SCREENING FOR TOLERANCE TO SALINITY: A CASE STUDY WITH SEVEN RICE VARIETIES FROM AFRICA RICE CENTER AND GHANA

A Thesis submitted to the Department of Crop and Soil Sciences,
Kwame Nkrumah University of Science and Technology in partial fulfillment of the requirement for the degree of

MASTERS OF SCIENCE

IN

AGRONOMY (PLANT BREEDING)

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August 2012

Abstract

An experiment was performed at the Soil Research Institute, Kwadaso, Kumasi-Ghana, to test the response of seven rice varieties from Ghana and Africa Rice Center to two levels of salinity in order to classify tolerant varieties. The soil used was silty-loam, classified as Feric acrisol, (FAO, 1990) from the arboretum at Soil Research Institute, Kwadaso, Kumasi, in the semi-deciduous forest agro-ecological zone of Ghana. Seeds were pre-germinated and two seedlings sown in pot filled with air-dry soil irrigated with water containing 0, 3 and 6 dsm^{-1} concentrations of sodium chloride respectively. Three indices were used to identify the tolerant rice varieties. They were (1) subjective index, (2) percent relative reduction and (3) salinity susceptibility index. Relative water content, shoot mass, plant height, root mass, flag leaf width and length were adversely affected by salinity. The 100-grain mass, spikelet fertility, number of filled grain, number of tillers and number of productive tillers were significantly ($p < 0.01$) decreased with increase in salinity levels. Salinity stress decreased yield components by 14 % to 91 % at low salinity level and 93 % to 100 % at high salinity level for all the varieties. Salinity stress also affected the mineral content of leaves with increase in both potassium and sodium uptake. The concentration of calcium and magnesium was reduced under salt stress. There were low water potentials in all the treatment pots which were evident by the reduction in plant relative water content. Among the rice varieties ITA 324, WITA 9 and BOUAKE 189 showed better performance under salinity stress and were therefore considered tolerant.

ACKNOWLEDGEMENT

It would not have been possible to write this thesis without the help and support of the kind people around me, to only some of whom it is possible to give particular mention here.

I would like to express my profound gratitude to my principal supervisor, Rev. Prof. Mensah Bonsu, for his excellent guidance, care, patience, and providing me with an excellent atmosphere for doing research. The good advice, support and friendship of my second supervisor and coordinator of program, Prof. Richard Akromah, has been invaluable on both an academic and a personal level, for which I am extremely grateful. I would like to acknowledge the financial support of AGRA, particularly in the award of a Postgraduate Research Scholarship that provided the necessary financial support for this research. I would like to thank Mr. Andrew Opuku and Mr. Ali Ibrahim, who, as good friends, were always willing to help and give their best suggestions. My appreciation to Mr. Maxwell Darko Asante of Crop Research Institute for providing the rice seeds for my research.

I would like to express my gratitude to the Executive Director General of the Soil Research Institute, Dr. J. O. Fening, for allowing me the use of the plant house for the experiment. My gratitude to Dr. Francis Tetteh, head of the soil science lab at the Soil Research Institute, for permitting me to use the facilities at the soil science lab. Many thanks to Mr. Anthony Abutiate for helping me with my soil analysis.

My sincere gratitude to the Ministry of Agriculture (MOA) and the administration of the Central Agricultural Research Institute (CARI) of Liberia for granting me the study leave to pursue my postgraduate studies abroad. I remember the generosity and encouragement of Dr. J. Q. Subah, for providing me the enabling environment to redevelop interest in the field of agriculture. I would like to thank Dr. Chakanda for his kindness, friendship and encouragement.

Finally, I would like to thank my loving wife, Matune J.C. Dolo for her patience in caring and looking after our household during my long absence. She has always cheered me up and stood by me through the good and bad times. My parents, Mr. & Mrs. Gorpu Dolo, in-laws, guardians, and siblings have given me their unequivocal support throughout, and I wish to express my sincere thanks to them.

For any errors or inadequacy that may remain in this work, the responsibility is entirely mine.

DEDICATION

This work is dedicated to the Almighty God, the Great "I AM" the LORD great in battle and fearful in praises, the giver of wisdom, my ever present help in time of need and the architect of the entire universe. To HIM alone be all honor and glory.

And also to my wife and our household for their tireless support.

CERTIFICATION

The work in this thesis was conducted by the undersigned candidate while enrolled in the Department of Crop and Soil Sciences at the Kwame Nkrumah University of Science and Technology-Ghana. No portion of this work has previously been submitted to another University for the award of a degree.

I certify that any help received in preparing this thesis and all sources used have been acknowledged.

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ACRONYMS

ANOVA	Analysis Of Variance
EC	Electrical conductivity
Dsm^{-1}	Decisiemens per meter
DW	Dry weight
TW	Turgid weight
EDTA	Ethylene Diamine Tetra-acetic acid
FW	Fresh Weight
RWC	Relative Water Content
SAR	Sodium Absorption ratio
OC	Organic carbon
ECEC	Effective Cation Exchange Capacity
WARDA	West African Rice Development Authority
IRRI	International Rice research Institute
FAO	Food and Agricultural Organization
TEB	Total Exchangeable Bases
BS	Base Saturation
ESP	Exchangeable Sodium Percentage
PS II	Photosystem II

TABLE OF CONTENTS

CHAPTER THREE

CHAPTER 4

CHAPTER 5

5.0 DISCUSSION

LIST OF TABLES

LIST OF FIGURES

APPENDICES

LIST OF PLATES

CHAPTER ONE

1.0 INTRODUCTION

Rice (*Oryza sativa*, L. and *O. glaberima*, Strudl) is one of the agronomically and nutritionally important cereal crops grown worldwide. It plays an important role as a staple food crop and is used to feed more than 3 billion people on a daily calorie intake of 50 to 80% (Khush, 2005). Rice is the single largest energy food to half of humanity; it is rich in genetic diversity in the form of thousand of land races and progenitor species (Nagaraju *et al*., 2002). Rice is one of the top five major carbohydrate crops for the world's population, especially in Asia. Rice is a semi-aquatic cereal, which originated in the tropics. It is the primary staple food for more than two billion people in Asia, the world's most densely populated region, and for hundreds of millions of people in Africa and Latin America (IRRI, 1985, Khush and Virk, 2000). A major limitation to rice crop production is abiotic stress, which can be induced by salinity, drought, extreme temperature, submergence, and heavy metal contamination. Salinity of arable land is one of the most important factors in retarding rice growth and development at both vegetative and reproductive stages (Shannon *et al*., 1998; Zeng and Shannon, 2000; Khan and Abdullah, 2003; Zeng *et al*., 2003).

Africa has become a big player in international rice markets, accounting for 32% of global imports in 2006, at a record level of nine million tonnes that year (Sohl, 2005). Africa's emergence as a big rice importer is explained by the fact that during the last decade rice has become the most rapidly growing food source in sub-Saharan Africa.

Indeed, due to population growth (4% per annum), rising incomes and a shift in consumer preferences in favor of rice, especially in urban areas (Balasubramanian *et al.*, 2007). The relative growth in demand for rice is faster in this region than anywhere in the world (WARDA, 2005). The production-consumption gap in this region is being filled by import, valued at over US$1.4 billion per year. This share of imports in consumption rose from an average of 43% from 1991 to 2000, to an average 57% by 2002–2004 (IRRI, 2002; WARDA, 2005).

The Food and Agriculture Organization of the United Nations (FAO) estimated in 2006 that current rice imports into the West and Central Africa sub-regions had grown to more than 6 million tonnes costing over $1 billion in scarce foreign exchange each year. The cost of importing rice therefore remains a heavy burden on trade balances in the region.

The cultivation of rice extends from dry lands to wetlands. Rice is also grown in cool climates at altitudes of over 2, 600 m above sea level in the mountains of Nepal, as well as in the hot deserts of Egypt. However, most of the annual rice production comes from tropical climate areas (Downing, 1992). Food security for more than half the world population depends on the ability of the world to supply and distribute rice. Rice supply depends on global rice production, while its distribution depends on the distance from production sites to consumers' residences as well as on transportation systems and facilitation.

Global food production will need to increase by 38% by 2025 and by 57% by 2050 (Wild, 2003) if food supply to the growing world population is to be maintained at current levels. Most of the suitable land has been cultivated and expansion into new areas to increase food production is rarely possible or desirable. The focus, therefore, should be an increase in yield per unit of land rather than in the area cultivated. More efforts are needed to improve productivity as more lands are becoming degraded. It is estimated that about 15% of the total land area of the world has been degraded by soil erosion and physical and chemical degradation, including soil salinization (Wild, 2003).

Temperature regimes greatly influence the growth duration, the growth pattern and the productivity of rice crops (Darwin *et al.*, 2005). Extreme temperatures – whether low or high – cause injury to the rice plant. In tropical regions, high temperatures are a constraint to rice production. Studies suggest that the temperature increase gives rise to rising sea level and changes in rainfall patterns and distribution as a result; global climate change could lead to substantial modifications in land and water resources for rice production as well as in the productivity of rice crops grown in different parts of the world (FAO, 2004).

Salinity is found to be the utmost importance in abiotic stresses because it affects the agriculture production adversely all over the world (Borsani *et al.,* 2003). Rice is found to be the salt sensitive crop (Mass and Hoffman, 1977) and in tropics there is no alternative of the rice crop to grow because it is only crop that will withstand in flooding (Shaheen and Hood-Nowotony, 2005). In south and Southeast Asia, 54 million hectares area is found to be effected from salinity (Akbar and Ponnamperuma, 1982) whereas in all over the Asia, 21.5 million hectares area is salt effected (Sahi *et al.*, 2006) which is found to be the major rice production region. Rice crop is observed to be very sensitive to salinity especially in early growth stages and it was observed that the salinity affects the kernel and aromatic characteristics of rice heavily. It also disturbs the antioxidants machanisms and osmoprotectants balance of the plant (Singh *et al.,* 2007). Salinity affects the osmotic and ionic balance of plant with soil (Greenway and Munns, 1980). Rice growth in salinity is dependent on the developmental stages of the plant as rice is relatively more resistant at germination, tillering and maturation whereas seedling stage, early reproduction stage, pollination and insemination are more sensitive stages (Babu, 1985). Furthermore it is also concluded that the plant physiological age also affects the salinity tolerance capability of plant and it varies from cultivar to cultivar even in one species (Shaheen and Nowotony, 2005).Scientists around the world have done great work both on the rice production as well as about the stages affected abruptly by the salinity.

The problem of salinity has been approached through better management practices and introduction of salt-tolerant varieties in the affected areas. Unfortunately, the use of improved irrigation management practices in salt-affected areas has generally proven to be uneconomical and difficult to implement on a large scale. Thus, genetic improvement of salt tolerance of major cereal crops like rice (*Oryza sativa*), wheat (*Triticum aestivum*), maize (*Zea mays*), and barley (*Hordeum vulgare*) appears to be the most feasible and promising strategy for maintaining stable global food production (Munns, 2002).

The rapidly growing demand for increased food, fibre, and fuel in the presence of rapidly declining availability of agricultural land due to increased soil salinity makes it imperative that crop production under saline conditions be significantly increased. It is thus essential to develop and employ technologies that will reduce the spread of salinization, reduce salinity levels in crop fields or increase salt tolerance of crops.

The general objective of this study was to assess salinity response of seven rice varieties to two levels of salinity in order to select varieties that are tolerant to salinity.

The specific objectives were:

To assess the effects of soil salinity on the growth and yield of rice.

To identify morpho-agronomic attributes of rice for salt tolerance.

To use salinity indices to screen seven rice varieties for their salinity tolerance

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Effect of salinity on soil properties

Salinity affects soil physical properties by causing fine particles to bind together into aggregates. This process, known as flocculation, is beneficial in terms of soil aeration, root penetration and root growth. This happens when calcium salt is the cause. Excessively high salinity conditions are, however, detrimental to plant growth. Especially where there are high sodium concentrations in the soil which generally cause soil dispersion as a result of breakdown of soil aggregates, which subsequently settle into soil pores. Soil dispersion causes soil pore blockage resulting in the reduction of soil permeability. Three main problems caused by sodium-induced dispersion are: reduced infiltration; reduced hydraulic conductivity and surface crusting (Frenkel *et. al.,* 1978; van de Graaf and Patterson, 2001). Surface crusting is a characteristic of sodium-affected soils (Frenkel *et. al.,* 1978; van de Graaf and Patterson, 2001). The hardened upper layers or surface crust are likely to restrict water infiltration and seed emergence. The primary causes of surface crusting are i) physical dispersion caused by raindrops or irrigation water, and ii) chemical dispersion. Surface crusting due to rainfall is greatly enhanced by sodium induced clay dispersion (Morin *et al.,* 1981). When clay particles disperse within soil water, the dispersed clay particles plug macropores in the soil surface, thereby, i) blocking avenues for water and roots to move through the soil, and ii) soil structure degradation due to dispersion and the development of a cement-like surface layer upon drying.

2.2 Effects of salinity rice growth and yield

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, low productivity 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).

Salinity has a negative impact on a number of yield components of rice including stand establishment; panicles, tillers and spikelets per plant; floret sterility; individual grain size; and even delayed heading. Maas and Grattan (1999) and Hanson *et al.* (1999)

indicated that rice yields decrease by 12 % for every unit (dsm^{-1}) increase in EC (average root-zone EC of saturated soil extract) above 3.0 dsm^{-1}. Salinity guidelines were first developed by Maas and Hoffman (1977). The major inhibitory effect of salinity on plant growth and yield 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 dSm^{-1} at 25 ^{O}C; sensitive ones are damaged even at 2 dSm^{-1} (Mass and Hoffman, 1977).

2.3 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). 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).

2.4 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 later 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).

2.5 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. 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 milligrams/kilograms of salt, the concentration of salt decreases with distance from the coast (Munns, 2002). If the concentration is 10 mg/kg, this would add 10 kg/ha 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).

2.5.2 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 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 adds 500 kg of salt to each hectare of land (Rhoades and Loveday, 1990).

2.6 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).

2.7 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 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 dsm^{-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.

2.7.1 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).

2.7.2 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 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).

2.7.3 Effects of salinity on plant relative water content

Salinity appears to affect two plant 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 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).

2.8 Criteria to assess salt tolerance

A great deal of research has provided a lot of information on salinity tolerance of plants with the main focus on water relations, photosynthesis and accumulation of various inorganic ions and organic metabolites (Munns, 2002; Ashraf, 2004). However, these determinants of salt tolerance vary amongst species and even among cultivars due to complex nature of the mechanism of salt tolerance (Ashraf, 1994; Flowers, 2004; Munns, 2007). In some comprehensive reviews Ashraf (2004) and Ashraf and Harris (2004) reported that metabolic sites at which salt stress damages plants are still not well understood and there are no well-defined plant physiological or biochemical selection criteria that could be used for improvement of salt tolerance in crops. However, there are a number of studies which show that intra-specific genetic variability for salt tolerance can be assessed by screening large number of lines/cultivars in saline conditions, using attributes such as growth, photosynthetic capacity, osmotic adjustment, ion homeostasis, antioxidant enzymes, cell membrane stability etc. (Ashraf, 2004; Munns, 2002; Ashraf *et al.*, 2006; Cuartero *et al.*, 2006). 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 in field conditions.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. Location of the study

This study was conducted in the green house at the Soil Research Institute, Kwadaso, Kumasi, Ghana. Kumasi is approximately located on latitude $6^0 41$ North and longitude $1^0 38$ West. Rainfall pattern in Kumasi is bimodal with a mean annual total of 1,302 mm. The major wet season is from March to July while the minor wet season is from September to November each year. The minor dry season occurs in August and the major dry season starts from mid November to end of February. Temperature is uniformly high throughout the year. The lowest mean monthly temperature of about 24.6^0C is usually recorded in August and the highest mean monthly temperature of about 28.8^oC is recorded in February. Morning relative humidity is uniformly high throughout the year. The annual evapo-transpiration in Kumasi is about 1234 mm with monthly value ranging from 107 to 144 mm in the major dry season and 71 to 118 mm in the rainy season (Mensah *et al.*, 2008).

3.2 Soil used for experiment

The soil used for the experiment was silty-loam classified as Feric Acrisol (FAO, 1990). The soil sample was collected near the arboretum at Soil Research Institute, Kwadaso, Kumasi, in the semi-deciduous forest agro-ecological zone of Ghana.

3.3 Soil sampling

The soil was sampled through the following procedure. The area to be sampled was cleared and all surface debris (rocks, twigs) removed. An area of approximately 40 cm by 40 cm was demarcated in the sampling site. The spade was inserted into the soil and the soil was collected and placed in an empty rice bag. The sampling was carefully done to prevent the brushing of loose materials back into the hole. Soil was collected from 0-20 cm depth.

3.4 Soil preparation and packaging

The sampled soil was brought from the field and placed on black polythene sheet for air drying. The soil was air-dried for 10 days and sieved through a 4 mm mesh. Eight kilograms (8 kg) of the air dry soil was placed in plastic buckets used for the experiment. The bucket dimension was 27 cm high, 20 cm wide at the bottom and 30 cm wide at the top.

3.5 Statistical analysis

Analysis of variance of all parameters (seedling, vegetative, reproductive and ripening stages) was done using the Genstat statistical package (9^{th} edition). Mean values of each attribute was compared using least significant difference (LSD) at 5 % probability**.**

3.6 Calibration of Electrical Conductivity (EC) against sodium chloride concentration

The calibration of NaCl against E.C was done by weighing 2.5 g of NaCl and adding it to 1000 ml of distilled water to obtain 1000 ppm Na. After that the conductivity meter was calibrated using 0.01M KCl to adjust the meter to read 14.13 µs/cm. From the 1000 ppm

NaCl solution, 25 ml was measured and diluted to various concentrations and the electrode of the conductivity meter inserted into the various solutions to read the different E.C levels. The various readings were used to determine the desired weight of NaCl for the desired salinity level for the treatments (3 ds/m and 6 ds/m).

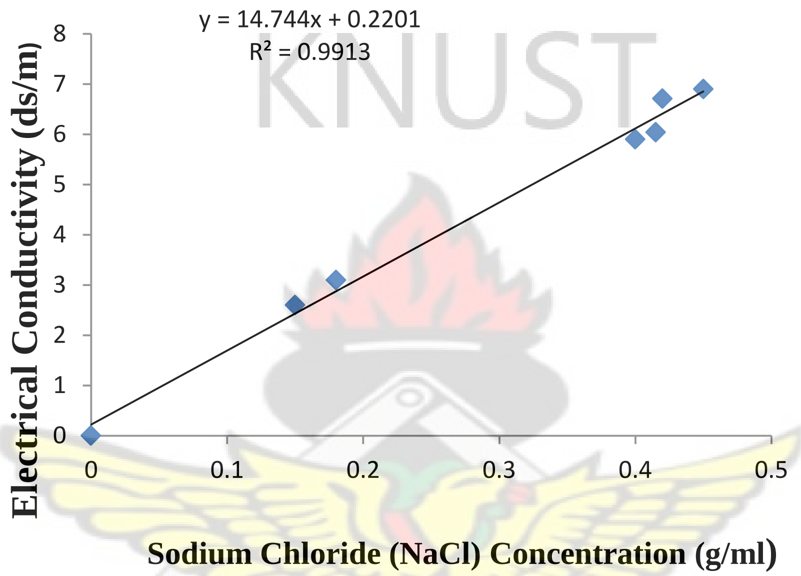

Figure 1.0: Calibration of Electrical Conductivity (EC) against Sodium Chloride (NaCl)

There was a linear relation between sodium chloride and electrical conductivity. This was used as the basis for selecting NaCl concentration that corresponded with EC (E.C 3 and E.C 6).

3.7 Laboratory analysis of soil samples

3.7.1 Soil pH

Soil pH was determined by calibrating the pH reader (Hanna Instrument pH Meter, Model Hi 9032), using buffer solutions; one buffer with neutral pH (7.0), and the other pH (4.0). Twenty-five (25) grams of soil sample were weighed into a 100 ml beaker and

25 ml distilled water was added to form a suspension medium and stirred intermittently with a glass stirring rod for 30 minutes. The electrode was inserted into the beakers containing the two solutions alternatively and the pH read from the digital display.

3.7.2 Electrical Conductivity

Electrical conductivity was determined by electrical conductivity meter (Hanna Instrument Conductivity Meter, Model Hi 9032) in 1:2 soil water ratios (Jackson, 1973). Twenty-five gram of soil sample was weighed into a 250 ml beaker and 50 ml of distilled water was added and stirred intermittently for one hour. The conductivity electrodes were then washed with distilled water and rinsed with standard KCL solution. The conductivity meter was adjusted to read 1.412 µs/cm, corrected to 25 ^{o}C. The electrodes were then washed and dipped into the soil extract and the digital display was recorded as the salt content in the extract, to indicate salinity of the soil sample.

3.7.3 Organic Carbon

Organic carbon was determined by using the modified walkley and Black (wet combustion) method (Nelson and Summers, 1982). One gram (1gm) of soil sample was weighed into a 500 ml Erlenmeyer flask and a blank sample included. Ten milliliters of 1.0 N potassium Dichromate ($K_2Cr_2O_7$) solution was added to the soil and a blank flask. Twenty millilitres of concentrated sulphuric acid was added and the mixture allowed to cool for thirty minutes on an asbestos sheet. Two hundred millilitres of distilled water and ten mililitres of concentrated Orthophosphoric acid (H_5PO_4) were added to the mixture. The excess dichromate ion ($Cr_2O_7^{-2}$) in the mixture was back titrated with 1 N ferrous sulphate using diphenylamine as indicator.

3.7.4 Particle size analysis

The particle size distribution was determined by the Bouyoucos Hydrometer Method. Fifty grams of air dried soil samples were weighed into 500 ml beakers into which 100 ml of calgon (sodium hexa-metaphosphate) and sodium hydrogen carbonate were added. The samples were then placed on an electric heater until they began to boil while stirring intermittently. The samples were then quickly transferred into a dispersion cup and placed on a mechanical mixer until the soil aggregates were broken (this usually takes about 3-4 minutes for coarse-textured soil and 7-8 minutes for fine-textured soil/clay). After the dispersion, the samples were then collected into a 1000 ml cylinder with the help of a funnel on which have been placed a sieve. The first hydrometer and temperature reading were taken at 40 seconds after vigorously shaking the cylinder and placing it on a flat table. After the first readings the suspension was allowed to stand for three hours and the second hydrometer and temperature readings taken. The first reading indicates the percentage of sand and the second reading percentage clay. The percentage of silt was determined by the difference between the sand and the clay. The percentage sand, clay and silt was calculated using the formula below:

% sand = $100-[H_1+0.2(T_1-20)-2.0]$ x2

% clay = $[H_2+0.2\ (T_2-20)-2.0]$ x2

% silt = 100 – (% sand + % clay)

Where:

H_1 = Hydrometer reading at 40 second

H_2 = Hydrometer reading at 3 hours

T_1 = Temperature at 40 seconds

T_2 = Temperature at 3 hours

0.2 (T-20) = Temperature correction factor to be added to hydrometer reading

-2.0 = Salt correction factor to be added to hydrometer reading

3.7.5 Determination of Total Nitrogen

Total nitrogen was determined using the Kjeldahl digestion method and distillation procedures as described by Bremner and Mulvaney (1982). Ten grams (10 g) of air dry soil sample was weighed into a 100 ml digestion flask then one (1) Kjeldahl tablet was added to the sample with 20 g of catalyst mixture after which 5 ml of concentrated H_2SO_4 was added to the sample to wet the soil. The mixture was then placed on a heating mantle under a fume chamber and left to stand for three hours (3 hrs) whereby the soil would have been clear or straw colored. The mantle was switched off and the sample left to cool. After cooling, 80ml of distilled water was added to the digested sample then mixed well and transferred into a distillation flask. Twenty mililiters of 10 N NaOH was added to the sample to commence distillation and then the distillate was immediately collected into a suitable receiver containing boric acid indicator mixture. The distillate was back-titrated with 0.02 N HCl to a grey end point. A blank sample was also run in a similar manner but without the soil sample.

Total nitrogen was calculated using the formula below:

$$\% \text{ N} = 14 \times (A-B) \times N \frac{100}{1000} \times 1$$

Where:

N = concentration of HCl used in titration

A = ml HCl used in sample titration

B = ml HCl used in blank titration

14 = atomic weight of nitrogen

1 = weight of soil sample

3.7.6 Determination of available Potassium

Potassium was determined by flame photometry. A standard series of potassium was prepared by diluting 100 mg/l of potassium to 100 mg/l. This was done by taking a 25 ml portion into 250 ml volumetric flask and made to volume with water. Portion of 0.5, 10, 15, and 20 ml of 100 mg/l standard solution was put into 200 ml volumetric flask. One hundred milliliters of 1.0 M NH4OAc solution was added to the flask and made to volume with distilled water. The standard series obtained was 0, 2.5, 5.0, 7.5, 10.0 mg/l for potassium. Potassium was measured directly by flame photometry at wavelength of 766.5.

Potassium was calculated as follows:

$$K(cmol/kgsoil) = \frac{(a\text{-}b) \times 250 \times mcf}{10 \times 39.1 \times S}$$

Where

A = mg/l of K in the diluted sample

B = mg/l of K in the diluted blank

S = air-dried sample weight of the soil in gram

Mcf = moisture correcting factor

39.1 = Molar mass for sodium

250 = volume of flask

3.7.7 Determination of Calcium and Magnesium

Calcium and magnesium were determined by Versanate Ethylene-Diamine Tetra-Acetic Acid (EDTA) method. Two and half grams (2.5 g) of soil sample were placed into a 100 ml flask and mixed with 50 ml of ammonium acetate solution. The mixture was shaken for five minutes, and filtered through No. 42 filter paper. An aliquot of 15 ml was pipette and 3-4 drops of EBT indicator were added. The solution was titrated with 0.02 N Versanate for a color change from blue to bright-blue or green. A blank titration was also done without soil.

Calcium and magnesium were calculated by the formula:

Ca + Mg (or Ca) (cmol/kg soil) = 0.02 x V x 1000/W

Where:

W = weight in grams of soil used for extraction

V= ml of 0.02 N EDTA used in titration

0.02 = concentration of EDTA used

3.7.8 Determination of Calcium

For the determination of calcium, 10 ml portion of the extract was transferred into an Erlenmeyer flask. To this, 10 ml of potassium hydroxide was added followed by 1 ml of Triethanolamine. Few drops of potassium cyanide solution and few crystals of cal-red indicator were added. The mixture was titrated with 0.02 N Ethylene Diamine Tetra-Acetic Acid (EDTA) solution from a red to blue end point.

3.7.9 Determination of Phosphorous

The readily acid-soluble forms of P were extracted with HCl: NH_4F mixture. The bray P1 method was used (Bray and Kurtz, 1945; Olsen and Sommers, 1982). Phosphorous in the extract was determined by the blue ammonium molybdate method with ascorbic acid as reagent.

Two grams of sample was weighed into a shaking bottle (50 ml) of extracting solution of Bray-1 (0.03 *M* NH_4F and 0.025 *M* HCl) was added. The sample was shaken for one minute by hand and immediately filtered through Whitman No. 42 filter. One milliliter of the standard series, the blank and the extract, two milliliters boric acid and 3ml of the colouring reagent (ammonium molybdate and antimony titrate solution) were pipette into a test tube and homogenized. The solution was allowed to stand for 15 minutes for the blue colour to develop to its maximum. The absorbance was measured on a spectronic 2ID spectrometer at 660nm wavelength.

A standard series of 0, 1,2, 2.4, 3.6, 4.8 and 6 mgP/l was prepared from a 12 mgP/l stock solution by diluting 0, 10, 20, 30, 40, 50 ml of the stock solution in 100 ml volumetric flask and made to volume with distilled water. Aliquots of 0, 1, 2, 4, 5, and 6 ml of the 100 mg/P/l of the standard solution were put in 100 ml volumetric flasks and made to 100 ml mark with distilled water.

$$P(mg/kg) = \frac{(a\text{-}b) \times 20 \times 6\ mcf}{S}$$

Where

a = mg/l P in sample extract

b = mg/l P in blank

S = sample weight in gram (g)

20 = ml extracting solution

6 = ml final sample solution

Mcf = moisture correction factor

3.7.10 Determination of exchangeable acidity

Exchangeable acidity consists of aluminum (Al^{+3}) and hydrogen (H^+). Form the soil sample, exchangeable acidity was extracted with 1.0 M KCl, and the sum of Al^{3+} + H^+ was determined by titration (McLean, 1965). Five grams of air dry soil sample was weighed into a 200 ml plastic bottle and 100 ml of 1.0 M KCl solution added. The mixture was shaken for one hour and then filtered. Fifty milliters of the filtrate was transferred into an Erlenmeyer flask and few drops of phenolphthalein indicator solution added. The solution was titrated with 0.05 N NaOH until the colour just turned permanently pink. The amount of base used was equivalent to total acidity (Al^{3+}+H^+). A few drops of 0.05 N HCl was added to the same mixture to bring the solution back to colourless condition and 10 ml of ammonium fluoride (NH_4F) solution added. The solution was then titrated with 0.05 N HCl until the colour turned pink. A blank was included in the titration. The milli-equivalent of acid used was equal to the amount of exchangeable Al and H determined by difference.

Calculation:

$$\text{Exchangeable acidity (cmol/kg soil)} = \frac{(a\text{-}b) \times 2 \times 100 \times mcf}{S}$$

Where:

A = ml NaOH used to titrate with sample

B = ml NaOH used to titrate with blank

M = molarity of NaOH solution

S = air-dried soil sample weight in gram

20 = 100/5 (titre/pipette volume)

Mcf = moisture correction factor (100 + % moisture)/100

3.7.11 Determination of Base Saturation

The base saturation was calculated by dividing the Total Exchangeable Bases by Effective Cation Exchangeable Capacity **(**ECEC) and the result expressed in percentage.

$$\%\ Base\ saturation = \frac{TEB}{ECEC} \times 100$$

3.7.12 Determination of Effective Cation Exchangeable Capacity

The effective Cation Exchangeable Capacity was determined by the summation of exchangeable bases (TEB) and exchangeable acidity (ex Al^{3+}+H) of the soil extracts.

3.7.13 Determination of Total Exchangeable Bases

Total Exchangeable Bases (TEB) was determined by the summation of all the Exchangeable Cations.

3.7.14 Determination of soil bulk density

Before the experiment, the air dried soil sample was packed into a container with a known volume. The soil was then oven dried at 105 ^{O}C and weighed to determine the dry mass. The dry mass and the volume of soil were used to calculate the bulk density.

The following formula was used to calculate the bulk density:

Bd = ms/vs

Where:

Bd = bulk density

Ms = dry mass of soil

Vs = volume of soil or container

3.8 Planting and planting materials

Five rice varieties (Sikamoo, Bouake 189, ITA 324, ITA 320 and Jasmine 85) were received from the Crop Research Institute, Fumesua, Kumasi Ghana and two (Wita-4 and Wita-9,) from the Central Agricultural Research Institute, Suakoko, Liberia. The experiment was conducted in plastic pots containing soil medium. Three salinity levels (0 ds/m, 3 dsm^{-1} and 6 dsm^{-1}) were used to investigate their effects on the growth and yield of rice under salinity stress. Eight kilograms of prepared (air-dry) soil was filled in each of 63 plastic pots. Seeds were pre-germinated for six days and two seedlings were sown per pot. Sixty-three pots were used for the experiment, and each pot had a dimension of 30 x 27 x 25 cm. Soil was watered with normal tap water for twenty-one (21) days until seedlings were fully established then salinity treatment was imposed. Sodium chloride (NaCl) salt was used to develop salinity (3 dsm^{-1} and 6 dsm^{-1}), while the rest of the pots were kept as control having no salinity (0 dsm^{-1}). The experiment was designed in a completely randomized design with three replications and in a 3x7 factorial arrangement.

3.9 Fertilizer application

Prior to sowing of seedlings, a basal dose of N, P_20_5 and K_2O (15:15:15) at 7 grams was mixed in the top 5 cm of soil as a means of providing essential nutrient for the plants.

3.10 Determination of morpho-agronomic attributes of rice

3.10.1 Plant height

In order to obtain an accurate data on the height of plants, all seedlings which survived after transplanting were tagged and the main culms were measured at different growth stages at 21 days interval using the meter rule. Plant height was measured from the base of the plant (top of the soil) to the tip of the tallest leaf.

3.10.2 Length and width of flag leaf

The main culms of seedlings were tagged before tillering. The length of flag leaf was measured in centimetre from the topmost blade below the flag leaf on the main culm to the tip of the leaf. The data were recorded at late vegetative stage. Leaf width was measured in millimeter at the widest portion of the blade on the flag leaf. The data were recorded at late vegetative stage.

3.10.3 Relative water content

Leaf relative water content (RWC) was measured by the method of Conroy *et al*. (1988). The leaf next to the penultimate leaf was sampled using the scissors. Then a cork borer was used to cut ten leaf samples from each treatment and then weighed immediately to determine the fresh mass. The samples were then soaked in water for four hours and weighed to determine the turgid mass, after which the samples were oven dried 50 ^{0}C for twelve hours and weighed to determine the dry mass (Fletcher *et al.*, 2006). Relative water content was determined at 17 (seventeen) day-interval.

Relative water content was calculated using the formula:

RWC = Fm-Dm/Tm-Dm x 100

Where:

RWC = relative water content

Fm = fresh mass of leaf samples

Dm = dry mass of leaf samples

Tw = turgid mass of leaf sample

3.10.4 Root and shoot masses

The root and shoot mass were measured by the following procedures: plant was removed from the soil and washed to remove loose soils and then placed on dry polythene sheets to allow any free surface moisture to dry out. Plant materials were placed in paper bags and oven dried at 100^{o}C for twelve hours and allowed to cool in a dry environment (in a paper bag to keep moisture out) and then weighed on an electronic balance. The roots were separated from the shoot/top and weighed separately to record the root mass and shoots were also weighed separately for each treatment.

3.11 Yield components

3.11.1 Spikelet fertility

The main-stem panicles were tagged at panicle emergence and harvested at physiological maturity separately from the tiller panicles. The main-stem filled grain number and unfilled grain number per panicle were determined at harvest. Spikelet fertility was defined as the ratio of filled grains to total number of grains in the panicle. Each floret was pressed between the thumb and forefinger to determine if the grain was filled or not. Spikelet fertility was expressed as percentage. The total number filled grains and unfilled grains were also recorded and divided by the number of main culms to get the mean number of spikelets.

3.11.2 100 grain weight

Filled grains were placed in paper bags and oven-dried at 100^{o}C for 12 hours to standard moisture content. From the dried seeds, 100 seeds of each variety were selected and weighed on an electric balance and the weight recorded as 100-grain weight.

3.11.3 Number of filled grains

The panicles of the main culms were harvested and each floret was pressed between the fingers and thumb to determine if the grain was filled. This was carried out for each variety per treatment. The filled grains were carefully counted and then the total number of filled grain recorded as the number of filled grain per panicle. This was done for all the treatments.

3.11.4 Number of tillers and productive tillers

Tillers were considered as the young plant arising from the main culm in an alternate pattern and typically including leaves, culm and roots, but which did or did not develop panicle. Productive tillers were considered as those tillers that produced spikelets with or without filled grains. These were counted separately and the numbers recorded.

3.12 Determination of nutrient content in leaves of rice

Plant samples were ashed at 450 ^oC in a muffle furnace. Five grams of finely ground dry leaf samples were weighed into silica crucible and placed in a muffle furnace at temperature of 450 ^oC for 3-4 hours. The ash residue was dissolved in 10 ml of dilute HNO3 (1:2) and filtered through acid-washed filter paper into 100 ml volumetric flask, and the volume was made to the mark. The estimation of K, Na was carried out using the flame photometer and Ca, Mg determined by EDTA complex metric titration.

3.13 Ranking rice varieties for their tolerance to salinity

3.13.1 Subjective approach

The subjective approach was used as an index to rank the varieties according to their tolerance to salinity. The rice varieties were ranked from 1 to 7 on the basis of the effects of salinity on the quantitative values of the physiological and yield parameters. The varieties were assigned values using the subjective scheme presented in (Table 4.11).

3.13.2 The Percent Relative Reduction Approach

The percent relative reduction was also used as index to compare to the result obtained from the subjective ranking of the seven varieties according to their levels of tolerance (Tables 4.5 and 4.8). To assess salinity tolerance, the percent relative reduction under saline conditions compare to control (RR %) was computed as:

RR% = 1 – (biomass under salinity/biomass under control).

3.13.3 Salinity Susceptibility Index

Salinity susceptibility index (SSI) was determined as, **SSI = Y_{W}-Y_{D} / SII (Y_{W}).** Where Y_W and Y_D are the mean biomass of a given accession in saline and non-saline conditions respectively, and SII was the salinity intensity index, calculated as **SII=1-X_S/X_N**.

Where: X_S and X_N, are the means of all accessions under salinity stressed and non - stressed environments respectively. The SSI provides an assessment of the relative performance of a given entry with regard to the mean performance of all the varieties. On the basis of SSI and RR% data for biomass under salinity compare to control, the mean was calculated for each of the physiological and yield parameters studied. The variety with the lowest RR% and SSI values were considered tolerant and those with higher values were the susceptible ones and ranked as such (Appendices 1& 2 and Table 4.10).

CHAPTER 4

4.0. RESULTS

The initial and final soil properties are presented below:

Table 4.1 Initial chemical properties of soil

pH 1:1 (H_2O)	Organic Carbon (%)	Total Nitrogen (%)	Organic Matter (%)	Exchangeable cation me/100g Ca	Mg	K	Na	TEB	Base sat. %
5.59	2.21	0.21	3.82	2.3	1.4	2.8	0.6	7.19	90.58

Table 4.1 continued. Initial chemical properties of soil and texture

Exch.A	E.C.E.C me/100g	P	Soil texture (%) sand	clay	silt	Textural Class
0.75	7.93	8.94	26.69	10.4	62.91	Silty-loam

Tables 4.1 show the initial chemical properties of soil used for the experiment in the green house, including soil texture. The Tables show that the initial soil reaction was moderately acid and nitrogen, magnesium, calcium, and phosphorous were low. The most deficient cations were potassium and sodium. The base saturation of the initial soil properties was 90.58%.

Table 4.2 Final soil chemical properties

					Exchangeable cations me/100g					
E.C- ds/m	pH(1:1) (H_2O)	Organic carbon %	Total Nitrogen %	Organic Matter %	Ca	Mg	K	Na	T.E.B	Base sat. %
0	5.67	1.89	0.13	3.25	6.9	2.97	0.59	0.28	10.68	93.21
3	4.87	2.25	0.16	3.86	2.38	2.46	1.21	8.49	14.8	92.07
6	4.93	2.17	0.16	3.74	1.64	2.35	3.19	1.74	6.38	80.26

The final soil properties are shown in (Table 4.2). There is a reduction in final soil pH, total nitrogen as compared to the initial soil properties. There was a decrease in calcium but an increase in potassium. In relation to treatments, there was an increase in potassium and sodium as salinity increased. On the other hand, calcium, and magnesium concentrations decreased as the level of salinity increased.

Table 4.3 Mean squares of treatments on various yield components of rice

Sources of variation	Df	100 grain weight	Number of filled grains	mean spikelet number	number of tillers	number of productive tiller	% spikelet fertility
Variety	6	0.01^{ns}	5036.7**	26760.1**	16.1*	10.4*	38.4**
Salinity	2	2.3*	271808.1**	908642.5**	416.1**	276.1**	3382.0**
Salinity x variety	12	0.006^{ns}	4819.6**	64763**	17.3**	11.7*	23.6**
Residual	42	0.02	18.9	6323	7.8	4.8	4
Total	62						

** Significant at 1% level of probability
*Significant at 5% level of probability
^{ns} Not significant

Table 4.4. Mean comparison of the effects of treatments on yield components of rice.

Variety	100 grain mass (g)	Number of filled grains	Mean number of spikelet	Number of tillers	Number of productive tillers	Percent spikelet fertility
BOUAKE 189	0.38	82.44	170.10	4.78	4.33	10.04
ITA 320	0.36	93.33	181	7.78	4.78	9.28
ITA 324	0.31	133.11	194.30	5.00	2.00	14.56
JASMINE 85	0.43	78.67	130.00	6.22	3.22	13.36
SIKAMOO	0.37	115.78	160.40	3.67	2.33	10.96
WITA 4	0.36	84.89	177.20	6.11	4.22	9.14
WITA 9	0.37	63.89	144.10	4.78	2.78	11.89
Lsd (5%)	0.14	4.14	8.38	2.66	2.09	1.908
Salinity levels						
0	0.64	219.95	296.80	10.43	7.43	25.04
3	0.48	59.52	192.70	4.19	2.29	8.91
6	-	-	6.50	1.81	0.43	-
Lsd (5%)	0.09	2.71	5.49	1.74	1.37	1.249
% CV	38.2	4.7	15.2	51.1	62.1	17.7

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(-) No data was recorded for these parameters

4.1 Effects of treatments on yield components of rice

Tables 4.3 and 4.4 show the effects of different levels of salinity on 100 grain mass, mean number of spikelet, number of filled grains, number of tillers, number of productive tillers, and percent spikelet fertility. Most of the yield contributing components measured were significantly ($p < 0.01$) reduced at 6 dsm^{-1} salinity level when compared with control (Table 4.3 and 4.4 and Appendix 1). These components were less affected at 3 dsm^{-1} in most of the varieties, but ITA 320 was the worst affected followed by WITA 4 and Jasmine 85. WITA 9, BOUAKE 189 and ITA 324 were the least affected varieties (Appendix 1). All plant properties related to yield did not produce any response at

6 dsm^{-1} due to increase in the salinity levels in all treatment pots. Therefore, in the analysis of ranking the rice varieties for tolerance to salinity, only the EC of 3 dsm^{-1} was used.

Table 4.5: Percent reduction of yield parameters as affected by level of salinity relative to control

	Salinity level (ds/m)	Bouake 189	ITA 320	ITA 324	Jasmine 85	Sikamoo	WITA 4	WITA 9
Yield parameters								
100 Grain mass	3	23	36	14	16	35	26	25
	6	100	100	100	100	100	100	100
Number of spikelet	3	28	50	38	27	52	40	13
	6	100	100	95	94	100	100	93
Number of filled grains	3	73	91	70	55	73	86	41
	6	100	100	100	100	100	100	100
Number of Tillers	3	48	77	56	76	24	52	48
	6	80	88	78	91	82	82	65
Number of productive tillers	3	75	87	20	55	69	73	61
	6	100	100	100	100	100	100	100
Percent spikelet fertility	3	62	83	51	39.7	82	78	57
	6	100	100	100	100	100	100	100

4.2 The percent reduction of yield parameters

The performance of all the rice varieties were affected by salinity levels (Tables 4.3 and 4.4). At 3 dsm^{-1} there was 14 %-36 % reduction in grain mass for the seven varieties. ITA 324 recorded the least reduction in grain mass followed by Jasmine 85 and WITA-9 (16 % and 25 % respectively) (Table 4.5). At low salinity level, 3 dsm^{-1}, the number of filled grains was reduced (41-91 %) in comparison with the control. The least affected variety was WITA 9 (41 %) while ITA 320 was severely reduced (91 %) as compared to the control. At higher salinity level (6 dsm^{-1}) there was 100 % reduction in number of filled grains, because three of the varieties flowered but died shortly after flowering (Table

4.5). The salinity x variety interaction affected the number of filled grains. There was significant ($p < 0.01$) reduction in the number of tillers. In the case of ITA 320, 87 % reduction of number of tillers was observed at 3 dsm^{-1} compared to control. WITA 9 had 65 % reduction in the number of tillers followed by ITA 324 with 78 %. JASMINE 85 had 91 % reduction compared to the control (Table 4.5 and Appendix 1). Induced salinity levels significantly reduced the number of productive tillers of all the varieties (20-87 %) in comparison to the control. ITA 320 was the most affected variety while ITA 324 was least affected (Table 4.5). In WITA 9 and ITA 324, 57 % and 51 % reduction respectively, were observed due to salinity level of 3 dsm^{-1}. However, WITA 9 gave better performance to salinity in terms of fertile spikelets (Table 4.5).

Table 4.6 Mean square values for the effects of treatments on different growth parameters of rice

Sources of variation	df	Relative water content	Plant height	Shoot weight	Root weight	Flag leaf width	Flag leaf length	Seedling survival
Variety	6	113.10**	514.62**	100.0**	23.51**	16.88*	36.87*	.16*
Salinity	2	433.40**	10122.87**	1368.0**	298.51**	2331.57**	6310.11**	16.78**
Salinity x Variety	12	52.60**	134.19**	53.7**	21.53**	13.48*	32.69*	0.15*
Residual	42	3.24	96.16	9.95	0.95	13.97	13.08	0.08
Total	62							

**Significant at 1% level of probability

*Significant at 5% level of probability

4.3. The mean squares values for the effects of treatments on different growth parameters of rice

The analysis of variance (Table 4.6) shows a significant ($p < 0.01$) effect on all growth parameters due to salinity stress. Salinity x variety interaction was significant ($p \leq 0.01$) for relative water content, plant height, shoot weight and root weight. In the case of flag leaf width, flag leaf length and seedling survival, the effects of salinity and variety interaction was significant at $p < 0.05$.

Table 4.7. Mean comparison of the effects of salinity levels on growth parameters of rice

Variety	Plant height	Relative water content	Shot mass	Flag leaf length	Flag leaf width	Root mass
BOUAKE 189	103.90	15.13	11.50	21.44	13.33	11.50
ITA 320	100.90	11.44	13.20	20.33	12.78	13.20
ITA 324	96.10	18.24	6.74	21.33	14.67	6.74
JASMINE 85	89.30	14.33	5.01	20.11	15.33	5.01
SIKAMOO	110.70	11.33	7.95	18.22	12.89	7.95
WITA 4	107.0	15.0	12.47	20.56	11.11	12.47
WITA 9	94.0	21.22	6.06	24.89	13.56	6.06
Lsd (5%)	9.33	1.12	3	3.44	3.56	3
Salinity level						
0	121.6	18.9	17.76	36.76	22.62	17.76
3	101.50	16.67	7.33	23.76	15.62	7.33
6	77.70	10.16	1.88	2.43	1.90	1.88
Lsd (5%)	6.11	1.71	1.97	2.25	2.33	1.97
% C V	9.8	11.8	35.1	17.2	27.9	29.8

4.4. Effects of salinity levels on growth parameters of rice

The mean comparison of plant height, relative water content, shoot mass, flag leaf length, flag leaf width and root mass as affected by salinity and varieties are presented in (Table 4.7). All growth parameters decreased with increasing salinity levels. With regard to mean comparison, there were no significant differences among the varieties for plant height and flag leaf width, however, SIKAMOO and WITA 9 recorded the highest for plant height and flag leaf width respectively. There were significant differences among the varieties for relative water content shoot mass, flag leaf length and root mass. The plant height was highest for the control (103.7-142 cm) followed by treatment one that is, 3 dsm^{-1} salinity (88-113.7 cm). On the other hand, the highest salinity level (6 dsm^{-1}) gave the lowest values for plant height (68.7-85 cm). Relative water content was lowest for control, but WITA 9 recorded the highest value for relative water content.

Table 4.8: Percent reduction in growth parameters as affected by level of salinity

Growth parameters	Salinity level ds/m	Bouake 189	ITA 320	ITA 324	Jasmine 85	Sikamoo	WITA 4	WITA 9
Shoot mass	3	52	72	37	81	56	54	44
	6	86	97	72	93	95	90	77
Plant height	3	15	18	24	21	20	13	2.3
	6	31	35	41	38	46	33	26
RWC	3	3	5	3	24	3.8	16	27
	6	44	66	20	37	70	28	11
Root weight	3	70	88	53	85	59	58	65
	6	97	99	90	98	95	93	89
Flag leaf length	3	36	45	26	31	42	48	16
	6	100	100	82	85	100	100	84
Flag leaf width	3	21	31	34	25	43	49	11
	6	100	100	80	83	100	100	75

4.5: Percent reduction in growth parameters as affected by level of salinity

There was a decrease in plant height (2.3-24 %) at 3 dsm^{-1} salinity level for all the varieties. At higher salinity level, 6 dsm^{-1}, there was 26-46 % reduction in plant height. There was a reduction in plant relative water content 3-27 % for low salinity level and 20-70 % for higher salinity level, respectively. Reduction in root mass was 53-88 % for low salinity level and 89-99 % for higher salinity level. Flag leaf length and width were reduced 16-49 % at 3 dsm^{-1}, and 80-100 % reduction at 6 dsm^{-1}.

Table 4.9. Scheme for quantitative ranking of rice varieties

Ranks	Points
1	14
2	12
3	10
4	8
5	6
6	4
7	2

The rice varieties were ranked from 1 to 7 on the basis of their quantitative values of the physiological and yield parameters (Appendices)

Table 4.10. Ranking rice varieties according to their tolerance to salinity.

Variety	Total scored points	Rank	Tolerance classification
ITA 324	80	1	Tolerant
WITA 9	76	2	Tolerant
BOUAKE 189	76	2	Tolerant
WITA 4	69	3	Susceptible
JASMINE 85	64	4	Susceptible
SIKAMOO	62	5	Susceptible
ITA 320	58	6	Highly Susceptible

70-79: Tolerant
60-69: Susceptible
50-59: Highly susceptible

4. 6. Ranking the rice varieties according to their tolerance to salinity.

Table 4.10 was used to rank the varieties according to their level of tolerance to salinity. This is the subjective index used for the classification of the seven rice varieties (see 3.13.1). The salinity susceptibility index calculated from the parameters (see 3.13.3) was also used to rank the varieties according to their susceptibility to salinity (Table 4.11).

Table 4.11 Salinity susceptibility index

Variety	% spikelet fertility	Mean spikelet	Filled grain	Grain mass	Tiller number	Productive tillers	Plant height	RWC	Total	Ranking
ITA 320	5	4	5	5	6	2	5	3	35	W- 9
WITA 4	3	5	7	6	1	6	4	4	36	ITA 324
Bouake 189	3	6	3	2	4	3	7	2	30	BOU-189
ITA 324	5	1	4	6	2	4	3	1	27	JAS- 85
Jasmine 85	1	7	2	3	7	4	2	5	31	ITA 320
WITA 9	2	1	1	1	3	5	1	6	20	W- 4
Sikamoo	4	3	6	4	5	1	7	7	36	SIKA

The indices show that WITA 9, ITA 324 and BOUAKE 189 ranked highest among all the varieties tested for their tolerance to salinity.

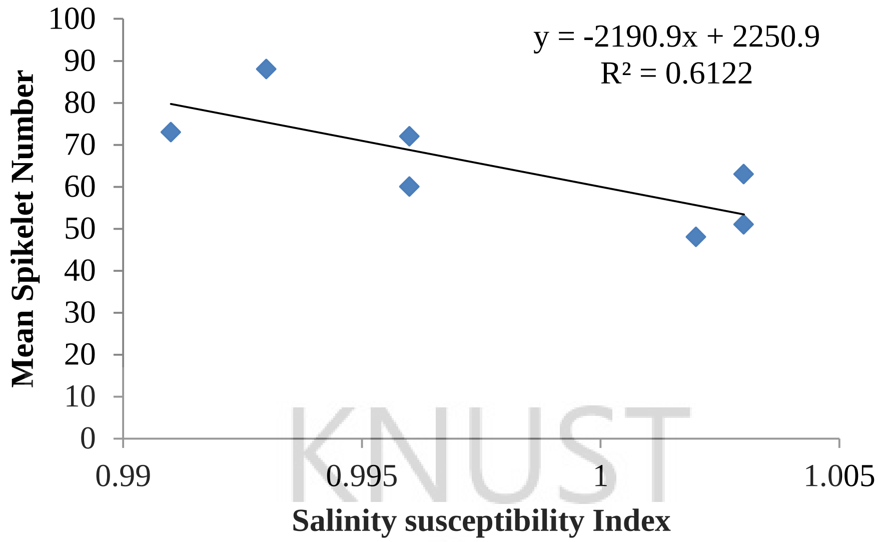

Figure 2. Relationship between the relative mean spikelet number under saline conditions and salinity susceptibility index.

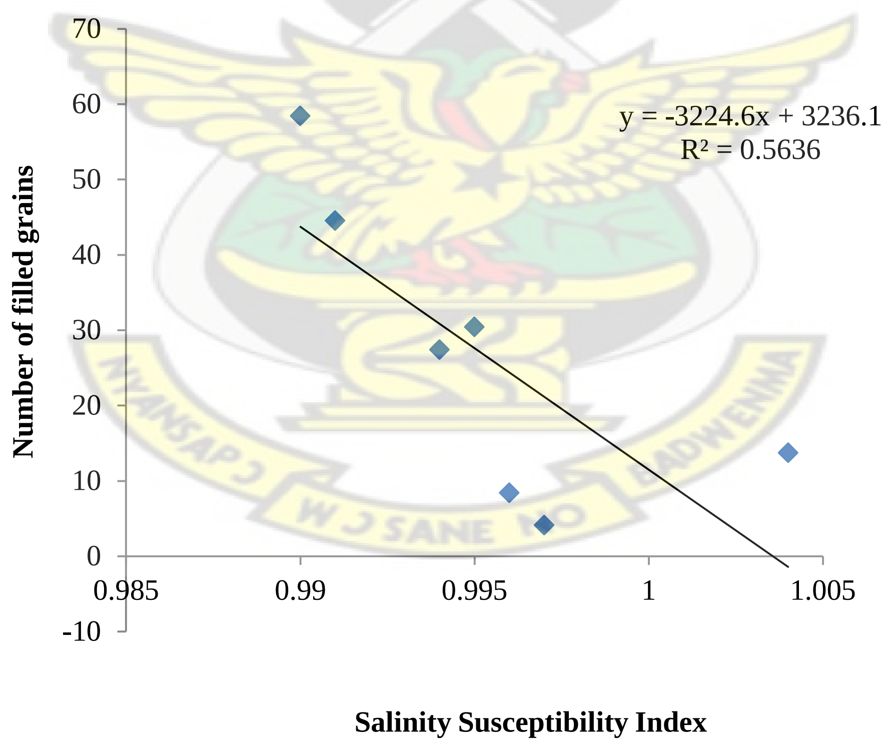

Fig.3. Relationship between the relative filled grain number under saline conditions and salinity susceptibility index.

4.7. Relationship between yield parameters and salinity susceptibility index (SSI).

Figures 2 and 3 show the relationships between Salinity Susceptibility Index (SSI) and the mean spikelet and number of filled grains respectively. A moderately strong relationship between the mean spikelet and SSI is shown in (figure 2). In the case of filled grains, there was also a moderately strong relationship with SSI. The co-efficient of determination shows that 61 % of variation in mean spikelet number can be attributed to salinity susceptibility index, and 56 % of variation in number of filled grains can also be attributed to salinity susceptibility index.

Table 4.12. Mean squares values for plant tissue analysis

Source of variation	d.f	Ca	K	Mg	Na
Variety	6	0.003^{ns}	0.08*	0.01*	0.13^{ns}
Salinity level	2	0.03*	0.77*	0.05*	5.58*
Variety x salinity	12	0.003^{ns}	0.16*	0.002*	0.15**
Residual	42	0.0017	0.0031	0.00027	0.06

**Significant at 1% level of probability
*Significant at 5% level of probability
^{ns} not significant

4.8. Mean squares values for plant tissue analysis

The analysis of variance for plant tissue indicated that there were no significant differences in calcium and sodium accumulation by the rice varieties, but potassium and magnesium accumulation was significant ($p < 0.05$) among all the varieties. Meanwhile, the effects of salinity on mineral uptake showed significant ($p < 0.05$) differences among the rice varieties. Variety x salinity interaction was not significant for calcium. There was significant ($p < 0.05$ & $p < 0.01$) among the varieties for potassium, Magnesium and sodium respectively.

Table 4. 13. Effects of salinity levels on nutrients concentration in leaves of rice

Variety	Ca	K	Na	Mg
BOUKE 189	0.24	0.51	0.62	0.21
ITA 320	0.25	0.42	0.62	0.12
ITA 324	0.23	0.55	0.63	0.19
JASMINE 85	0.21	0.59	0.41	0.19
SIKAMOO	0.22	0.66	0.66	0.22
WITA 4	0.26	0.48	0.39	0.19
WITA 9	0.21	0.69	0.41	0.17
Lsd. (5%)	0.04	0.05	0.24	0.02
Salinity level				
0	0.26	0.36	0.02	0.22
3	0.25	0.58	0.54	0.2
6	0.19	0.74	1.05	0.13
Lsd. (5%)	0.03	0.04	0.16	0.01
CV %	18.0	10.0	46.6	8.8

Figures having the same letters in a column do not different at 5 % probability

4.9. Effects of salinity levels on Ca, K, Na and Mg concentration in leaves of rice

The mean comparison shows that the concentrations of Ca, K and Mg were significantly reduced by induced salinity for all the rice varieties, but Na concentration was increased in the leave tissues of the varieties (Table 4.13). Among the varieties, there were no significant differences in the concentration of Ca and Na, however, WITA 4 recorded the highest accumulation of Ca while SIKAMOO recorded the highest value for Na. There were significant differences among all the rice varieties for K and Mg accumulations in leaf tissues.

Table 4.14 EC values at the end of the experiment

EC before the experiment		EC values after the experiment			
Variety	**EC dsm^{-1}**	**Variety**	**control**	**3 dsm^{-1}**	**6 dsm^{-1}**
ITA 320	0.41	ITA 320	0.45	5.97	5.34
WITA 4	0.41	WITA 4	0.43	5.01	6.18
BOUAKE 189	0.41	BOUAKE 189	0.47	6.54	7.98
ITA 324	0.41	ITA 324	0.42	4.56	6.99
JASMINE 85	0.41	JASMINE 85	0.44	6.72	7.35
WITA 9	0.41	WITA 9	0.44	6.87	6.48
SIKAMOO	0.41	SIKAMOO	0.46	7.59	7.47

4.10. EC values after and before the experiment (Table 4.14).

The values increased with increasing salinity level of the irrigation water. In the control pots treated with tap water, the final EC value slightly increased which implied that the underground water used was slightly saline. The EC value of 3 dsm^{-1} increased to 7.59 dsm^{-1}, whereas EC value of 6 dsm^{-1} increased to 7.98 dsm^{-1}. This was a clear indication of the cumulative effects of the addition of sodium chloride on soil salinity.

CHAPTER FIVE

5.0 DISCUSSION

The response of each of the seven rice varieties used for the study was assessed as discussed below. The seven rice varieties, Bouake 189, ITA 320, ITA 324, Jasmine 85, Sikamoo, WITA 4 and WITA 9, produced different responses to the different levels of salinity. The effects of salinity on the varieties varied among the different varieties with increasing salinity levels. Salinity levels affected the soil chemical properties as well as the mineral contents in the leaves of the rice varieties.

5.1. The effects of salinity on soil properties

5.1.1 Electrical conductivity (EC) of soil

The E.C values of the different salinity levels were increased at the end of the experiment due to the cumulative inputs of sodium chloride. Similar results were reported by Kayamanidou and Xanthoulis (1998) and Massena (2001). At the end of the experiment, there was increase in the EC values in the upper layer of the soil and this could be attributed to the movement of water upward due to evaporation. Salt-laden water will move laterally in the highly permeable soil layer until it reaches the soil surface (Doering and Sandoval, 1976). The water then evaporates, leaving behind the salts to accumulate.

5.1.2 Soil pH

The initial analysis recorded a pH value of 5.9. There was a decrease in pH value for the various treatments at the end of the experiment. Sodium accumulation in soil should increase soil pH, but this result shows otherwise. The decrease in the value of pH could be attributed to the application of nitrogen fertilizer to the various treatments, including the control as reported by other researchers (Narwel *et al.*, 1993; Singh and Verloo, 1996).

5.1.3 Phosphorous and potassium

The result from the final soil analysis shows that there was an increase in potassium and phosphorous concentration with increase in salinity levels. Increase salinity in the rhizosphere prevents water and nutrients uptake by plant. Phosphorous were also added to all the treatments. Hence, it was observed that phosphorous accumulation in the soil increased with increasing salinity levels. This result could be supported by similar reports by Pescod (1992) and Heidarpour *et al.* (2007)).

5.1.4 Nitrogen

Total nitrogen was decreased at the end of the experiment for all treatments including the control. The control recorded the least total nitrogen, which indicates that N uptake was higher in the non-saline soil than the saline soils. Nitrogen uptake adversely affected by soil salinity. In plants, nitrogen comprises 80% of the total absorbents (Marschner, 1995). In general, salinity reduces nitrogen accumulation and saline environments are associated with nitrogen deficiency (Siddiqui *et al.*, 2010). Tuna *et al.* (2007) suggested the decrease in nitrogen in saline environment may be due to the inhibition of nitrogen uptake by NO^{-}_{3}/Cl^{-} interaction at the ion transport sites.

5.2. Effects of salinity on growth and yield components of rice

5.2.1 100 grain mass

Among the seven varieties there was no significant differences observed in 100-grain mass, but there were significant ($p < 0.01$) differences in 100-grain mass observed among the varieties at different salinity levels. However, variety x salinity interaction showed no significant differences among the varieties as it relates to 100-grain mass. At higher salinity level (6 dsm^{-1}) there was 100% reduction in grain mass therefore, there was no grain recorded. Reduction in crop yield as a result of salt stress has also been reported for rice (Mahmood *et al*., 2009). According to Munns (2002), salt stress decreases growth in most plants and these plants are not able to produce their maximum biomass. This stress at grain filling stage can cause a decrease in the photosynthates mobilization to grains and thereby decreasing grain mass (Sadeghipour, 2008).

5.2.2 Number of filled grains

Different physiological and yield components of rice had different sensitivity to salinity the number of filled grain per panicle was significantly ($p < 0.01$) reduced as salinity increased. High effectiveness of salinity on number of grains has been reported by many researchers (Beatriz *et al.*, 2001). The control treatment had the most amount filled grains and after that was treatment at 3 $dS\ m^{-1}$. The least number of filled grains per panicle was at 6 $dS\ m^{-1}$ salinity which showed 100 % decrease compared with the control treatment. Therefore increasing salinity caused decrease in number of filled grains per panicle of rice. Filled grain number per panicle had high effect on yield. Salinity decreased yield through decreasing number of filled grains per panicle. Mahmood *et al.*

(2009) studied the effect of salinity on rice and stated that increasing salinity significantly reduced grain filling capacity. Increased number of incompletely filled grains might be a result of assimilate shortage during grain filling, brought about by early leaf senescence (Sheehy *et al.*, 2001; Murchie *et al.*, 2002) which in this case was caused by salinity (Shannon, 1998; Zeng, 2000).

5.2.3 Number of spikelet

Different growth stages showed different sensitivity to salinity considering effect on number of spikelets per panicle. Effectiveness of different growth stages and also effect of salinity on number of spikelets was significant ($p < 0.01$). High influence of salinity on spikelet number per panicle and rice sensitivity to salinity of irrigating water was reported by many researches (Scardaci *et al.*, 1996; Shannon *et al.*, 1998; Zeng and Shannon, 2000). Decrease in number of spikelets per panicle is one of the major factors of reduction in rice yield due to salinity (Scardaci *et al.*, 1996; Shannon, *et al.*, 1998). IRRI in (1978) reported that during the reproductive stage, salts adversely affect the number of spikelet per panicle.

5.2.4 Number of tillers

Salinity stress greatly affected the development and viability of tillers in this experiment. All the rice varieties in the experiment were significantly ($p < 0.01$) influenced by levels of salinity in terms of tillers production. Tillers production gradually decreased with the increase levels of salinity, however, mean comparison shows that there was no significant difference among the seven varieties, but there were significant differences at the different salinity levels. There were no productive tillers for all the varieties at higher salinity level (6 ds/m). The result can be supported by Zeng and Shannon (2000). It was also reported that the number of tillers decreased progressively with increase in salinity levels (Desai *et al*., 1975 and Sexena and Pandey, 1981). (Ling *et al*., 2000) and (Young *et al*., 2003) also stated that the number of tillers hill^{-1}decreased with increasing salinity levels in rice. The decrease in number of tillers might be due to the toxic effect of salt on plant growth.

5.2.5 Productive tillers

Rice grain yields are highly dependent upon the number of panicle-bearing tillers produced per plant. There were significant differences among the varieties as well as the variety x salinity interaction. Mean comparison of productive tillers under stress showed that there were no significant differences among the varieties, however, the productive tillers decreased with increasing salinity levels. Khatun *et. al.* (1995); Lutts *et al.* (1995) reported that Salinity's effect on rice resulted in a decrease in the number of productive tillers and fertile florets per panicle and a reduction in individual grain mass.

5.2.6 Spikelet sterility

Spikelet sterility is the opposite of spikelet fertility. In this experiment, spikelet sterility increased with increase in salinity levels. Zeng and Shannon (2000), reported that grain yield per plant was reduced primarily by a reduction in the number of tillers per plant, number of spikelets per panicle, and the grain weight per panicle. Further they reported that substantial reduction in filled grains at 6 dSm^{-1} and higher suggesting that high salinity was causing some sterility. Abdullah *et al.* (2001), reported that sterility and reduction in seed set were primarily due to reduced translocation of soluble carbohydrates to primary and secondary spikelet, accumulation of more sodium and less potassium in all floral parts and inhibition of the specific activity of starch synthesis in developing rice grains, thus reducing seed set.

5.2.7 Spikelet fertility

Spikelet fertility is an important contributory factor to grain yield. In this experiment spikelet fertility was greatly influenced by salinity levels. According to the analysis of variance, there were significant differences among the varieties as well as the interactions. At the different salinity levels, there were significant differences observed. However, the mean comparison shows that there were no significant differences among the varieties. Among rice varieties, ITA 324 and WITA 9 show some minimum response to salinity in decreasing spikelet fertility. As salinity increased to 6 dsm^{-1}, there was a drastic decline of spikelet fertility for all the varieties used in the experiment, however at 6 dsm^{-1} Jasmine 85, WITA 9 and BOUAKE 189 produced spikelet but these varieties died before reaching the grain filling stage. The reduction in spikelet fertility could be attributed to failure of grain formation in rice which was caused by lack of pollen viability. Grattan *et al.* (2002), reported that under saline condition, spikelet fertility is inversely proportional to sterility; this was originally adapted from Zeng and Shannon, (2000). Khatun *et al.* (1995) earlier reported that salinity reduces pollen viability and seed set.

5.2.8 Plant height

According to the analysis of variance, plant height of the different rice varieties were significantly affected by levels of salinity. In case of all the varieties it was observed that plant height decreased as the salinity level increased. The mean comparison shows that there was not significant differences in plant height among the varieties, though the control recorded the highest followed by Sikamoo and Bouake 189. However, there was a significant difference at the different levels of salinity. The result indicates that the effects of salinity on plant elongation of different varieties were different, which might be due to genetic potentiality of the varieties. It is reported that soil salinity suppresses shoot growth more than the root growth (Maas and Hoffman 1977; Ramoliya *et al.,* 2004). Islam *et al.* (2007) also observed the differences in plant height of rice varieties with different salinity levels. Javed and Khan (1975), Sexena and Pandey (1981) also reported that plant height progressively decreased with increase in salinity levels, because high concentration of soluble salt in soil and osmotic pressure creates disturbance in uptake of water and other nutrients.

5.2.9 Relative water content, root and shoot mass, flag leaf length and width

Salinity stress had a significant effect ($p < 0.01$) on all growth parameters of the seven rice varieties. In addition, comparison of means by Duncan's Multiple Range Test demonstrated that, salinity stress decreased root dry mass. The decrease in root mass was parallel with the increase in salinity levels. The highest root dry mass was obtained in the control treatment while the lowest one was observed in 6 dsm^{-1} salinity treatment. Similar result was achieved when shoot dry mass was measured. The highest and the lowest shoot mass was related to the control treatment and 6 dsm^{-1} salinity treatment, respectively. Salinity stress diminished plant growth and significantly decreased total dry mass. There was a downward trend in shoot mass because of deterrent effects of salinity on plant height. Salt stress adversely affects the growth and development of rice, and the results of this study confirm that all growth variables of rice drastically decreased with salinity treatment. Plants had the reduction in their dry mass because of the proportional increase in sodium concentration, which could imply that an ionic effect was being manifested. It is also assumed that in addition to toxic effects of NaCl, higher concentration of salt reduced the water potential in the medium which hindered water absorption and thus reduced plant growth. High salt content decreased the osmotic potential of soil water; this reduces the availability of soil water for plants (Aşik *et al.*, 2009). Sagi *et al.* (1997) also found the adverse effects of salinity stress on shoot and root growth. It has been reported that, decline in plant biomass may be due to excessive accumulation of NaCl in chloroplasts of plant, which affects growth rate, and is often associated with a decrease in the electron transport activities of photosynthesis (Kirst, 1989) and inhibition of PSII activity (Kao *et al.*, 2003). In general, salinity reduces leaf length, leaf width, shoot and root dry mass leading to low yields (Hamdy *et al.*, 1993; Essa, 2002; Li *et al.*, 2006 and Sharifi *et al.*, 2007).

5. 2.10 Effects of salinity on uptake of nutrients into leaves of rice

The cation potassium (K) is essential for cell expansion, osmoregulation and cellular and whole–plant homeostasis (Schachtman *et al.*, 1997). During salt stress, plants need a mechanism to regulate turgidity. Many studies have shown the importance of K in regulating turgidity (Guardia and Benlloch, 1980; Mengel and Arneke, 1982; Hsiao and Lauchli, 1986). According to the analysis of variance there was no significant difference in calcium concentration in leaf but the interaction was significant. Analysis of variance results for potassium and magnesium showed significant differences among the varieties and interaction in the uptake or concentration of minerals. However, sodium concentration was not significantly different among the varieties but the interaction showed differences. Mean separation of K and Na concentration in leaf showed significant increase among all the rice varieties. High stomatal potassium requirement is reported for photosynthesis (Chow *et al.,* 1990). The role of potassium in response to salt stress is also well documented, where Na and K exchange during salt uptake (Fox and Guerinot, 1998). In the present study, high value of potassium content in all tissues of the varieties recorded with increasing soil salinity suggests that Na increased potassium uptake. Moreover, the significant high value of sodium in leaves and stem tissues of the varieties suggests that this mechanism to block Na transfer to growing tissues was not effective at high salt concentration. It is reported that uptake mechanisms of both potassium and sodium are similar (Watad *et al.,* 1991, Schroeder *et al.,* 1994). Calcium is important during salt stress, e.g., in preserving membrane integrity (Rengel, 1992), signaling in osmoregulation (Mansfield *et al.,* 1990) and influencing K/Na selectivity (Cramer *et al.,* 1987). In the present study there was a low value of Calcium content in leaves at increasing salinity levels. Excessive Na^+ concentration inhibits Ca^{2+} uptake in many plants (Grieve and Fujiyama, 1987; Dobermann *and* Fairhurst, 2000). It is reported that uptake of calcium ion from the soil solution may decrease because of ion interactions, precipitation and increases in ionic strength that reduce the activity of Ca^{2+}

(Janzen and Chang, 1987; Garg and Gupta, 1997). Low levels of Ca in growth media cause defects, such as deterioration of the cell membrane, loss of cellular components, and eventually cell and tissue death. Kaya *et al.* (2003) showed that Ca deficiency induced high concentration (NaCl) in strawberry. Calcium ions ameliorate the effects of salt stress by competing with sodium ions for membrane-binding sites. Patel *et al.* (2011) tested the effects of supplemental Ca on the nutrient levels in casalpinia crista L. (Fabaceae) in salinized soil in a green house. They demonstrated that salt reduced N, P, K and Ca content in tissues, however, the addition of Ca restored the levels of these nutrients.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

It is evident from the result of the present work on the response of rice to salinity that, for most of the yield and physiological parameters, ITA 324, BOUAKE 189 and WITA 9 were tolerant. The salinity susceptibility index used for the screening of the varieties consistently showed the three varieties as the first three with the highest tolerance to salinity as compared to the rest. The number of tillers, number of productive tillers, plant height, mean spikelet, spikelet fertility, number of filled grains and relative water content, were the yield and physiological parameters used to compute the salinity susceptibility index for the seven rice varieties. The index showed that BOUAKE 189, WITA 9, and ITA 324 performed better under saline condition. Yield attributes and physiological growth attributes were important criteria for selecting a tolerant variety. Varieties with an average good yield attributes and physiological growth attributes were considered important for salt tolerance efficiencies.

6.2 Recommendation:

Further studies by using both the greenhouse and field conditions should be carried out to compare the performance of the varieties under both conditions.

Agricultural extension staff should be made aware of these rice varieties for introduction to rice farmers in salinity prone areas.

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APPENDICES

Appendix 1 Effects of salinity on yield parameters of rice

Variety

	Salinity levels	Bouake 189	ITA 320	ITA 324	Jasmine 85	Sikamoo	WITA 4	WITA 9
100 Grain weight	0	0.65	0.66	0.5	0.7	0.68	0.62	0.64
	3	0.5	0.42	0.43	0.59	0.44	0.46	0.48
	6	*	*	*	*	*	*	*
Mean spikelet number	0	297	361	348	219	326	332	194
	3	213.3	182	217	159	155	200	222
	6	*	*	17	12	*	*	16
number of tillers	0	8.33	17.33	9	14	5.67	11	7.67
	3	4.33	4	4	3.33	4.33	5.33	4
	6	1.67	2	2	1.33	1	2	2.67
Number of filled grain	0	194	258	306	163	273	224	121
	3	53	22	93	73	75	31	71
	6	*	*	*	*	*	*	*
Productive tiller	0	8	12.67	3.33	6.67	5.33	10	6
	3	2	1.67	2.67	3	1.67	2.67	2.33
	6	*	*	*	*	*	*	*
Percent spikelet fertility	0	21.8	23.83	29.33	25	27.87	22.43	25
	3	8.33	4	14.33	15.07	5	5	10.67
	6	*	*	*	*	*	*	*

(*) No data collected for these parameters

Appendix 2. Effects of salinity on growth parameters of rice

	Salinity levels	Variety						
		BOUAKE 189	ITA 320	ITA 324	JASMINE 85	SIKAMO O	WITA 4	WITA 9
Relative water content	0	18	17.67	19.33	18	17.33	17.67	24.33
	3	17.33	16.67	20	13.67	16.67	14.67	17.67
	6	10.06	6.01	15.4	11.33	5.17	12.67	21.67
Shoot weight	0	21.3	30.33	10.61	11.96	15.94	24.05	10.14
	3	10.15	8.44	6.66	2.26	7.04	11.07	5.68
	6	3.05	0.82	2.94	0.81	0.86	2.29	2.37
Plant height	0	122.7	122.7	122.7	111.3	142	126	103.7
	3	104	100.3	93.3	88	113.7	110	101.3
	6	85	79.7	72.3	68.7	76.3	85	77

Appendix 3. Salinity in Saturated Soil Extract Based on Scale of EC

Relative Salt Level	EC dS/m	Plant Condition
Low	0 - 2.5	Salinity Effects Mostly Negligible
Medium	2.5 - 5.0	Very Sensitive Plants Affected
High	5.0 - 7.5	Many Plants Affected
Excessive	Above 7.5	Only Salt Tolerant Plants Grow

Source: USDA Information Bulletin, 194. 1994

Appendix 4. Analysis of variance for relative water content of rice leaves

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	6	678.302	113.05	34.9	0.001**
Salinity level	2	866.716	433.358	133.8	0.001**
Variety x Salinity	12	630.685	52.557	16.23	0.001**
Residual	42	136.033	3.239		
Total	62	2311.737			

Standard Errors of Differences of means (SED)	
Variety	0.85
Salinity levels	0.56
Salinity x Variety	1.47
Least Significant Difference Means (LSD)	
Variety	1.71
Salinity levels	1.12
Salinity x Variety	2.97
Coefficient of Variation (CV %)	11.8

Appendix 5. Analysis of variance table for the effects of salinity on root dry mass

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	6	141.0514	23.5086	24.7	0.001**
Salinity level	2	597.0091	298.5045	313.68	0.001**
Variety x Salinity	12	258.3719	21.531	22.63	0.001**
Residual	42	39.9684	0.9516		
Total	62	1036.4008			

Standard Errors of Differences of means (SED)	
Variety	0.46
Salinity levels	0.30
Salinity x Variety	0.80
Least Significant Difference Means (LSD)	
Variety	0.93
Salinity levels	0.61
Salinity x Variety	1.61
Coefficient of Variation (CV %)	29.8

Appendix 6. Analysis of variance table for the effects of salinity on shoot dry mass

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	6	600.135	100.022	10.05	0.001**
Salinity	2	2736.003	1368.001	137.46	0.001**
Variety x Salinity	12	643.846	53.654	5.39	0.001**
Residual	42	417.989	9.952		
Total	62	4397.971			

Standard Errors of Differences of means (SED)	
Variety	1.49
Salinity levels	0.77
Salinity x Variety	2.58
Least Significant Difference Means (LSD)	
Variety	3.00
Salinity levels	1.97
Salinity x Variety	5.20
Coefficient of Variation (CV %)	35.10

Appendix 7. Analysis of variance for the effects of salinity on the number of filled grains

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	6	30219.97	5036.66	266.2	0.001**
Salinity	2	543616.22	271808.11	14365.7	0.001**
Variety x Salinity	12	57835.56	4819.63	254.73	0.001**
Residual	42	794.67	18.92		
Total	62	632466.41			

Standard Errors of Differences of means (SED)	
Variety	2.05
Salinity levels	1.34
Salinity x Variety	3.60
Least Significant Difference Means (LSD)	
Variety	4.14
Salinity levels	2.71
Salinity x Variety	7.17
Coefficient of Variation (CV %)	4.70

Appendix 8. Analysis of variance for the effects of salinity on mean spikelet number

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	6	26760.1	4460	7.05	<.001
Salinity	2	908642.5	454321.3	718.48	<.001
Variety x Salinity	12	64763	5396.9	8.53	<.001
Residual	42	26558	632.3		
Total	62	1026723.7			

Standard Errors of Differences of means (SED)	
Variety	11.85
Salinity levels	7.76
Salinity x Variety	20.53
Least Significant Difference Means (LSD)	
Variety	23.92
Salinity levels	15.66
Salinity x Variety	41.43
Coefficient of Variation (CV %)	15.20

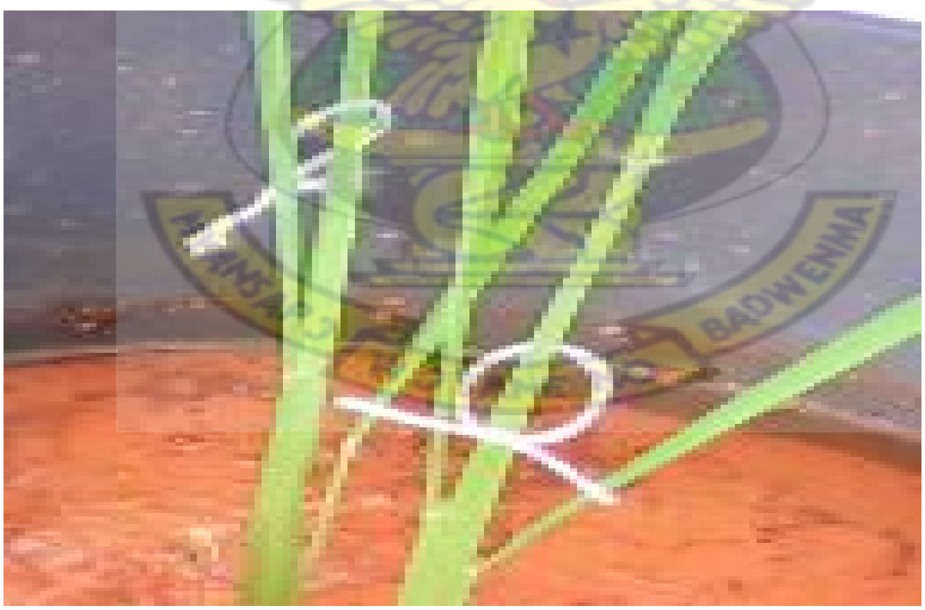

Plate 2. Rice seedlings tagged prior to tillering

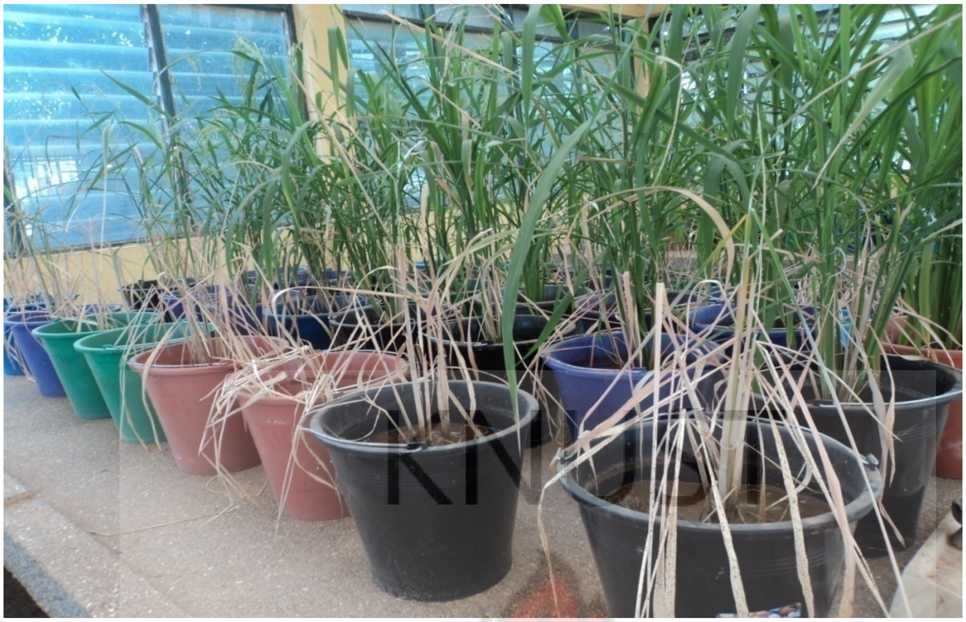

Plate 3. Effects of salinity on the vegetative growth of rice