in attention to increase of global population and food sources deficiency (Kumar 2007) identified that the Rice is susceptible to salinity, specifically, at the early vegetative and later reproductive stages (Shannon 1998, Mass 1977) observed that the rice genotypes show wide variations in salinity tolerance due to additive gene effects (Sahi 2006) studies indicated that rice is more resistant at reproductive and grain filling than at germination and vegetative stages (Heenan 1988), as well as low levels of salinity can increase the resistance of rice to higher and lethal salinity levels (Djanaguiraman 2006) at present, salinity is the second type of stress and is the most predominant hindrance to rice production after drought (Gregorio 1997) identified that the effects of salinity on the growth and yield of rice in field have been well studied including the study of genotypic variance for salt tolerance amongst the paddy germplasms (Akbar 1986, Khatun 1995) reported that the rice is generally considered to be a salt-tolerant crop. Maas and Hoffman (1977) showed that rice threshold EC is 3 dS m^{-1} and a 1dS m^{-1} increase in salinity reduces yield by 12%. Moorman and Breeman (1978) reported that EC value of 6-10 dS m^{-1} is associated with a 50% decrease in yield. Pearsons and Ayres (1960) found that salt tolerance of rice varied with its growth stages. The plant is tolerant during germination, but young seedlings are sensitive until the age of four weeks. An increase in salt tolerance occurs up to tillering, but the plant again becomes sensitive during flowering. Sensitivity again diminishes during the maturation period. IRRI in 1978 reported that during the reproductive stage, salts adversely affected the number of spikelets per panicle. Further, yield reduction under salinity was due to adverse effects on panicle formation and grain setting, which can reduce the yield of even the more tolerant crops.

2.2.1 Effect of salinity stress on germination

Seed germination is one of the most fundamental and vital phases in the growth cycle of plants that determine plant establishment and the yield of the crops. 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* (Carpıcı *et al.* 2009; Khodarahmpour *et al.* 2012), *Brassica* spp. (Ibrar *et al.* 2003; Ulfat *et al.* 2007), *Glycine max* (Essa 2002), Vigna spp., (Jabeen *et al.* 2003) and *Helianthus annuus* (Mutlu and Buzcuk 2007). It is well established that salt stress has negative correlation with seed germination and vigor (Rehman *et al.* 2000) reported that the higher level of salt stress inhibits the germination of seeds while lower level of salinity induces a state of dormancy (Khan and Weber 2008). Salinity have many-fold effects on the germination process: it alters the imbibition of water by seeds due to lower osmotic potential of germination media (Khan and Weber 2008) listed that the causes toxicity which changes the activity of enzymes of nucleic acid metabolism (Gomes-Filho *et al.,* 2008), alters protein metabolism (Yupsanis *et al.,* 1994; Dantas *et al.,* 2007) , disturbs hormonal balance (Khan and Rizvi 1994), and reduces the utilization of seed reserves (Promila and Kumar 2000 ; Othman *et al.*2006) it may also negatively affect the ultrastructure of cell, tissue and organs (Koyro 2002; Rasheed 2009) however, there are various internal (plant) and external (environmental) factors that affect seed germination under saline conditions which includes nature of seed coat, seed dormancy, seed age, seed polymorphism, seedling vigor, temperature, light, water and gasses (Wahid *et al.,* 2011) the germination rates and percentage of germinated seeds at a particular time varies considerably among species and cultivars. Lauchli and Grattan (2007) proposed a generalized relationship between percent germination and time after adding water at different salt levels in Solanum lycopersicum, high concentrations of salt (150 mM NaCl) in the germination media signi fi cantly delays onset and reduced the rate of germination (Foolad and Lin 1997, 1998) further investigation in *S. lycopersicum*, Kaveh *et al.* (2011) found a signi fi cantly negative correlation between salinity and the rate and percentage of germination which resulted in delayed germination and reduced germination percentage. Cuartero and Fernandez-Munoz (1999) reported that seeds need 50% more days to germinate at 80 mM NaCl and about 100% more days at 190 mM NaCl than control. Neamatollahi *et al.* (2009) reported that increasing of NaCl concentration in priming treatments reduced germination percentage due to higher osmotic pressures. Lombardi and Lupi (2006) reported that an increase in NaCl concentration progressively retarded and decreased germination of *Hordeum secalinum*, where 10-day treatment with 400 and 500 mM NaCl caused 40% and 38% reductions in germination rate, respectively. Bordi (2010) reported that the germination percentage in *B. napus* significantly reduced at 150 and 200 mM NaCl. Germination rate also decreased on increasing concentration of salinity levels. Compared with control, germination percentage and germination speed were decreased by 38% and 33%, respectively at 200 mM NaCl. This was caused due to ionic imbalance, osmotic regulation disorders and fi nally decreased water absorption by seeds. While studying with four rice cultivars, we observed a signi fi cant reduction in germination rate when exposed to various concentration of salt (30–150 mM). However, the sensitive cultivars were more prone to germination reduction under salt stress (Hasanuzzaman *et al.,* 2009) suggested 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 vigor (95%) in *Zea mays* seeds exposed to 240 mM NaCl.

2.2.2 Effect of growth on parameters

One of the 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 uptake capacity of the plant, and this causes quick reduction in the growth rate. This fi rst 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 2002b) the mechanisms by which salinity affects growth of a plant depend on the time scale over which the plant is exposed to salt Munns (2002b) summarized the sequential events in a plant grown in saline environment. He stated that "In the fi rst few seconds or minutes, water is lost from cells and shrinked. Over hours, cells recover their original volume but the elongation rates are still reduced which led to lower growth rates of leaf and root. Over days, cell division rates are also affected, and contribute to lower rates of leaf and root growth. Over weeks, changes in vegetative development and over month's changes in reproductive development can be seen". Later on, Munns (2005) developed the 'two-phase growth response to salinity' for better understanding the temporal differences in the responses of plants to salinity the fi rst phase of growth reduction is a quicker process which is due to osmotic effect. The second phase, on the other hand, is much slower process which is due to the salt accumulation in leaves, leading to salt toxicity in the plants. The later one may results in death of leaves and reduce the total photosynthetic leaf area which reduce the supply of photosynthate in plants and ultimately affect the yield. With annual species, the timescale is day or week, depending on species and salinity level. With perennial species, the timescale is months or year. During phase 1, growth of both genotypes is reduced due to the osmotic effect of the saline solution adjacent to roots. During phase 2, leaves of more sensitive genotype are died and the photosynthetic capacity of the plant is greatly reduced which imposes an additional effect on growth. Upon addition of salt at one step, the growth rate plummets to zero or below and takes 1–24 h to regain the new steady rate, depending on the extent of the osmotic shock (Munns, 2002a) studied that in plants, where Na^+ and Cl^- build up in the transpiring leaves over a long period of time, resulting in high salt concentration and leaf death. Leaf injury and death are attributed to the high salt load in the leaf that exceeds the capacity of salt compartmentation in the vacuoles, causing salt to build up in the cytoplasm to toxic levels (Munns 2002a, 2005; Munns *et al.* 2006) there are abundant literature indicating that plants are particularly susceptible to salinity during the seedling and early vegetative growth stage. In our study, we observed a remarkable reduction in plant height and tiller number and leaf area index in O. sativa plants grown in saline soil (Hasanuzzaman *et al.,* 2009) under saline condition, some crops are most sensitive during vegetative and early reproductive stages, less sensitive during fl owering and least sensitive during the seed fi lling stage. In all these studies, seed weight is the yield component of interest but similar conclusions regarding growth stage sensitivity were obtained with both determinate crops (the grain crops) and indeterminate (cowpea) crops (Lauchli and Grattan 2007) . Khatun and Flowers (1995) studied the effect of salinity on sterility and seed set in O. sativa. Salinity increased the number of sterile fl orets and viability of pollen, becoming more pronounced with increased salinity. Seed set was reduced by 38% when female plants were grown in as low as 10 mM NaCl. In Suaeda salsa, plant height, number of branches, length of branches and diameter of shoot were significantly affected by salt stress which was due to the increased content of Na^+ and Cl^- (Guan *et al.,* 2011) while studying with G. max, Dolatabadian *et al.* (2011) observed that salinity stress significantly decreased shoot and root weight, total biomass, plant height and leaf number. However, leaf area was not affected by salinity stress.

2.2.3 Effects of water relation

According to Romero-Aranda *et al.* (2001) 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 plant's 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) according to Katerji *et al.* (1997) , a decrease in RWC indicates a loss of turgor that results in limited water availability for cell extension processes. Steudle (2000) reported that in transpiring plants, water is thought to come from the soil to the root xylem through apoplastic pathway due to hydrostatic pressure gradient. However, under salt stressed condition, this situation changes because of the restricted transpiration. Under these situations, more of water follows cell-to-cell path, flowing across membranes of living cells (Vysotskaya *et al.,* 2010).

2.2.4 Nutrient Imbalance

It is well-established that crop performance may be adversely affected by salinity induced nutritional disorders. However, the relations between salinity and mineral nutrition of crops are very complex (Grattan and Grieve 1999) identified that the nutritional disorders may result from the effect of salinity on nutrient availability, competitive uptake, transport or distribution within the plant. Numerous reports indicated that salinity reduces nutrient uptake and accumulation of nutrients into the plants (Rogers *et al.* 2003; Hu and Schmidhalter 2005) however, very few evidences exist that addition of nutrients at levels above those considered optimal in non-saline environments, improves crop yield (Grattan and Grieve 1999). In fact, these processes may occur simultaneously and whether they affect the crop yield or quality depends on the toxic level, composition of salts. The crop species and surrounding environment (Grattan and Grieve 1999) numerous plant studies have demonstrated that salinity could reduce N accumulation in plants. Decreased N uptake under saline conditions occurs due to interaction between Na^+ and NH_4^+ and/or between Cl^- and NO_3^- that ultimately reduce the growth and yield of the crop (Rozeff 1995) this reduction in NO_3^- uptake is associated with Cl^- antagonism (Bar *et al.,* 1997) or reduced water uptake under saline conditions (Lea-Cox and Syvertsen 1993) the availability of P was reduced in saline soils due to (a) ionic strength effects that reduced the activity of PO_4^{3-}, (b) phosphate concentrations in soil solution was tightly controlled by sorption processes and (c) low solubility of Ca-P minerals. Hence, it is noteworthy that phosphate concentration in fi eld grown agronomic crops decreased as salinity increased (Qadir and Schubert 2002) different plant studies indicated that high level of external Na^+ caused a decrease in both K^+ and Ca^{2+} concentrations in plant tissues of many plant species (Hu and Schmidhalter 1997, 2005; Asch *et al.,* 2000) this reduction in K^+ concentration in plant tissue might be due to the antagonism of Na^+ and K^+ at uptake sites in the roots, the influence of Na^+ on the K^+ transport into xylem or the inhibition of uptake processes (Suhayda *et al.,* 1990) in another study, Hu and Schmidhalter (1997) also stated that Mg^{2+} concentration decreased due to salinity in *T. aestivum* leaves. The availability of micronutrients in saline soils is dependent on the solubility of micronutrients, the pH of soil solution, redox potential of the soil solution and the nature of binding sites on the organic and inorganic particle surfaces. In addition, salinity can differently affect the micronutrient concentrations in plants depending upon crop species and salinity levels (Oertli 1991) micronutrient deficiencies are very common under salt stress because of high pH (Zhu *et al.,* 2004).

2.3 Effects of salinity on rice growth

Generally, rice has an average life span of 3-7 months, depending on the climate and the variety. It is not a water plant but substantial amounts of water are required for growing rice. Cultivated species of rice are considered to be semi-aquatic annuals. The height of the plant can range from 0.4 m to over 5 m in some floating rice. Salt affected soils are enriched with salts that is, sodium chloride (NaCl), sodium sulphate (Na_2SO_4), calcium chloride ($CaCl_2$) and magnesium chloride ($MgCl_2$). Sodium chloride is a major salt contaminant in most saline soils. The effects of sodium ions are well established as this ion can cause damage to plant cells by both ionic and osmotic effects, leading to growth retardation and eventually cell death (Hasegawa *et al.,* 2000; Munns *et al.,* 2002; Mansour and Salama, 2004; Chinnusamy *et al.,* 2005). The damaging effects of salt injury on rice plant have been extensively reviewed. It is well established that the excess of NaCl alone can cause more toxicity to the rice plant than mixed salts (Ashraf and Yousef, 1998). Breeding programs for enhanced salt tolerance in rice crop are meaningful means of overcoming the salinity problem (Gregorio *et al.,* 2002; Senadhira *et al.,* 2002; Flowers and Flowers, 2005).

Mass and Hoffman (1977) reported the major inhibitory effect of salinity on plant growth has been attributed to: i) osmotic effect ii) ion toxicity iii) nutritional imbalance leading to reduction in photosynthetic efficiency and other physiological disorders. Most rice cultivars are severely injured in submerged soil cultured on EC of 8-10 dS m^{-1} at 25 ^oC; sensitive ones are damaged even at 2 dS m^{-1}. Salinity is a single word; nevertheless its effect on plants and plants reaction toward it needs pages to be discussed, The salinity stress triggered initially two main harmful effects, namely, i) osmotic (reduced water uptake) and ii) specific ion toxicity stress (mainly Na^+ ad Cl^-), which leads to the third generative stress – iii) oxidative stress- where uncontrolled production of ROS (Reactive Oxygen Species) as superoxide radicals (O_2), hydrogen peroxide (H_2O_2) and hydroxyle radicals (OH^-). Those unstable molecules accumulated to toxic levels and trigger an oxidative damage effect on the valuable biochemical molecules as proteins, enzymes, DNA and RNA. Sharma, *et al.,* (2012) on the level of morphology, the plants under salinity stress showing many external symptoms. The tip of the affected leaves turn white, chlorotic patches appear on some leaves and plant stunting and reduced tillering. Growth inhibition is the primary injury that leads to other symptoms although programmed cell death may also occur under severe salinity shock.

2.4 Screening for salinity tolerance

Rice possesses quite low salt tolerance than other crops (Mass and Hoffman, 1977), thus being one of the contributory factors for lower production on saline soil. Breeding salt tolerant crop varieties is considered to be the most pragmatic approach for better yield under saline conditions (Shannon *et al.,* 1998) studied breeding for salinity tolerance in rice requires reliable screening techniques. These techniques must be rapid to keep pace with the large amount of breeding material generated. Screening under field conditions is difficult due to stress heterogeneity, presence of other soil related stress and significant influence of environmental factors such as temperature, relative humidity and solar radiation. These complexities together with the degree of salinity and reproducibility, cause difficulties in developing and using reliable methods of screening voluminous materials. Although the selection criteria for salt tolerance should be based on field performance of plants during full growing season (Sammons *et al.,* 1978), it is well evidenced that salt tolerant plants tested under greenhouse conditions also exhibit salt tolerance under field conditions. Furthermore, because in field conditions soil salinity is more heterogeneous and occurs in patches, it is more suitable to screen plants in greenhouse conditions where saline conditions are reasonably controlled and uniform (Munns and James, 2003).

2.5 Causes and Types of Salinity

Among the abiotic stresses, salinity is the most destructive factor which limits the crop productivity considerably. A large area of land in the world is affected by salinity which is increasing day by day. Salinity is more prominent problem in irrigated crop lands. Worldwide, around 17% of the cultivated land is under irrigation and irrigated agriculture contributes more than 30% of the total agricultural production (Hillel 2000) it is estimated that at least 20% of total irrigated lands in the world is salt-affected (Pitman and Lauchli 2002) however, the statistics varies depending on sources. According to the FAO Land and Nutrition Management Service (2008), 6.5% of the total land in the world is affected by salt (either salinity or sodicity). There are different causes of the development of soil salinity. The major forms are viz. (i) natural or primary salinity and (ii) secondary or human-induced salinity. Primary salinity is occurred due to the long-term natural accumulation of salts in the soil or surface water. This is a natural process which is caused mainly by weathering of parent materials containing soluble salts through break down of rocks containing Cl^- of Na^+, Ca^{2+} and Mg^{2+} and sometimes SO_4^{2-} and CO_3^{2-}. In addition, deposition of sea salt carried by wind and rain is also a reason, which varies with the types of soil. Secondary salinity occurs due to anthropogenic activities that disrupt the hydrologic balance of the soil between water applied (irrigation or rainfall) and water used by crops (transpiration) (Munns 2005 ; Garg and Manchanda 2008) in many irrigated areas, the water table has raised due to excessive amounts of applied water together with insufficient drainage. Most of the irrigation systems of the world have caused secondary salinity, sodicity or waterlogging (Garg and Manchanda 2008) in irrigated lands, after irrigation, the water applied to the soil is consumed by the crop or evaporates directly from the moist soil. The excess salt is remained and accumulated in the soil which is called salinization it is sometimes recognizable by a whitish layer of dry salt on the soil surface. In addition, salted groundwater may also contribute to salinization. Due to excessive irrigation and improper drainage the water table rises which allow the salty groundwater to reach in the upper soil layers and rhizosphere based on the nature, characteristics and plant growth relationships in salt affected soils, two main types of soils have been coined by Szabolcs (1974). These are: A) Saline soils-The soluble salts are chiefly NaCl and Na_2SO_4 and sometimes also contain appreciable quantities of Cl^- and SO_4^- of Ca^{2+} and Mg^{2+}. These soils contain suf fi cient neutral soluble salts to pose negative effect on growth of most crop plants. B) Sodic soils – These soils contain Na^+ salts capable of alkaline hydrolysis, mainly Na_2CO_3. Previously these soils have also been termed as ‘Alkali’.Further categories of salt-affected soils which, though less extensive, are commonly found in different parts of the world are: C) Acid-sulfate soils: These soils have pH below 3.5 to 4.0 and found within a 50 cm depth that is directly or indirectly caused by H_2SO_4 formed by the oxidation of pyrite (FeS_2) or other reduced S compounds which is accelerated by brackish and saline mangrove swamps. Apart from high salinity, this soil also responsible for iron (Fe) and aluminium (Al) toxicities and de fi ciency of phosphorus (P) (Pons 1973; Abrol *et al.* 1988). D) Degraded sodic soils: These soils are an advanced stage of soil development coming from the washing out of salts. In this process there is a af fi nity for the dispersed clay and organic matter to move down the profile resulting in the formation of a dark, extremely compact layer having a sharply defined upper surface and merging gradually into the subsoil with increasing depth. These soils originally had enough exchangeable Na^+ but that most of this Na^+ has been lost through leaching (Abrol *et al.,* 1988).

2.5.1 Types of salinity

There are two main types of salinity which can occur namely: primary and secondary either naturally or resulting from human activities.

2.5.1.1 Primary salinity

Primary salinity results from the accumulation of salts over long periods of time, through natural processes in the soil or groundwater. It is caused by two natural processes. The first is the weathering of parent materials containing soluble salts. Weathering processes break down rocks and release soluble salts of various types, mainly chlorides of sodium, calcium and magnesium, and to a lesser extent, sulphates and carbonates. Sodium chloride is the most soluble salt. The second is the deposition of oceanic salt carried in wind and rain. 'Cyclic salts' are ocean salts carried inland by wind and deposited by rainfall, and are mainly sodium chloride. Rainwater contains 5 to 50 mg kg^{-1} salt, the concentration of salt decreases with distance from the coast (Munns, 2002). If the concentration is 10 mg kg^{-1}, this would add 10 kg ha^{-1} of salt for 100 mm of rain per year. Accumulation of this salt in the soil would be considerable over millennia (Ghassemi *et al.,* 1995; Munns, 2002).

2.5.1.2 Secondary salinity

Secondary salinity is caused by poor irrigation water, land clearing, sea water intrusion and large levels of salt in effluent from intensive agriculture and industrial wastewater. Soil salinity build-up also takes place as a consequence of irrigation. An irrigation water containing 100 mg/L total dissolved solid will deposit an amount of 0.136 tons of salt in 11 the soil for each acre-foot per acre of water applied (Biggar *et al.,* 1984). An application of a 100 mm depth of irrigation containing 500 g salt/L-1 adds 500 kg of salt to each hectare of land (Rhoades and Loveday, 1990).

2.6 Salinity built-up

The main obstacle to intensification of crop production in the coastal areas is seasonally high content of salts in the root zone of the soil. The salts enter inland through rivers and channels, especially during the latter part of the dry (winter) season, when the downstream flow of fresh water becomes very low. During this period, the salinity of the river water increases. The salts enter the soil by flooding with saline river water or by seepage from the rivers, and the salts become concentrated in the surface layers through evaporation. The saline river water may also cause an increase in salinity of the ground water and make it unsuitable for irrigation. In addition, during years of low rainfall the volume of fresh water that drains from the watershed into rivers reduces and thus salt water from the ocean intrudes much farther inland inundating rice fields and subjecting them to salt stress. Destruction of natural vegetation such as mangroves from coastal regions and river deltas either by severe flooding or human activities has led to intrusion of saline water into productive croplands (WARDA, 2007). These problems are expected to be aggravated by climate change which is predicted to bring about increases in sea level rise, frequency of storms and rising temperatures (Yeo, 1999).

2.7 Nature and Mechanisms of Salt Stress

Most crops do not grow well on soils that contain salts. One reason is that salt causes a reduction in rate and amount of water that plant roots can take up from the soil. Also, some salts are toxic to plants when present in high concentration. The highly tolerant crops can withstand a salt concentration of the saturation extract up to 10 g L^{-1}. The moderately tolerant crops can withstand salt concentration up to 5 g L^{-1}. The limit of the sensitive group is about 2.5 g L^{-1} (Brouwer *et al.,* 1985) stated some plants are more tolerant to a high salt concentration than others. Some of the negative effects of salinity have been caused mainly by Na^+ and Cl^- ions in plants and these ions produce the decisive conditions for plant survival by intercepting different plant mechanisms. Plant roots are generally affected due to Na^+ and Cl^- along with other cations present in the soils in different concentration (1–150 mM for glycophytes; more for halophytes). However, the uptake of these ions depends on the plant growth stage, genetic characters and environmental factors like temperature, relative humidity and light intensity. Excessive amount of salt in cultivated soils retards the growth, limits economic yield and even lead plants to death. There are some points at which salt transport is regulated. These are: (i) selective uptake from the soil solution, (ii) loading of xylem, (iii) removal of salt from the xylem in the upper part of the plant, (iv) loading of the phloem and (v) excretion through salt glands or bladders (Munns *et al.* 2002) for a salt tolerant plant growing for some time in a soil solution of 100 mM NaCl, the root concentrations of Na^+ and Cl^- are typically about 50 mM, the xylem concentration about 5 mM, and the concentration in the oldest leaf as high as 500 mM (Munns 2002a) stated that the toxic ions move into the plant with the water flow. The ions move from soil to the vascular system of the root by symplastic and apoplastic pathways. In symplastic pathway, water enters into the roots through plasma membranes of epidermis and further cell-to-cell movement occurs through plasmodesmata until the xylem becomes saturated. In apoplastic pathway, water enters through intracellular spaces to unload the salt in differential osmotic potential is the dynamic force of energy driven pathways, i.e. symplastic, while apoplastic is a non-energy driven pathway. Hence, based on osmotic potential, plant can control the toxic ions like Na^+ to enter into the cell through energy driven pathway (Garciadeblas *et al.,* 2003).

2.8 The extent of salinity problem

According to a report published by FAO in 2000, the total global area of salt-affected soils including saline and sodic soils was 831 million hectares (Martinez-Beltran and Manzur, 2005), extending over all the continents including Africa, Asia, Australia and the Americas. Salinity is a major problem limiting rice production in Africa. Approximately 650,000 ha of rice production land in West Africa are threatened by salinization, particularly within the Sahel (arid or semi-arid region) where rainfed rice production is not feasible Africa Rice Centre (WARDA, 2007). This problem is expected to be aggravated by climate change which is predicted to bring about increases in sea level rise. However, not all salinity problems are confined to the semi-arid regions of the world. Ponnamperuma and Bandyopadhya, (1980) reported that some 20% of the potentially exploitable saline soils of the world are in the humid regions of South and Southeast Asia and about half of these (30 million ha) are coastal saline soils. Salinization of soil and water is a common problem in arid and semiarid regions around the world (Ghassemi *et al.,* 1995). Soil salinization diminishes crop yields, increases runoff and soil erosion, and contributes to desertification (Banin and Fish, 1995). Water salinization degrades surface water and groundwater supplies and limits irrigation. Sodium ions are well known as causing toxic damage to plant cells by both ionic and osmotic effects, causing growth retardation, low productivity and eventually, cell death (Hasegawa *et al.,* 2000; Munns *et al.,* 2002; Mansour and Salama, 2004; Chinnusamy *et al.,* 2005). Excess salinity in soil water can decrease plant available water and cause plant stress. High concentrations of salts have detrimental effects on plant growth (Garg and Gupta, 1997; Mer *et al.,* 2000) and excessive concentrations kill growing plants (Donahue *et al.,* 1983).

2.9 Salinity problem and its effects on agricultural productivity

Salinity is one of the major abiotic stresses that affects crop productivity and quality and has been described as one of the most serious threats to agriculture and the natural status of the environment (Chinnusamy *et al.,* 2005). Increased salinization of arable land is expected to have devastating global effects, resulting in a 30% land loss within the next 25 years and up to 50% by the year 2050 (Wang *et al.,* 2003). Earth is a salty planet, with most of its water containing about 30 g of sodium chloride per litre. This salt solution has affected, and continues to affect, the land on which crops grow or might be grown; its extent is sufficient to pose a threat to agriculture (Flowers and Yeo, 1995; Munns, 2002). Approximately 7 % of the world's land area, 20 % of the world's cultivated land and nearly half of the irrigated lands are affected with high salt contents (Szabolcs, 1994; Zhu, 2001). In view of another projection, 2.1% of the global dry land agriculture is affected by salinity (FAO, 2003). Effects of salinity are more obvious in arid and semi-arid regions where limited rainfall, high evapo-transpiration and high temperature associated with poor water quality and soil management practices are 13 the major contributing factors (Azevedo Neto *et al.,* 2006). One-fifth of irrigated agriculture is negatively affected by high soil salinity. The expected population growth, over 9 billion by 2050, will increase the pressure for agricultural production on marginal saline lands. Salt affected soils can be divided into three main groups: (1) saline soils, (2) sodic soils, and (3) saline-sodic soils (Reeve and Fireman, 1979). Saline soils have excessive accumulations of soluble salts whereas sodic soils have high concentrations of exchangeable sodium (ES). Saline-sodic soils have a combination of both properties. Saline soils have an electrical conductivity (E.C.), of saturated paste extract, greater than 4 dS m^{-1} and the pH does not exceed 8.5. Calcium and magnesium are the dominant exchangeable cations. These soils are usually stable and have good structure. The good structure is caused by the flocculating effect of calcium and magnesium. If adequate drainage is established, the excessive soluble salts may be removed by leaching and they become non-saline soils again. The exchangeable sodium percentage (ESP) exceeds 15.

2.9.1 Effects of salinity on nutrient uptake

Excessive amount of soluble salts in the root environment causes osmotic stress, which may result in the disturbance of the plant water relations in the uptake and utilization of essential nutrients, and also in toxic ion accumulation (Munns, 2002; Lacerda *et al.,* 2003). The interactions of salts with mineral nutrients may result in considerable nutrient imbalances and deficiencies (McCue and Hanson, 1990). Ionic imbalance occurs in the cells due to excessive accumulation of sodium ion (Na^+) and Chlorine (Cl^-) ion and reduces uptake of other mineral nutrients, such as potassium, Calcium, and Manganese (Karimi *et al.,* 2005). It has been documented that many plant species have the ability to compartmentalize and accumulate Na^+ and Cl^- in older leaves. Only at high salinity levels, or in sensitive species which cannot control Na^+ transport or compartmentalize the ions, the ionic effect dominates the osmotic effect (Munns and Tester, 2008), toxic ionic effects of excess Na^+ and Cl^- uptake, and reduces nutrient uptake (K^+, Ca^{2+}) because of antagonistic effects of salinity on rice growth (Dobermann and Fairhurst, 2000). The high salinity increases sodium concentration and sodium uptake. During a long time in salinity, therefore, the sodium toxicity causes reduction in the yield (Castillo *et al.,* 2003).

2.10 Effects of salinity on crop physiological processes

Soil salinity causes adverse effects on different physiological processes (nitrogen fixation, photosynthesis, Osmosis) which are responsible for the reduction of growth of plants (Ashraf, 2004; Munns *et al.,* 2006). Adverse changes in morphological structures associated with physiological modifications due to salinity may be the main factors of growth decline under salt stress. Salt accumulation in the expanding leaves has been correlated with photosynthetic decline and with ultra-structural and metabolic damages and sequential death of leaves (Yeo and Flowers, 1986), and growth vigour may be related to the survival efficiency of different varieties (Yeo *et al.,* 1990). So, leaf characters and physiological growth attributes may be important criteria for a tolerant variety. High salt contents reduce the growth and production by affecting physiological processes, including modification of ion balance, water status, mineral nutrition, stomatal behaviour, and photosynthetic efficiency (Munns, 1993). Most plants are salt sensitive with either a relatively low salt tolerance or severely inhibited growth at low salinity levels so differ in 15 the growth response to salinity (Moisender *et al.,* 2002; Sheekh and Omer, 2002). Salt stress affects plant physiology at both whole plant and cellular levels through osmotic and ionic stress (Murphy and Durako, 2003). High concentration of salts in the root zone decreases soil water potential and the availability of water (Lloyd *et al.,* 1989). This deficiency in available water under saline condition causes dehydration at cellular level and ultimately osmotic stress occurs. The excessive amounts of toxic ions like Na^+ and Cl^- create an ionic imbalance by reducing the uptake of beneficial ions such as K^+, Ca^{2+} and Mn^{2+} (Hasegawa *et al.,* 2000).

2.11 Effects of salinity on plant relative water content

Salinity appears to affect two processes: water relations and ionic relations. During initial exposure to salinity, plants experience water stress, which in turn reduces leaf expansion. During long-term exposure to salinity, plants experience ionic stress, which can lead to premature senescence of adult leaves. The problem is compounded by mineral deficiencies (Zn, P) and toxicities (Fe, Al, and organic acids), submergence, deep water and drought (Gregorio, *et al.,* 2002). There are antagonistic effects on nutrient uptake by plants that cause nutrient disorders particularly of potassium (K^+) and calcium (Ca^{2+})

under salinity conditions. Excessive Na^+ concentration inhibits Ca^{2+} uptake in many plants (Grieve and Fujiyama, 1987; Dobermann and Fairhurst, 2000). Rice as a salt-sensitive crop is a species native to swamps and freshwater marshes and its cultivated varieties provide one of the world's most important food crops. Salinity leads to dehydration and osmotic stress, resulting in stomatal closure, reduced supply of carbon dioxide and a high production of reactive oxygen species, causing irreversible cellular 16 damage and photoinhibition (Darwish *et al.,* 2009). The effect of salinity on the rice depends on several factors: (i) the intensity of the stress, (ii) the climatic conditions and (iii) the resistance level of the genotype (Asch and Wopereis, 2001).

2.12 Effects of soil salinity on plant growth

Moradi *et al.* (2003) reported that the saline soils are characterised by white surface crust, good soil tilth, high fertility and poor yield. The white surfaces occur as water evaporates from saline soil salts which were in the water are left behind and accumulate on the soil surface. The excess of these salts keep the clay in a flocculated state so that these soils generally have good physical structure, tillage characteristics and permeability to water, even better than those of non-saline soils (Siyal *et al.,* 2002). In these soils, the salinity does not affect the physical properties of soils but it is harmful because elevated soluble salts in the soil solution reduce the availability of soil water to plants (Moradi *et al.,* 2003; Siyal *et al.,* 2002). Salts tie up high amount of water in the soil and prevent plantsfrom absorbing it by osmotic pressure. Seedlings are the most sensitive to water stress and crop stand is reduced because of seedling death and poor yield (FAO, 2003). Manney (2004) reported salts accumulate to toxic levels thereby enhancing the rate of leaf senescence. Manney (2004) reported further that both initial and long term effects of salt stress lead to a reduced photosynthetic capacity of the plant through a reduction in leaf area, chlorophyll content and stomatal conductance, and eventually low biomass production. Moreover, processes leading to seed germination, seedling growth, flowering and fruit set are also negatively affected (FAO, 2003). However, the lower the salts content of the soil the lower the dS m^{-1} rating and the less effect on plant growth (FAO, 2005). Studies by Maas (1986) reported that crop yields are not significantly affected where the salt level is 0 to 2 dS m^{-1}. A level of 2 to 4 dS m^{-1} restricts some crops. Levels of 4 to 5 dS m^{-1} restrict many crops and above 8 dS m^{-1} restrict all but very tolerant crops. According to Chinnusamy *et al.* (2005) most grain crops and vegetables are glycophytes and are highly susceptible to soil salinity above EC of 2 dS m^{-1}. They reported further that a threshold above which reduction in rice yield is anticipated ranges from 1.9 to 3.0 dS m^{-1} (Chinnusamy *et al.,* 2005). Furthermore, FAO (2005) highlighted yield losses for most salt sensitive crops. The report showed that if the EC is less than 4, the yield loss will be less than 10%; if the EC is more than 6, the yield loss will be 20 – 50% and when the EC is more than 10, then yield loss will be more than 50%. Most crops are adversely affected by salinity. In general, cereals are more tolerant than legumes (Reynolds *et al.,* 2005). Many wild relatives of crop plants show greater tolerance than their domesticated descendents (Blum, 2004). Salt has both osmotic and salt-specific effects on plants, (Munns, 2002; Munns, 2005), impacting at different times (Shannon, 1997). Rapid changes can occur in field salinity (Richards, 1984). The effects of these changes are increased by plants preferentially extracting water from less saline areas of the root zone, by drought in rainfed environments (Srivastava and Jana, 1984), and by water logging in irrigated environments. This can be compounded by additional stresses which vary with time (Gregorio *et al.,* 2003). Among cereals, rice (*Oryza sativa*) is the most sensitive (Munns and Tester, 2008). The general effect of salinity is to reduce the growth rate resulting in smaller leaves, shorter stature, and sometimes fewer leaves (Munns and Termaat, 1987; Jacoby, 1994). Rajendran *et al.* (2009) reported that salinity stress affects crop growth, yield and productivity. The reduction in shoot growth occurs in two phases: a rapid response to the increase in external osmotic pressure, and a slower response due to the accumulation of Na^+ in leaves (Munns and Tester, 2008). Roots are also reduced in length and mass but may become thinner or thicker. Maturity rate may be delayed or advanced depending on species (Shannon and Grieve, 1999). Salinity effects on plants are complex (Greenway and Munns, 1980). The initial and primary effect of salinity, especially at moderate salinity concentrations, is due to its osmotic effects (Munns and Termaat, 1986). At the whole plant level, ion concentrations in plant tissues increase as a result of salinity stress. The measurable or visible effects of salinity on plants can include reduced growth rate, damage of meristems in growing shoots, reductions in yield components, or typical symptoms of nutritional disorders under osmotic and ionic stress. Grain yield reduction of rice under stress of University of root zone salinity can be caused by injuries at both seedling and maturity stages. In most commonly cultivated rice cultivars, young seedlings were very sensitive to root zone salinity (Zeng and Shannon, 2000a). The degree to which growth is reduced by salinity differs greatly with species and to a lesser extent with varieties within a species. The severity of salinity response is also mediated by environmental interactions such as relative humidity, temperature, radiation and air pollution (Shannon *et al.,* 1994).

2.13 Characteristics of salt affected soils

Salinity refers to the increase in the soil surface of dissolved salts, mostly sodium chloride or common table salt, but calcium, magnesium, sulphates and bicarbonates are also implicated in soil contamination by salts (Mba *et al.,* 2007). Oldeman *et al.* (1991) had estimated that 19.5% of irrigated land was salt-affected soils and that 2.1% of dryland agriculture, was salt-affected soils. All estimates indicate a disturbing trend for this major constraint to irrigated and dry land agriculture. A distinction can be made between primary salinization that involves salt accumulation through natural processes and secondary salinization processes caused by human interventions such as inappropriate irrigation practices (Gergely, 2012). There are two main categories of salt affected lands, the saline, sodic (alkali). This classification is based on the electrical conductivity (EC); soil pH and exchangeable sodium percentage or sodium adsorption ratio (SAR) (Lamond and Whitney, 1992; Flowers and Flowers, 2005).

2.14 Harmful effects of salt stress on plants

A soil is considered saline if it contains soluble salts in sufficient quantities that can interfere with the growth of most crop species (Elphick *et al.,* 2001; Werner and Finkelstein, 1995). The harmful effects of salts in inhibiting plant growth can broadly be discussed in two categories namely, osmotic stress and ionic toxicity. Due to the presence of high salt under salt stress, the osmotic pressure in the soil solution exceeds that of plant cells reducing the plant's ability to take up water and essential minerals nutrients like K^+ and Ca^{2+} (Glenn, Brown and Khan, 1997; Munns, James and Läuchli, 2006). In extreme cases, the soil solution may become hyper-osmotic causing the root cells to lose water instead of absorbing it. Water scarcity disrupts maintenance of cell turgor and cell elongation causing wilting and ultimately death of a plant. This effect of salt stress can thus be described as the drought effect of salt stress (Zhang *et al.,* 2001; Apse and Blumwald, 2002; Munns *et al.,* 2002). The sodiumion (Tuteja 2007) on the other hand, potassium (K^+), if present in the cytosol at a concentration higher than the adequate level can be very harmful for most plants (Na^+) is an essential macronutrient and should be abundant in the cell for efficient metabolic functioning as it is involved in osmo-regulation, maintenance of membrane potential and turgor and enzyme activation (K^+) is the co-factor for more than 50 enzymes (Fox and Guerinot, 1998; Maathius and Amtman, 1999; Mäser *et al.,* 2002a; Cuin *et al.,* 2003). Since Na^+ and K^+ are physicochemically similar monovalent cations when fully hydrated, they compete in saline conditions for uptake through shared transport systems (Schachtman and Liu, 1999). Moreover, at a high concentration, Ca^{2+}can be displaced from the plasma membrane by Na^+ which negatively impacts intracellular K^+ influx in the cell and changes the homeostasis of other ions such as NO_3^- (Cramer, Epstein and Lauchli, 1989). Thus excessive uptake of Na^+ alters (mainly elevates) Na^+/K^+ ratios and exerts metabolic toxicity as Na^+ and K^+ competes for the binding sites of many enzymes and disrupts many crucial processes in which K^+ is involved (Bhandal and Malik, 1988; Zhang *et al.* 2001; Tester and Dabenport, 2003; Munns, James and Läuchli, 2006). High Na^+ on the other hand can dissipate the membrane potential and facilitate the uptake and buildup of Cl^- in the cytosol and exert a direct toxic effect on cell membranes and on metabolic activities in the cytosol (Greenway an Munss, 1980; Hasegawa *et al.,* 2000; Zhu, 2001; Tuteja, 2007). Consequences of these primary effects cause some secondary effects like reduced cell expansion and membrane function, assimilate production, photosynthesis as well as decreased cytosolic metabolism and production which ultimately causes growth inhibition and eventual death of plants.

2.15 Mechanisms of Na^+ uptake in plants

In saline soil, epidermal cells of root tips including root hairs are the primary sites for the uptake of inorganic ions through the plasma membrane (Golldack *et al.* 2003; Horie *et al.,* 2012). The uptake of salts into roots and translocation into shoots can primarily be attributed to the transpirational flux of the plants (Amtmann and Sanders 1998; Yeo 1998; Blumwald 2000). The H^+-ATPases of plasma membrane acts as the primary pump and generates a proton motive force which drives the transport of Na^+ and K^+ along with other solutes (Braun *et al.,* 1986; Craig Plett and Møller 2010; Kronzucker and Britto 2011). The transport proteins spanning the lipid bilayer of the plasma membrane which is usually impermeable to solutes facilitate the movement of solutes in and out of the cytosol. Sodium (Na^+), a positive ion mainly enters the cytosol passively as the potential in plant cells is negative inside the plasma membrane (Blumwald 2000; Hasegawa 2013; Yamaguchi *et al.,* 2013; Adams and Shin, 2014). Once it enters epidermal cells or cortical cells, Na^+ may follow a symplastic (connected by plasmodesmata) or apoplastic pathway (bypass flow) before it encounters the Casparian bands in endodermis layer (Yeo, Yeo and Flowers 1987; Yadav, Flowers and Yeo, 1996; Ochiai and Matoh 2002; Gong, Randall and Flowers 2006; Krishnamurthy *et al.,* 2009). Apoplastic enzymes shows more tolerance to salts than cytoplasmic enzymes, in both halophytes and glycophytes, indicating the ability of apoplast to withstand relatively high concentrations of Na^+ (Thiyagarajah *et al.,* 1996; Adams and Shin, 2014).

2.15.1 Na^+ toxicity on plants

Growth inhibition by Na^+ toxicity is one of the principal adverse effects of salt stress in plants. The sodium ion (Na^+) is very harmful in cell for most plants when it is present in the cytosol at a concentration higher than the adequate level (1-10 mM). The potassium ion (K^+), on the other hand, is one of the essential and most abundant monovalent cations in cells, and needs to be maintained within 100-200 mM range in the cytosol for efficient metabolic functioning (Walker, Sanders and Maathuis, 1996, Taiz and Zeiger, 2002; Cuin *et al.,* 2003). As a co-factor in cytosol, K^+ activates more than 50 enzymes, which are very susceptible to high cytosolic Na^+ and high Na^+/K^+ ratios (Munns, James and Läuchli, 2006). Therefore, apart from low cytosolic Na^+, maintenance of a low cytosolic Na^+/K^+ ratio is also critical for the function of cells (Rubio, Cassmann and Schroeder, 1995; Zhu, Liu and Xiong, 1998). At saline conditions, Na^+ competes with K^+ for uptake through common transport systems, since Na^+ and K^+ are physicochemically similar monovalent cations. Thus, elevated levels of cytosolic Na^+, or in other way high Na^+/K^+

ratios, exert metabolic toxicity by a competition between Na^+ and K^+ for the binding sites of many enzymes (Bhandal and Malik, 1988; Tester and Davenport, 2003). Moreover, at a high concentration, Na+ can displace Ca^{2+} from the plasma membrane, resulting in a change in plasma membrane permeability. This can be reflected by a leakage of K^+ from the cells (Cramer, Epstein and Läuchli, 1989). This high uptake of Na^+ and leakage of K^+ result in an imbalance in the Na^+/K^+ ratio in the cytosol, which, in turn, leads to many imbalances in enzymatic reactions of the cell. As a consequence of these primary effects, secondary stresses, such as oxidative damage, often occur. In extreme cases these adverse effects contribute to plant growth inhibition and even plant death.

2.16 Salinity a tough enemy to the agriculture

Salinity is defined as the presence of excessive amount of soluble salt that hinder or affect negatively the normal function needs for plant growth. It is measured in terms of electroconductivity (EC), or exchangeable Na^+ percentage (ESP) or with the Na^+ absorption ratio (SAR) and pH of the saturated soil past extract. Therefore saline soil are those with EC more than 4 dS m^{-1} equvilant to 40 mM NaCl, ESP less than 15% and pH below 8.5 (Waisel, 1972 and Abrol, 1986). Table 1 showing the three different level of salinity and its relations to the value of EC as a common factor for salinity degree classifications. Approximately 20% of the irrigated lands in the world are presumably affected by soil salinization (yeo, 1999). Saline lands are not only distributed in desert and semi-desert regions but also frequently occurs in fertile alluvial plains, rivers, valleys, and coastal regions, close to densely, populated areas and irrigation systems. For example, the agricultural Egyptian economy suffers from severe salinity problems 33% of the cultivated lands are already salinized. The costs of salinity to agriculture are estimated conservatively to be about $US 12 billion a year and it is expected to increase as soil are further affected (Ghassemi *et al.,* 1995). Salinity also could be severely destructive to the agricultural economy as a result of natural causes. For instance, recent deposition of toxic salt sediments and sea intrusion in tsunami-affected areas of Maldives damage >70% of agriculture land, destroyed >370,000 fruit tree and affected around 15,000 farmers, with cost estimated at around AU$ 6.5 million (FAO, 2005). Most of grain crops and vegetables are glycophytes (salt-sensetive flora), therefore are highly susceptible to soil salinity even when the soil EC is < 4 dS m^{-1}. Different threshold tolerance EC and different rate of reduction in yield beyond threshold tolerance indicates variations in salt tolerance among those crops (Chinnusamy *et al.,* 2005).

2.17 The impact of salinity stress on the water status of plants

In fact, in saline soils, although water is present it is unavailable to plants because it retained by the ions in the soil, such as Na^+ and Cl^-. Under non-stress conditions, intracellular osmotic potentials (Ψ) is generally more negative than that of the soil solution, resulting in water influx into roots according to water potential gradient. Because of dissolved ions that decrease extracellular Ψosm, salinity stress immediately reduce $\Delta\Psi$ thus water influx. If the water potential gradient is reversed due to sever salinity / osmotic stress, water efflux from roots (dehydration) can occur (Horie et al, 2012). Typically, as the water content of the plant decreased, its cell shrink and the cell wall relax. This decrease in cell volume results in lower turgid pressure, additionally the plasma membrane become thicker and more compressed because it covers a smaller area than before. Removal of water from the membrane disturbs the normal bi-layer structure and results in the membrane becoming exceptionally porous when desiccated (Taiz and Zeiger, 2002). Stress within the lipid bi-layer may also results in displacement of membrane proteins and this contributes to loss of membrane integrity, selectivity, disruption of cellular compartmentalization and a loss of enzyme activity, which are primarily membrane based. In addition to membrane damage, cytosolic and organelle proteins may exhibit reduction activity or may even undergo complete denaturation when severely dehydrated. The high concentration of cellular electrolytes due to the dehydration of protoplasm may also cause disruption of cellular metabolism (Mahajan and Tuteja, 2005).

2.18 Ecology of rice

The rice plant is an annual grass with round, hollow, jointed culms, flat leaves, and a terminal panicle. It is the only cultivated cereal plant adapted to growing in both flooded and non-flooded soils. Rice is grown under a wide range of climatic and geographical conditions on all five continents (Toriyama *et al.,* 2005). Rice is grown in widely diverse production environments. Five major rice growing environments can be broadly identified based on water regime: irrigated, rainfed lowland, tidal wetland, deepwater, and upland (Khush, 1984).

CHAPTER III

MATERIALS AND METHODS

Laboratory and pot experiments were conducted to study the effect of different salinity levels on germination, and growth, performance of some local and exotic rice varieties. The detailed experimental methodology used in each experiment is as outlined below:

3.1 Laboratory experiment: Effect of salinity on germination and early growth response of rice varieties

3.1.1 Experimental site

The germination test on rice seeds was conducted in Petri-dishes at the laboratory, Department of Agricultural Chemistry, of Hajee Mohammad Danesh Science and Technology University (HSTU), at room temperature (27 ±2^{o}C) with 12 hour light.

3.2 Period of study

The experiment was conducted during the period of July to December 2017.

3.3 Experimental treatments and layout

The factorial combinations of treatments were laid out in a completely randomized design (CRD) with four replications. Treatments comprised of 3 rice varieties, namely Nonabokra, Botessor, and BRRI dhan28, and four different salinity levels *viz*., 0 (distilled water), 3, 6, and 9 dS m^{-1}.

3.3.1 Selection of rice varieties

Three varieties

a) Nonabokra,

b) Botessor, and

c) BRRI dhan28

3.3.2 Preparation of salinity treatments

Six salinity levels *viz.* 0 (control), 3, 6 and 9 dS m^{-1} were used in this experiment. Different salinity levels were prepared by dissolving commercial salt (NaCl, Batch# 088K0089, SIGMA-ALDRICH Co., USA) at the rate of 640 mg per litre distilled water for 1 dS m^{-1} salinity level. Distilled water was used as the control i.e. 0 salinity. After preparation the salinity levels were checked by electrical conductivity meter (model: Z 865/SCHOTT Instruments, Germany) and necessary adjustment was made.

3.3.3 Germination test

Seeds were surface sterilized with 1% sodium hypochlorite solution, rinsed with sterile water, and germinated in 11.4 cm petri-dishes lined with filter paper. Twenty-five seeds were placed on the filter paper in each petri-dish, and 10 mL of treatment solution of different salinity was used in each petri-dish to immerse the seeds partially. Six petri-dishes were placed in plastic trays and kept enclosed in a polythene bag, and the seeds were allowed to germinate at room temperature (27 ±2^{o}C). Distilled water was added to each petri-dish everyday as necessity. Seeds were considered germinated when both the plumule and radicle were extended more than 2 mm (ISTA, 1999), and the number of seeds germinated were recorded daily up to 9th day. Plumule and radicle lengths were measured by centimetre scale on the 9th day. The plumule and radicle samples were oven-dried to a constant weight at 65 ^{o}C and dry weights were recorded for each treatment.

3.3.4 Germination index, final germination percent and germination energy

At 9th day after final count, germination index (GI) was calculated as described in the hand book of AOSA (1983).

$$GI = \frac{\text{No. of germinated seeds}}{\text{Day of first count}} + \ldots\ldots\ldots\ldots + \frac{\text{No. of germinated seeds}}{\text{Day of final count}}$$

Final germination percent (FGP) at 4 DAS were calculated using the following formulae (Ellis and Robert, 1981; Ruan *et al.,* 2002).

$$\mathrm{FGP} = \frac{\text{Number final germinated seeds}}{\text{Total number of seed tested}} \times 100$$

Germination percentage with formula:

$$\text{Germination (\%)} = \frac{\text{No of seeds germinated}}{\text{Number of seeds placed}} \times 100$$

Varieties were classified as tolerant (T, with 0-20% reduction), moderately tolerant (MT, with 21-40% reduction), moderately susceptible (MS, with 41-60% reduction) and susceptible (S, with >60% reduction) based on total dry matter (plumule and radicle) reduction due to salt impositions (Fageria, 1985).

3.4 Pot experiment: Effect of salinity on growth performance of selected rice varieties

3.4.1 Experimental site

The experiment was conducted in pots (20cm diameter × 17 cm depth) at a pots in Department of Agricultural Chemistry, Hajee Mohammad Danesh Science and Technology University (HSTU). The temperature was measured using a thermometer and light intensity was measured with a heavy duty light meter (Extech ® model 407026).

3.5 Period of study

The experiment was conducted during the period July to December 2017.

3.6 Experimental treatments and layout

The factorial combination of treatments was laid out in a randomized complete block design (RCBD) with four replications. Treatments included 3 rice varieties namely Nonabokra, Botessor, and BRRI dhan28, and four different salinity levels *viz.*, 0 (distilled water), 3, 6, and 9 dS m^{-1}.

3.6.1 Selection of rice varieties

Three rice varieties were selected for use in the present study based on the results of the earlier germination experiment. Nonabokra, Botessor and BRRI dhan28.

All rice seeds were collected from Bangladesh Rice Research Institute (BRRI).

3.6.2 Preparation of growth media

Soil for the pot experiment was collected from HSTU field. The soil was pulverized, and inert materials, visible insect pests and plant debris were removed. The soil was then crushed, mixed thoroughly, and dried in the sun. Three kg of soil was placed in each pot. Soil was analyzed for total nitrogen using the Kjeldahl method and soil organic carbon was determined according to Walkley and Black (1934). Available phosphorus was determined by Murphy and Riley method (Bray and Kurtz, 1945) and exchangeable K, Ca, Mg and Na were determined by ammonium acetate extraction method (Benton Jones, 2001). The stretches from latitude 3° 59' N to 2°44' N and longitude 100°29' E to 101°48' E. The soil series, physical and chemical properties were recorded.

3.6.3 Preparation of salt treatments

Four salinity treatments *viz.*, 0 (control), 3, 6 and 9 dS m^{-1} were used in this experiment. Salt treatments preparation has been described in section earlier.

3.6.4 Rice seedling establishment and application of salinity treatments

The pots were filled with 3 kg soil well mixed with urea, triple supper phosphate (TSP), muriate of potash (MOP) and gypsum as sources of N, P, K and S at the rate of 60 kg N, 80 kg P_2O_5, 150 kg K_2O and 20 kg S ha^{-1}, respectively. The rice seeds were soaked in water for 24 hours followed by incubation for 12 hours to allow sprouting**.** Three week-old rice seedlings were transplanted into the pots with three seedlings per pot. Two weeks after transplanting the salt treatments were applied. To avoid osmotic shock, salt solutions were added in three equal portions on alternate days until the expected conductivity (0, 3, 6 and 9 dS m^{-1}) was reached. Urea was top dressed twice at 30 and 45 days after transplanting at 60 kg N ha^{-1}. Standard agronomic practices were adopted and crop protection measures were carried out as necessary. Leachates of salt solutions were collected daily from each pot, monitored for electric conductivity (EC) measurements and necessary adjustments were made. Conductivity of soil was determined using conductivity meter (Model: ECTestr, Spectrum Technologies, Inc.).

3.7 Parameters measured

3.7.1 Growth parameters

Plant height (cm) was measured from the ground level to the tip of the longest leaf just before harvesting. Shoot and root samples were carefully separated and rinsed in distilled water. The root and shoot samples were oven-dried to a constant weight at 70°C for 72 hours. The mean root dry weight $hill^{-1}$ was calculated for each treatment.

3.7.2 Biochemical constituents

Leal samples were collected from three plants of each treatment at 45 days after transplanting for biochemical test.

3.7.2.1 Proline content

Proline was estimated according to the method of Bates *et al.* (1973). About 45 days old fresh rice leaf tissue (0.5 g) was homogenized in 10 mL of 3% sulfo-salicylic acid, and filtered through Whatman No. 2 filter paper. Two mL of the filtrate was brought to reaction with 2 mL acid ninhydrin solution (1.25 g ninhydrin in 30 mL glacial acetic acid), 20 mL 6M orthophosphoric acid, and 2 mL of glacial acetic acid for 1 hour at 100°C. The reaction was then terminated in an ice bath, and extracted with 4 mL toluene, mixed vigorously by passing a continuous stream of air for 1-2 min. The chromophore containing toluene was aspirated from the aqueous phase, warmed at room temperature and the absorbance was read using scanning spectrophotometer (Model UV-3101PC, UV-VIS NIR) at 520 nm. The proline concentration was determined from a standard curve and calculated on a fresh weight basis as follows:

µmol proline g^{-1} fresh weight = (µg proline mL^{-1} ×mL of toluene/115.5)/ (g of sample).

3.7.2.2 Chlorophyll content

Leaf samples were collected at 45 days after transplanting from each treatment. Three cm^2 fresh leaves were transferred into small vials containing 20 mL of 80% acetone, covered with aluminum foil, and kept in the dark for 7-10 days to ensure release of all the chlorophyll from the tissues. A 3.5 ml of supernatant was then sampled to measure the absorbance using a spectrophotometer (Systronics UV- VIS 118) at 664 and 647 nm wave lengths. The chlorophyll content was calculated using the following formulae (Coombs *et al.,* 1987):

Chlorophyll-a (mg/cm^2) = 3.5/3(13.19A_{664}—2.57A_{647})

Chlorophyll-b (mg/cm^2) = 3.5/3 (22.10A_{647}—5.26A_{664})

3.7.2.1.1 Mineral elements

After harvest the root and shoot samples were ground using a Wiley Hammer Mill, passed through 40 mesh screens, mixed well and stored in plastic vials until analysis. The samples were analyzed separately to determine the Na, K, Ca and Mg contents. All elemental analyses were conducted on acid digested materials using the micro-Kjeldahl digestion system (Thomas *et al.,* 1967). The content of Na, K, Ca and Mg was measured by Atomic Absorption Spectrophotometer (AAS) (Clesceri *et al.,* 1988). The Na/K, Na/Ca, Na/Ca, K/Ca, K/Mg and Ca/Mg ratios were calculated from concentrations of Na, K, Ca, and Mg in both shoot and root tissues.

3.7.2.1.2 Carotene and Chlorophyll from plant sample

Preparation of solvent: Acetone: n-Hexane (4:6).

Procedure: 1 g sample was taken in a test tube with stopper about 15 ml solvent was added to it. Then it was heated for 15 minutes, then it was filtered and measured absorbance at 663 nm, 505 nm and 453 nm. Beta carotene content was estimated in mg/g or mg/100ml by using the following equation.

Calculation: β-Carotene = 0.216x A_{663}- 0.304 x A_{505} + 0.452 x A_{453} (mg/g)/ (mg/100ml if the sample is liquid).

$$\text{Vitamin A (I.U)} = \frac{\text{beta} - \text{carotene(mg/g)}}{0.6}$$

Ash content: Ash content was determined by the AOAC (1990). 5 g of sample was weighed accurately in the pre-weighed crucible (w_1). The crucible was heated in a furnace at 650^0 C for maximum 4 hrs. Then cooled the crucible in a desiccators and weighed (w_2). Ash content was calculated using the following equation.

$$\%\text{Ash}=\frac{W1}{W2}\times 100$$

3.7.2.1.3 Estimation of Calcium (Ca) from plant extract by Complexometric Method of Titration Using Na_2-EDTA as a Complexing Agent

Procedure

Take 5 ml of plant extract solution in a 250 ml conical flask. Then added 20 ml distilled water into the conical flask and shake thoroughly. And added10 drops of each masking agent of a) Hydroxylamine hydrogen chloride. b) Potassium ferocyanide c) Triethanolamine (TEA). After that added 5 ml NaOH buffer solution and shake thoroughly. And added 5-6 drops calcon indicator into the conical flask (depending on the concentration of the indicator solution) and shake the flask thoroughly. Then titrate against 0.01 M Na_2-EDTA solution from burette. Continue the titration until pink color of the solution completely turns to pure blue color. And repeat the experiment at least 3 times. And Conduct a blank experiment also by taking all the reagents as above except calcium stock solution. Take 5 ml more distilled water into conical flask instead of calcium solution. Finally tabulate the data and calculate the amount of calcium present in the prepared solution.

Formula of Calculation: 1 ml 1M EDTA ≡ 1ml 1M Ca = 40.08 mg Ca

3.7.2.1.4 Estimation of Magnesium from plant extract by Complexometric Method of Titration Using Na_2-EDTA as a Complexing Agent

Procedure

Take 5 ml of plant extract solution in a 250 ml conical flask. And added 20 ml distilled water into the conical flask and shake thoroughly. And added 10 drops of each masking agent of (a) Hydroxylamine hydrogen chloride (b) Potassium ferocyanide (c) Triethanolamine (TEA) (d) Sodium tungstate. And added Ammonia ammonium buffer solution and shake thoroughly. And added 5-6 drops EBT indicator into the conical flask (depending on the concentration of the indicator solution) and shake the flask thoroughly. Then titrate against 0.01 M Na_2-EDTA solution from burette. Continue the titration until pink color of the solution completely turns to pure blue color. And repeat the experiment at least 3 times. And Conduct a blank experiment also by taking all the reagents as above except plant extract solution. Take 5 ml more distilled water into conical flask instead of extract solution. Finally tabulate the data and calculate the amount of calcium present in the prepared solution.

Formula of Calculation: 1 ml 1M EDTA ≡ 1ml 1M Mg = 24.305 mg M

3.7.2.1.5 Estimation of Sulfur from the Plant/Fertilizer Samples Turbidimetrically Using Barium Chloride as Turbidimetric Reagent

Procedure:

Add about 0.3 g (1 scoop) barium chloride to each standard series, and added about 0.3 g (1 scoop) barium chloride to 20 ml of unknown test solution, and Mix it until barium chloride dissolves completely, then allow to stand it for 30 minutes before reading, after that take spectrophotometer reading at 425 nm wavelength putting the cuvette in the cuvette chamber against the blank one, Finally find out the concentration of sulfate from the standard curve.

Standard curve preparation

A standard curve was prepared using X-axis for S concentration (ppm) and Y-axis for absorbance. The obtained values of absorbance for S standard series solution are plotted against their respective concentration (ppm) in the mm graph paper. The points are added using straight line. The standard curve should be straight line. The standard curve can be easily prepared using the Microsoft Excel software. The absorbance data put against its concentration of S solution. The graph prepared and a straight line equation is obtained according to the data input, ie., Y= a+bx, where x is S concentration and Y is absorbance, and be are constant values. a and b values are obtained from the graph. Then unknown concentration of S sample can be obtained using the straight line equation.

Result:

By plotting the absorbance of unknown test sample on the standard curve, the concentration of S in the unknown test sample is computed from the mm graph paper as ppm.

$$\text{mg \% Sulfur of plant/fertilizer sample} = \frac{\text{ppm}}{\text{weight of sample} \times 1000} \times 100$$

3.7.2.1.6 Estimation of phosphorus from water soluble triple super phosphate by stannous chloride method with the help of a spectrophotometer

Procedure

a) Development of molybdophosphoric blue color

5 ml of plant extract /4th TSP working solution was taken in a 100 ml volumetric flask. About 50 ml distilled water was added to it. Then 4 ml of sulphomolybdic acid solution was added and 5-6 drops of stannous chloride solution was added to it and was shaken thoroughly. Then the volume was made up to the mark with distilled water. Then the absorbance was taken in a spectrophotometer at 660 nm wavelength within 5 minutes. Prepare a blank solution also by taking all the reagents as described except phosphorus solution.

b) Preparation of standard or calibration curve

Prepare a standard or calibration curve by plotting the absorbance (optical density) of light in Y axis and concentrations of the solutions in X axis in a graph paper. By plotting the spectrophotometer reading on the standard curve, the concentration of test sample (TSP working solution) is easily obtained

Calculation

$$\text{Mg\% Phosphorus} = \frac{\text{ppm} \times 100 \times 100}{\text{weight of sample} \times 5 \text{X}1000} \times 100$$

Result

Z mg % P is present in the supplied plant extract /TSP fertilizer sample.

Relationship: $\%P = \%P_2O_5 \times 0.43$

$\%P_2O_5 = \% P \times 2.29$

3.7.2.1.7 Estimation of Potassium from plant extract/water soluble muriate of potash with the help of a flame emission spectrophotometer

a) Preparation of standard series solutions of potassium (5, 10, 15, 20, 25, 30, 40, 50 and 60 ppm K)

Prepared a series of potassium standard solutions containing 5, 10, 15, 20, 25, 30, 40, 50 and 60 ppm K by pipeting 5, 10,15, 20, 25, 30, 40, 50 and 60 ml of 100 ppm K solution in 9 (nine) different 100 ml volumetric flasks, respectively. Made the volume up to the mark with distilled water in each flask and shaked the flasks thoroughly. Thus, a series of potassium standard solution are prepared.

b) Preparation of standard or calibration curve

Take the readings of flame emission spectrophotometer (% emission) for the potassium standard series solutions along with the unknown MP fertilizer sample. Prepare a standard or calibration curve in a graph paper by plotting the concentrations of different K standard solutions on X axis and corresponding percent of emission on Y axis. By plotting the flame emission spectrophotometer reading (% emission) on the standard curve, the concentration of test sample (unknown) can be easily obtained.

Calculation

By plotting this % emission of unknown plant extract /fertilizer sample on the standard curve, the concentration of K in the plant extract /4th MP solution is **X** ppm.

$$\text{Mg\% K} = \frac{\text{PPm} \times 100}{\text{wt. of sample} \times 1000} \times 100$$

Result

Mg % K is present in the supplied plant sample/ MP fertilizer.

3.7.2.1.8 Estimation of Iron from plant extract

Procedure

Place 0.5 to 0.75 grams of the unknown compound into a clean 125 ml Erlenmeyer flask. And added 50 ml of the Sulfuric Acid to the flask. And swirl the flask until the iron compound is dissolved. Then titrate the iron compound with the Potassium Permanganate solution to the colorimetric endpoint. And perform a total of three trials. Finally calculate the % iron in the unknown compound for each trial.

Calculation: 1ml 1N $KMnO_4$ solution ≡ 0.05585g Fe^{2+}

Determination of starch from plant sample

One of oven dried plant sample was taken in conical flask and 50 ml of cold water was added. The content of flask allowed to stand for one hour with occasional stirring. It was then filtered and residue was washed with 5o ml of distilled water. The sample was hydrolyzed with 10% HCl for 2.5 hours under reflux. Then it was neutralized with dilute sodium hydroxide solution and filtered. The filtrate was transferred in a 100 ml volumetric flask and volume was made up to the mark. The reducing sugar in the filtrate was determined by the Fehling's titration method and the amount of glucose was calculated.

%Starch= % Glucose × 0.9

3.8 Technique for shoot and root growth measurement

Five healthy seedlings were taken from each replication of all treatments for measurement of shoot and root length. Each replication of individual treatments was averaged the root and shoot lengths measured individual treatment finally.

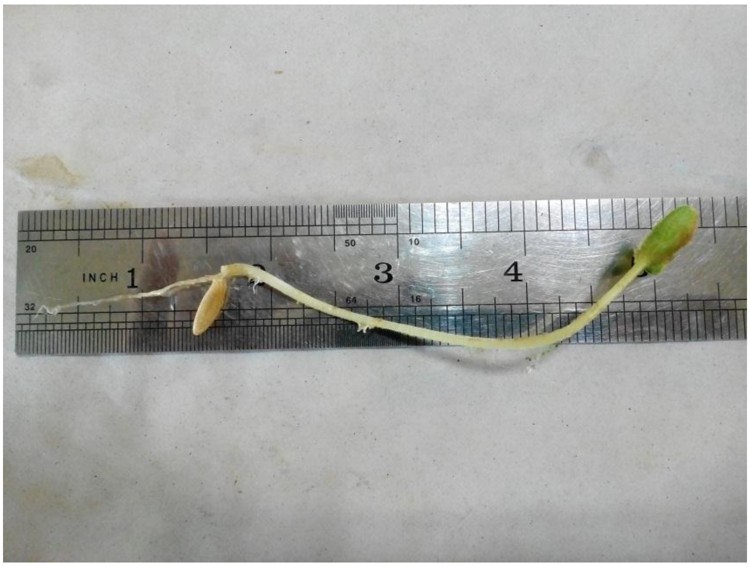

Fig 1: Technique for root and shoot growth measurement of rice seedling

3.9 Statistical analysis

Statistical analysis of the data generated out of the chemical analysis of waste samples, were done with the help of a computer (MS excel) following the standard procedure as described by Gomez and Gomez (1984). Correlation studies were also computed following the procedure described by aforesaid authors. MSTAT-C software was also used for analysis of data. The data were subjected to analyze the co-efficient of variance and means were compared by the DMRT method.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Laboratory experiment: Effect of salinity on germination and early growth response of rice varieties

4.1.1 Germination Percentage

The effects of salinity on final germination percentage (FGP) were significant (Table 4.1). The FGP of different varieties also differed at all salinity levels. At 9 dS m^{-1} salinity, germination was more suppressed in all varieties i.e. Germination percentages were inversely related to salt concentration level. The percentage of germination significantly decreased in all varieties due to increasing salinity (Table 4.1). At the salinity level 3 dS m^{-1} the highest germination percentage 90% was recorded in Nonabokra, the lowest germination percentage 86.67% was recorded in Botessor, which was statistically similar with BRRI dhan28. At the salinity level 6 dS m^{-1} the highest germination percentage values was recorded in Nonabokra with more than 83.33% germination percentage, the medium germination percentage was recorded in Botessor with values of less than 80% (Table 4.1), the lowest germination percentage was recorded in BRRI dhan28 with less than value 67.33% germination percentage. At the salinity level 9 dS m^{-1} the highest germination percentage 66.33% was recorded in Nonabokra, the FGP medium was recorded in Botessor with value of 52.33%, the lowest germination percentage was recorded in BRRI dhan28 with values 50.33% germination percentage, Nonabokra were superior in germination at 3 dS m^{-1} compared to others, which are salt tolerant check Nonabokra. Overall, the results revealed that seed germination of the tested rice varieties decreased with increasing salt concentration. However, the effect of salt stress on seed germination varied among the varieties. The reduced in germination might be due to disturbance of ionic homeostasis since salinity alters membrane selectivity (Na^+ over K^+). This physiological process may bring at least two challenges to the young plant (embryonic development); (i) Na^+ toxicity and (ii) severe K^+ deficiency. Being an inorganic osmolyte, K^+ also requires for preventing osmotic challenge (so-called plasmolysis) of embryonic cell. So, germination decreased due to physiological affect as well as nutritional imbalance. The results are in agreement with the findings of Akbar and Ponnamperuma (1982; Mondal *et al.,* (1988); Jamil and Rha (2007); Momayezi *et al.,* (2009a).

Table 4.1. Effects of different salinity level on final germination percentage of rice varieties

Varieties	Salinity level (dS m^{-1})			
	0	3	6	9
Nonabokra	100.0 a	90.00 ab (10%)	83.33 a (14%)	66.33 a (29%)
Botessor	100.0 a	86.67 a (13%)	80.00 a (18%)	52.33 b (34%)
BRRI dhan28	100.0 a	86.67 a (13%)	67.33 b (22%)	50.33 b (45%)
LSD	13.03	13.03	13.03	13.03
CV (%)	9.34 %	9.34 %	9.34 %	9.34 %

Means within columns with the same letters are not significantly different (LSD, $p \leq 0.05$).

4.1.2 Effect of salinity on shoot and root length

The main effect of salinity levels on shoot length of different varieties was found to be significant and shoot length showed significant reduction with increased salt levels (Table 4.2). At 3 dS m^{-1}, the longest shoot (5.527 cm) was found in Nonabokra, while the shortest (5.387 cm) was observed in BARRI dhan28. At salinity levels of up to 6 dS m^{-1}, the check variety Nonabokra showed better performance, followed by Botessor, and BRRI dhan28. Further increase in salinity to 6 dS m^{-1}, resulted in Nonabokra showing better performance with shoot length of (4.913 cm), followed by Botessor (4.573 cm) and BARRI dhan28 (3.817 cm), while the most affected varieties were BRRI dhan28 with shoot lengths of less than 4 cm. At 9 dS m^{-1}, the maximum length (3.75 cm) was observed in Nonabokra, while the minimum length (3.687 cm) was in BARRI dhan28. Root lengths were also similarly decreased with increased salinity (Table 4.3). Salinity depressed root lengths much more than shoot. At all salinity levels Nonabokra, had significantly higher root lengths compared to the other varieties, while BRRI dhan28 produced the lowest root length. In the present study, the shoot and root lengths were significantly reduced by salinity. Actually, salt stress significantly reduced young shoot and young root lengths in all rice varieties. The results indicated that salt stress affected not only germination but also the growth of young seedlings.

Table 4.2. Effects of different salinity levels on shoot length of rice varieties

Varieties	Salinity level (dS m^{-1})			
	0	3	6	9
Nonabokra	7.407 a	5.527 a (16%)	4.913 a (20%)	3.750 a (35%)
Botessor	7.167 a	5.490 a (23%)	4.573 a (27%)	3.723 a (40%)
BRRI dhan28	7.027 a	5.387 a (31%)	3.817 b (38%)	3.687 a (55%)
LSD	0.5055	0.5055	0.5055	0.5055
CV (%)	5.80%	5.80%	5.80%	5.80%

Means within columns with the same letters are not significantly different (LSD, $p \leq 0.05$).

Table 4.3. Effects of different salinity level on root length of rice varieties

Varieties	Salinity level (dS m^{-1})			
	0	3	6	9
Nonabokra	5.60 a	4.33 a (12%)	3.77 a (18%)	2.75 a (29%)
Botessor	5.13 b	4.22 a (20%)	3.17 b (25%)	2.18 b (35%)
BRRI dhan28	5.21 b	3.61 b (24%)	3.04 b (28%)	2.16 b (50)
LSD	0.5718	0.5718	0.5718	0.5718
CV (%)	8.84 %	8.84 %	8.84 %	8.84 %

Means within columns with the same letters are not significantly different (LSD, $p \leq 0.05$).

The results could be explained in the following ways: (i) the young plant may suffers from water, which is known as water deficit effect of salinity (Munns 2005), (ii) the young plant may be unable to maintain cell turgor, which is required for expanding tissues and finally, affect shoot and root growth as a whole, (iii) excess Na^+ and Cl^- may also affect shoot and root growth, which is known ion-specific effect or ion toxicity of salinity (Rahman *et al.,* 2001, Djanguiraman *et al.,* 2003 and Jamil and Rha, 2007). The results also corroborate with those of Momayezi *et al.* (2009) who also observed shoot and root length reductions in rice genotypes due to increasing salt levels.

4.1.3 Effects of salinity shoot and root dry weight

Shoot dry weights were inversely related to salt concentration (Table 4.4). Shoot dry weight was relatively less sensitive to salt than root dry weight. Rice varieties differed in shoot dry weight response to salt concentration. At the lowest salt concentration (3 dS m^{-1}), the greatest reduction in shoot dry weight was observed in BRRI dan28 (0.11), the lowest reduction in shoot dry weight was observed in Nonabokra (0.13). At the salinity level 6 dS m^{-1} the more reduction in shoot dry weight was founded in BRRI dhan28 (0.07), the lowest reduction in shoot dry weight was founded in Nonabokra (0.10). At the salinity level 9 dS m^{-1} the greatest reduction in shoot dry weight was founded in BRRI dhan28 (0.04), and the lowest reduction in shoot dry weight was founded in Nonabokra (0.07). However, shoot dry weight of BARRI dhan28 was more affected in comparison to the salt tolerant check Nonabokra (Table 4.4).

Table 4.4. Effects of different levels of salinity on shoot dry weight of rice varieties

Varieties	Salinity level (dS m^{-1})			
	0	**3**	**6**	**9**
Nonabokra	0.17 a	0.13 a (13%)	0.10 a (16%)	0.07 a (24%)
Botessor	0.16 b	0.13 a (15%)	0.07 b (19%)	0.06 b (32%)
BRRI dhan28	0.14 c	0.11 b (18%)	0.07 b (22%)	0.04 bc (51)
LSD	0.05342	0.05342	0.05342	0.05342
CV (%)	15.88 %	15.88 %	15.88 %	15.88 %

Means within columns with the same letters are not significantly different (LSD, $p \leq 0.05$). Values in parenthesis indicate percent reduction relative to the respective controls.

Root dry weights also decreased with increasing salinity levels (Table 4.5). At the lowest salt concentration (3 dS m^{-1}) the greatest reduction in root dry weight was observed in BRRI dan28 (0.08), but the lowest reduction was recorded Nonabokra (0.10). At the salinity level 6 dS m^{-1}, the more reduction in root dry weight was recorded in BRRI dhan28 (0.04), while the lowest reduction was recorded in Nonabokra (0.07). At the salinity 9 dS m^{-1}, the reduction of root dry weight of all verities were some (0.02). However, root dry weight of BRRI dhan28 was more affected in comparison to the tolerant check Nonabokra.

Table 4.5. Effect of different salinity level on root dry weight of rice varieties

Varieties	Salinity level (dS m^{-1})			
	0	3	6	9
Nonabokra	0.14 a	0.10 a (14%)	0.07 a (18%)	0.02 a (29%)
Botessor	0.13 ab	0.09 ab (16%)	0.06 ab (22%)	0.02 a (35%)
BRRI dhan28	0.11 b	0.08 b (18%)	0.04 b (25%)	0.01 b (49%)
LSD	0.1048	0.1048	0.1048	0.1048
CV (%)	19.23 %	19.23 %	19.23 %	19.23 %

Means within columns with the same letters are not significantly different (LSD, $p \leq 0.05$). Values in parenthesis indicate percent reduction relative to the respective controls.

Produced the second highest root dry weights at this salinity level. The salinity stress was recorded to affect dry matter production of the rice seedlings, which also indicates disturbance of photosynthetic ability. Similar results were recorded by Jamil and Rha (2007) and Momayezi *et al.* (2009a)

4.2 Pots experiment: Effect of salinity on germination and growth performance of selected rice varieties

4.2.1 Plant height

The plant height of different rice varieties was significantly influenced by salinity. The average plant height of different varieties at 3, 6 and 9 dS m^{-1} salinity were recorded to be 35.31, 34.33 and 34.03 cm, respectively (Table. 4.6). At 3 dS m^{-1}, the plant height of Nonabokra were significantly higher compared to Botessor and BRRI dhan28. However, plant height was slightly reduced in Botessor, but was more reduced in BRRI dhan28 compared to the control. At 6 dS m^{-1}, Nonabokra and Botessor showed the more decrease compared to the control, but at the higher salinity level of 9 dS m^{-1}, BRRI dhan28 were seriously reduced and were dead, while Nonabokra and produced higher plant height compared to the other varieties. The result indicated that there was a genotypic variation on plant growth during salinity stress. Reduced photosynthesis may be one of the reasons for reduced plant growth as well as plant height under salinity stress. Several investigators also reported that photosynthesis seriously decreased onset of salinity (Khan *et al.,* 1997; Choi *et al.*, 2003; Alam *et al.*, 2004; Motamed *et al.,* 2008; Mahmood *et al.,* 2009) which corroborated our findings.

Table 4.6. Effect of salinity on plant height 30 days after transplanting of selected rice varieties

Varieties	Salinity level (dS m^{-1})			
	0	3	6	9
Nonabokra	35.77 a	33.50 a (9%)	26.87 a (12%)	24.80 a (17%)
Botessor	34.43 a	31.10 a (11%)	26.77 b (15%)	23.27 a (20%)
BRRI dhan28	34.03 a	26.10 b (16%)	17.60 b (19%)	16.17 b (35%)
LSD	9.845	9.845	9.845	9.845
CV (%)	5.13 %	5.13 %	5.13 %	5.13 %

Means within columns with the same letters are not significantly different (LSD, $p \leq 0.05$). Values within parenthesis indicate percent relative to the control.

Table 4.7. Effect of salinity on plant height at 45 days after transplanting of selected rice varieties

Varieties	Salinity level (dS m^{-1})			
	0	3	6	9
Nonabokra	39.83 a	35.70 a (7%)	32.93 a (10%)	30.17 a (13%)
Botessor	39.07 a	34.97 a (9%)	28.83 ab (14%)	24.60 b (19%)
BRRI dhan28	35.87 b	34.90 a (12%)	23.13 b (18%)	19.67 b (28%)
LSD	4.669	4.669	4.669	4.669
CV (%)	2.08 %	2.08 %	2.08 %	2.08 %

Means within columns with the same letters are not significantly different (LSD, $p \leq 0.05$). Values within parenthesis indicate percent relative to the control.

4.3 Biochemical constituents

4.3.1 Proline content

The accumulation of proline was significantly influenced by different levels of salinity. The proline content increased with increasing the salinity level in all varieties (Table. 4.8). At 3 dS m^{-1}, there was a slight increased in proline content in all varieties, except Nonabokra. At 6 dS m^{-1}, the highest proline accumulation was found in Nonabokra, while the lowest accumulation was in BRRI dhan28. The accumulation increased sharply in all varieties at 9 dS m^{-1}, but the highest increment (1.09 µmol g^{-1} fw) was recorded in Nonabokra, while the lowest (0.55 µmol g^{-1} fw) was in BRRI dhan28. The accumulation of compatible solutes such as proline is an important mechanism in higher plants under salt-stress. Proline accumulation in salt stressed plants is a primary defence response to maintain osmotic pressure in a cell. Many researchers have reported the significant role of proline in osmotic adjustment, and protection of cell structure in many crops (Desingh and Kanagaraj, 2007).

Table 4.8. Effect of salinity on proline content of selected rice varieties

Varieties	Salinity level (dS m^{-1})			
	0	3	6	9
Nonabokra	3.48 a	3.19 a (12%)	2.49 a (20%)	1.09 a (57%)
Botessor	3.00 a	2.60 ab (20%)	1.81 b (24%)	0.66 b (63%)
BRRI dhan28	2.99 b	2.48 b (22%)	1.71 b (27%)	0.55 b (85%)
LSD	1.629	1.629	1.629	1.629
CV (%)	11.97 %	11.97 %	11.97 %	11.97 %

Chutipaijit *et al.* (2009) reported that free proline content of rice varieties was significantly increased with increasing salinity levels. The proline accumulation of rice genotypes was significantly influenced by the application of different concentration of salt (Momayezi *et al.,* 2009b). Wanichananan *et al.* (2003) found that the proline content of rice seedlings was affected by the presence of NaCl in the growth medium and the proline content positively correlated with the NaCl. The proline content in rice genotype of KDML105 seedlings was enriched and higher than those of HJ seedlings under salt stressed environment (Cha-um *et al.,* 2009). Similar result was observed by Moradi and Ismail (2007) where proline concentration increased significantly in all three rice lines with increasing salinity levels.

4.3.2 Chlorophyll content

Chlorophyll-a in the three rice varieties was significantly affected by salinity (Table 4.9). The highest chlorophyll-a was recorded in Nonabokra (14.54), while the lowest was recorded in BRRI dhan28 at 9 dS m^{-1} (3.32). At 3 dS m^{-1}, the highest values were founded in Nonabokra with value (12.49) and lowest was founded in BRRI dhan28 (11.79) relative to the control. At 6 dS m^{-1}, Nonabokra produced maximum chlorophyll-a value (9.44) and the lowest was founded in BRRI dhan28 (8.00) relative to the control. However, at 9 dS m^{-1}, all varieties were more influenced by salt stress, but more chlorophyll-a was recorded in Nonabokra. However, the lowest chlorophyll-a was obtained in BRRI dhan28 (3.32) relative to the control.

Table 4.9 Effect of salinity on chlorophyll-a content of selected rice varieties

Varieties	Salinity level (dS m^{-1})			
	0	3	6	9
Nonabokra	14.54 a	12.49 a (15%)	9.44 a (20%)	3.95 a (58%)
Botessor	14.18 b	12.01 ab (18%)	9.15 ab (25%)	3.48 ab (75%)
BRRI dhan28	14.12 b	11.79 b (25%)	8.00 b (40%)	3.32 b (85)
LSD	0.3852	0.3852	0.3852	0.3852
CV (%)	3.37 %	3.37 %	3.37 %	3.37 %

Means within columns with the same letters are not significantly different (LSD, $p \leq 0.05$). Values within parenthesis indicate percent relative to the control.

The effect of salinity on chlorophyll-b was significant (Table 4.10). The highest chlorophyll-b content (4.22) was recorded in Nonabokra at control level, while the lowest chlorophyll-b content (0.25) was obtained in BRRI dhan28. At 9 dS m^{-1} (Table 4.10). At 3 dS m^{-1}, the highest amount of chlorophyll-b content (3.16) was noted in Nonabokra, while the lowest amount (2.68) was recorded in BRRI dhan28 relative to the control. At 6 dS m^{-1}, a higher chlorophyll-b content (2.28) was obtained in Nonabokra, and the lowest value (1.69) was found in BRRI dhan28. At the salinity level of 9 dS m^{-1}, the highest amount of chlorophyll-b content (0.67) was noted in Nonabokra, while the lowest amount (0.25) was recorded in BRRI dhan28 relative to the control.

Table 4.10 Effect of salinity on chlorophyll-b content of selected rice varieties

Varieties	Salinity level (dS m^{-1})			
	0	**3**	**6**	**9**
Nonabokra	4.22 a	3.16 a (23%)	2.28 a (25%)	0.67 a (55%)
Botessor	4.00 a	3.10 b (25%)	2.17 a (30%)	0.61 a (71%)
BRRI dhan28	3.50 b	2.68 b (30%)	1.69 b (35%)	0.25 b (90%)
LSD	0.4354	0.4354	0.4354	0.4354
CV (%)	10.23%	10.23%	10.23%	10.23%

Means within columns with the same letters are not significantly different (LSD, $p \leq 0.05$). Values within parenthesis indicate percent relative to the control.

4.3.3 Carotene Content

The effected of salinity on carotene was significant (Table 4.11). The highest carotene conten (4.30) was recorded in Nonabora while the lowest carotene content (0.56) was observed BRRI dhan28 at 9 dS m^{-1} (Table 4.11). At 3 dS m^{-1} the highest amount of carotene content (3.67) was noted in Nonabokra, while the lowest amount was recorded in BRRI dhan28 with (2.29) relative to the control. At 6 dS m^{-1}, a higher carotene content was obtained in Nonabokra (2.76) and the lowest value was found in BRRI dhan28 (2.21). At 9 dS m^{-1}, a higher carotene content was obtained in Nonabokra (1.91) and the lowest value was found in BRRI dhan28 (0.56).

Table 4.11 Effect of salinity on carotene content of selected rice varieties

Varieties	Salinity level (dS m^{-1})			
	0	**3**	**6**	**9**
Nonabokra	4.30 a	3.67 a (9%)	2.76 a (16%)	1.91 a (55%)
Botessor	3.94 a	2.28 ab (12%)	2.21 a (23%)	0.57 b (60%)
BRRI dhan28	3.85 a	2.19 b (14%)	2.21 a (34%)	0.56 b (75%)
LSD	1.476	1.476	1.476	1.476
CV (%)	8.40%	8.40%	8.40%	8.40%

Means within columns with the same letters are not significantly different (LSD, $p \leq 0.05$). Values within parenthesis indicate percent relative to the control.

4.4 Effect of salinity levels on mineral constituents

4.4.1 Effects of salinity levels on sodium in shoot and root

Varietal differences for sodium concentrations in shoots and roots were pronounced ranging from 401 to 71.67 under control conditions. Sodium concentrations in the three selected rice varieties were observed to increase progressively with increasing salinity (Table 4.12). At 3 dS m^{-1}, the highest Na content was found in Nonabokra with relative values of (401) compared to the control, while the lowest was in BRRI dhan28 with relative values of (383.3) compared to the control. At 6 dS m^{-1}, sodium content was founded in Nonabokra (334.3), while the lowest sodium content was founded in BRRI dhan28 (289.0) At 9 dS m^{-1}, sodium contents were found to increase sharply in all varieties. The highest value was recorded in Nonabokra with (95.33) relative to the control, while the lowest value was found in BRRI dhan28 (70.61).

Table 4.12. Effect of salinity on sodium content in shoots and roots of selected rice varieties

Varieties	Salinity level (dS m^{-1})			
	0	**3**	**6**	**9**
Nonabokra	495.0 a	401.0 a (11%)	334.3 a (14%)	95.33 a (60%)
Botessor	458.7 b	386.3 ab (15%)	306.7 b (20%)	84.67 a (72%)
BRRI dhan28	458.0 b	383.3 b (18%)	289.0 bc (27%)	70.67 b (81%)
LSD	146.7	146.7	146.7	146.7
CV (%)	7.06%	7.06%	7.06%	7.06%

Means within columns with the same letters are not significantly different (LSD, $p \leq 0.05$). Values within parenthesis indicate values in percent relative to the control.

These results may be explained in the following ways: At high Na, HKT may be relevant for Na rather than K uptake (Maathuis and Amtmann, 1999); massive influx of Na^+ into the cells via non-selective cation channels (NSCCs) which occurs in the presence of excess Na in typical saline environments (Rahman *et al.,* 2008). Similar findings are also reported that sodium ions in rice shoot and root generally increased in salt-stressed conditions, but the rate of increase was dependent on salt concentration (Djanaguiraman *et al.,* 2006; Ahmad *et al.,* 2007; Momayezi *et al.,* 2009b; Mahmood *et al.,* 2009; Ikram-ul-Haq *et al.,* 2010; Amirjani, 2010).

4.4.2 Potassium in shoot and root

Potassium (K) concentration in shoot and root of rice varieties ranged from 1180.0 to 1074.0 in control plants. Potassium concentrations of the three selected rice varieties were found to decrease significantly with increasing salinity (Table 4.13). At 3 dS m^{-1}, the highest amount of K (947.7) was found in Nonabokra, with relative to the control, while the lowest was recorded in BRRI dhan28, (906) relative to the control. At 6 dS m^{-1}, Nonabokra performed better followed by Botessor (887.3) relative to the control, but BRRI dhan28 was affected more, (625) relative to the control. However, at 9 dS m^{-1}, severe reductions in K concentrations was found in BRRI dhan28 (276.7) relative to the control, while Nonabokra were less affected (128.3) relative to the control.

Table 4.13 Effect of salinity on potassium in shoots and roots of selected rice varieties

Varieties	Salinity level (dS m^{-1})			
	0	3	6	9
Nonabokra	1180.0 a	947.7 a (11%)	887.3 a (19%)	276.7 a (50%)
Botessor	1103.0 a	944.0 a (14%)	729.7 ab (21%)	275.0 a (69%)
BRRI dhan28	1074.0 a	906.0 a (19%)	625.0 b (29%)	128.3 b (75%)
LSD	166.4	166.4	166.4	166.4
CV (%)	3.19 %	3.19 %	3.19 %	3.19 %

Means within columns with the same letters are not significantly different (LSD, $p \leq 0.05$). Values within parenthesis indicate value in percent relative to the control.

K^+ concentrations were reduced with increasing salinity levels. The possible causes are (i) high external Na negatively affects K acquisition due to similar physiochemical properties of Na and K; (ii) KUP (potassium uptake permease)/HAK (High Affinity K) transporters are extremely selective for K and they are blocked by Na under salt stress

(Santa-Maria *et al.,* 1997). The present results are in accordance with the research reports of Mahmood *et al.,* 2009; Ikram-ul-Haq *et al.,* 2010; Amirjani, 2010; Summart *et al.,* 2010.

4.4.3 Calcium in shoot and root

Calcium concentrations in shoots and roots across varieties were observed in the range of 1077.0 to 1028.0 in untreated rice plants. Calcium content was affected by salinity with clear differences among varieties (Table 4.14). At 3 dS m^{-1}, the highest amount of calcium content was found in Nonabokra (857.7), the lowest amount of calcium content was found in BRRI dhan28 (845.3). At 6 dS m^{-1}, the highest amount of calcium content was found in Nonabokra (707.7), the lowest amount of Ca content was found in BRRI dhan28 (677) relative to the control. However, at 9 dS m^{-1}, severe reduction in Ca^{+} was found in BRRI dhan28, (140.3) relative to the control treatment, while the less affected varieties were Nonabokra (148.3) relative to the control.

Table 4.14 Effect of salinity on calcium in shoots and roots of selected rice varieties

Varieties	**Salinity level (dS m^{-1})**			
	0	**3**	**6**	**9**
Nonabokra	1077.0 a	857.7 a (12%)	707.7 a (15%)	248.3 a (49%)
Botessor	1034.0 a	856.0 a (17%)	586.7 b (19%)	243.3 a (65%)
BRRI dhan28	1028.0 b	845.3 a (20%)	577.0 b (27%)	140.3 b (89%)
LSD	116.3	116.3	116.3	116.3
CV (%)	2.57 %	2.57 %	2.57 %	2.57 %

Means within columns with the same letters are not significantly different (LSD, $p \leq 0.05$). Values within parenthesis indicate values in percent relative to the control.

Calcium is essential for the maintenance of cell membrane integrity. Calcium plays an important role in the synthesis of new walls in cell, particularly the middle lamellae that separate newly divided cells (Taiz and Zeiger, 2006). The rice membrane damage and enhanced Permeability due to affected by the displacement of Ca^{2+} by increasing Na^{+} from the binding sites of phospholipids of membranes. In our study, calcium ion decreased with increased the salinity levels. It seemed that seedlings of rice plant were unable to uptake the required quantities of Ca^{2+} from the medium and finally growth and development of seedlings were severely affected (Ahmad *et al.,* 2006). Momayezi *et al.* (2009b) reported that calcium ion content in shoot and root of rice plant significantly decreased with increasing salinity level. The results are in accordance with the findings was found by Amirjani (2010) and Summart *et al.,* (2010).

4.4.4 Effects of salinity levels on Mg content in shoot and root

Magnesium content in shoots and roots was significantly affected by salinity levels (Table 4.15). At 3 dS m^{-1}, the highest Mg content was recorded in Nonabokra (939.7) relative to the control, while the lowest was in BRRI dhan28, (717.3) relative to the control. At a salinity of 6 dS m^{-1}, the highest amount of Mg^{+} content was recorded in Nonabokra (795) relative to the control, while the lowest amount was recorded in BRRI dhan28, (741) relative to the control. A at 9 dS m^{-1}, the highest amount of Mg^{+} content was recorded in Nonabokra (392.3) relative to the control, while the lowest amount was recorded in BRRI dhan28, (165.0) relative to the control. Momayezi *et al.* (2009b) noted that the amounts of Mg^{2+} in shoot and root tissues of rice plant remained almost constant with salinity levels up to 3 dS m^{-1} and decreased significantly thereafter. Similar result was observed by Amirjani (2010) where the magnesium ion in soybean crop significantly decreased by increasing salt concentration.

Table 4.15. Effect of salinity on magnesium in shoots and roots of selected rice varieties

Varieties	Salinity level (dS m^{-1})			
	0	3	6	9
Nonabokra	1158.0 a	939.7 a (13%)	795.0 a (15%)	392.3 a (58%)
Botessor	1061.0 a	908.3 a (15%)	773.7 a (18%)	168.3 ab (75%)
BRRI dhan28	1012.0 a	717.3 b (18%)	741.0 a (25%)	165.0 b (88%)
LSD	219.9	219.9	219.9	219.9
CV (%)	4.53%	4.53%	4.53%	4.53%

Means within columns with the same letters are not significantly different (LSD, $p \leq 0.05$). Values within parenthesis indicate values in percent relative to the control

4.4.5 Effects of salinity levels on shoot and root S content

Sulfur concentrations in shoots and roots across varieties were observed in the range of 752.7% to 801.0% in untreated rice plants. Sulfur content was affected by salinity with clear differences among varieties (Table 4.16). At 3 dS m^{-1}, the highest sulfur content was recorded in Nonabokra (690) relative to the control, while the lowest recoded was in BRRI dhan28, (455.3) relative to the control. At 6 dS m^{-1}, the highest sulfur content was founded in Nonabokra (564.3) relative to the control, while the lowest amount was recorded in BRRI dhan28, (377.7) relative to the control. However, at 9 dS m^{-1}, severe reduction in S was found in BRRI dhan28 (262.7) relative to the control treatment, while the less affected varieties were Nonabokra (133.7) relative to the control.

Table 4.16. Effect of salinity on sulfur in shoots and roots of selected rice varieties

Varieties	Salinity level (dS m^{-1})			
	0	**3**	**6**	**9**
Nonabokra	801.0 a	690.0 a (9%)	564.3 a (13%)	262.7 a (52%)
Botessor	781.3 a	667.3 a (11%)	500.7 a (15%)	153.0 a (69%)
BRRI dhan28	752.7 a	455.3 b (14%)	377.7 ab (18%)	133.7 b (73%)
LSD	243.7	243.7	243.7	243.7
CV (%)	6.96 %	6.96 %	6.96 %	6.96 %

Means within columns with the same letters are not significantly different (LSD, $p \leq 0.05$). Values within parenthesis indicate values in percent relative to the control

4.4.6 Effects of salinity level on shoot and root phosphorus content

Phosphorus concentrations in shoots and roots across varieties were founded in the range of 11.94 to 12.86% in untreated rice plants. Phosphorus content was affected by salinity with clear differences among varieties (Table 4.17). At 3 dS m^{-1}, the highest phosphorus content was redoded in Nonabokra (11.75) relative to the control, while the lowest amount was recorded in BRRI dhan28, (10.10) relative to the control. At 6 dS m^{-1}, the highest P content was recorded in Nonabokra (9.32) relative to the control, while the lowest amount was recorded in BRRI dhan28, (3.06) relative to the control. However, at 9 dS m^{-1}, severe reduction in P was found in BRRI dhan28 (1.95) relative to the control treatment, while the less affected varieties were Nonabokra with (3.06) relative to the control.

Table 4.17. Effect of salinity on phosphorus in shoots and roots of selected rice varieties

Varieties	Salinity level (dS m^{-1})			
	0	3	6	9
Nonabokra	12.26 a	11.75 a (9%)	9.32 a (12%)	3.06 a (55%)
Botessor	12.73 a	10.40 a (12%)	8.73 ab (15%)	2.81 a (69%)
BRRI dhan28	11.94 b	10.10 b (14%)	3.06 b (18%)	1.95 b (87%)
LSD	1.899	1.899	1.899	1.899
CV (%)	3.26 %	3.26 %	3.26 %	3.26 %

Means within columns with the same letters are not significantly different (LSD, $p \leq 0.05$). Values within parenthesis indicate values in percent relative to the control

4.4.7 Effects of salinity level on shoot and root Zinc content

Zinc concentrations in shoots and roots across varieties were recorded in the range of 3.11 to 3.66% in untreated rice plants. Zinc content was affected by salinity with clear differences among varieties (Table 4.14). At 3 dS m^{-1}, the highest amount of Z content was recoded in Nonabokra (2.89) relative to the control, while the lowest amount was recorded in BRRI dhan28, (2.29) relative to the control. At 6 dS m^{-1}, the highest amount of Z content was founded in Nonabokra (1.90) relative to the control, while the lowest amount was founded in BRRI dhan28, (1.58) relative to the control. However, at 9 dS m^{-1}, severe reduction in Z was found in BRRI dhan28 (0.35) relative to the control treatment, while the less affected varieties were Nonabokra (0.48) relative to the control.

Table 4.18. Effect of salinity on zinc in shoots and roots of selected rice varieties

Varieties	Salinity level (dS m^{-1})			
	0	3	6	9
Nonabokra	3.66 a	2.89 a (12%)	1.90 a (21%)	0.48 a (45%)
Botessor	3.37 ab	2.52 b (18%)	1.77 a (29%)	0.46 a (64%)
BRRI dhan28	3.01 b	2.29 b (25%)	1.58 b (34%)	0.35 b (77%)
LSD	0.3890	0.3890	0.3890	0.3890
CV (%)	7.64%	7.64%	7.64%	7.64%

Means within columns with the same letters are not significantly different (LSD, $p \leq 0.05$). Values within parenthesis indicate values in percent relative to the control

CHAPTER V

SUMMARY AND CONCLUSION

Saline areas in Bangladesh are predicted to increase due to sea level rise as a consequence of global climate change. To ensure food security under this situation rice cultivation has to be expanded into saline areas. Glycophyte being in nature most rice varieties cannot grow satisfactorily under saline condition. Therefore, saline tolerant rice varieties need to be selected for these areas. With this view, the present study was undertaken with the aim of genotypic variations in salt tolerance of rice at germination and early seedling stage of growth.

Laboratory studies were conducted to identify salt tolerant rice variety. Three rice varieties were used in the study of which two (Nonabokra. and Botessor) were colected from Gazipur and One (BRRI dhan28) were from dinajpur local area. Rice varieties were tested in this study had a range of tolerance to salinity. Germination and early growth responses of these varieties under different salinity levels viz. 0, 3, 6, and 9 dS m^{-1} were studied in the laboratoty. The results showed that germination percentage between salinity levels, and were reduced with increasing salinity. Highest germination percentage (90.00%), while lowest germination percentage (50.33%) were recorded under control condition. Among the tested varieties, Botessor, and BRRI dhan28 were inferior in germination and Nonabokra showed higher germination percentage values at 9 dS m^{-1} compared to other varieties. Based on seedling dry weights, Nonabokra were salt tolerant at 3 dS m^{-1}, moderately salt tolerant at 6 dS m^{-1}, and susceptible at 9 dS m^{-1}.

All the varieties (Nonabokra, Botessor and BRRI dhan28) were then selected for further testing of salinity tolerance at growth stage in pots experiments. By subjecting them to salinity levels of 0, 3, 6 or 9 dS m^{-1}. Nonabokra produced higher plant height, shoot and root dry matter compared to other varieties. Biochemical constituents, *viz.* clorophyll contents, proline, were also influenced by salinity levels. Salinity severely affected the synthesis of leaf chlorophyll in all tested varieties. Chlrophyll a/b ratio increased but chlorophyll a, b and total chlorophyll decreased significantly with increasing the salinity levels. The decreasing pattern of chlorophyll-b were greater than chlorophyll-a. Higher chlorophyll contents were observed in Nonabokra at all salinity levels. The proline content was also influenced by increasing the salinity. The highest proline accumulation was found in Nonabokra while the lowest accumulation was in BRRI dhan28. The highest increment (3.19 µmol g^{-1} fw) was in Nonabokra, while the lowest (0.55 µmol g^{-1} fw) was in BRRI dhan28. The highest carotene conten (4.30) was recorded in Nonabora while the lowest carotene content (0.56) was observed BRRI dhan28 at 9 dS m^{-1}. The sulfur concentration were found from 801 to 133.7 unit (mg l^{-1}). The phosphors concentration were ranged from 12.86 to 1.95. The zinc concentration were found from 3.66 to 0.35. The concentration of Na+ in both rice shoots and roots significantly increased with increasing the salinity level. The highest concentration of this ion was recorded at the higher salinity level (9 dS m^{-1}) and lowest was in the control treatment. K^+, Ca^{2+} and Mg^{2+} content significantly decreased with increasing salinity. Highest concentration of these ions was recorded in the control treatment and lowest was at the higher salinity level (9 dS m^{-1}). Considering overall growth response, and reprted of biochemical constituants and mineral contents. Nonabokra were found to be tolerant, while Botessor were observed to be moderately tolerant.

The overall results revealed that the variety Nonabokra proven as salt tolaerance, Botessor as moderately tolerant and BRRI dhan28 as salt susceptible variety.

The present study revealed that germination and seedling growth of rice varieties were suppressed with increasing salt concentration under laboratory conditions. With respect to final germination percentage, one varieties *viz.* Nonabokra were found to perform better than other varieties at 9 dS m^{-1} salt concentration. Based on seedling dry weights, Nonabokra were salt tolerant at 3 dS m^{-1}. Among the varieties tested in the pots study, BRRI dhn28 were found to be more affected, followed by BRRI dhan28 with respect to vegetative growth, physio-biochemical constituents, mineral composition and yield performance. Finally, based on the overall results on growth and yield performance, it is concluded that varieties Nonabokra were salinity tolerant, while Botessor were observed to be moderately tolerant, and BRRI dhan28 were susceptible varieties.

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