EFFECTS OF SALINITY ON GROWTH AND YIELD OF RICE (*Oryza sativa* L.) AND DEVELOPMENT OF TOLERANT GENOTYPES IN KILOSA DISTRICT, TANZANIA

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EXTENDED ABSTRACT

Salinity is an ever increasing problem that reduces rice yield in many rice fields around the world. Soil salinity contributes to one of the most serious ecological and environmental problems in most of the irrigation schemes in Tanzania. Developing a salt tolerant rice genotype is one of the solutions to the problem of salinity. Marker assisted selection (MAS) is an indirect selection process where a trait of interest is selected based on a marker (morphological, biochemical or DNA/RNA variation) linked to a trait of interest rather than on the trait itself. Thus, the MAS technique was used in this study because it is very reliable in the selection of several traits associated with salinity and can also accelerate the breeding process and increase selection efficiency. Therefore, the objectives of the study were: i) to evaluate the responses of eight rice genotypes at various levels of salinity for the identification of parents for breeding program. ii) to assess farmer's perceptions about salinity problems occurring in their fields in the study area. iii) to estimate heritability of *saltol* in the new lines, and iv) to assess the marker-trait association and segregation ratio of new rice (*Oryza sativa*, L) lines.

A study was conducted under a controlled environment in the Department of Crop Sciences and Horticulture of the Sokoine University of Agriculture, to evaluate the responses of eight rice genotypes at three levels of NaCl concentrations (0 mM NaCL, 50 mM NaCl and 100 mM NaCl) and identify parental materials for breeding program. This was followed by a study on farmer's perception about the occurrences of salinity problems in their irrigated schemes. The study on farmer's perception was conducted in two villages (Ilonga and Chanzuru) in Kilosa District, Morogoro region involving rice farmers. The other study was conducted during April to September 2016 at Chanzuru irrigation scheme in Kilosa District to determine the characteristics of soil, and evaluate segregating

populations in selected saline environment at Chanzuru irrigation scheme. The heritability and genetic advance as well as the association of marker with quantative traits were determined during the study.

During the controlled experiment, three salinity indices namely salinity tolerance index, salinity susceptibility index and % relative reduction were used along with the IRRI standard evaluation score for salinity tolerance to rank the rice genotypes in terms of their tolerance and susceptibility. The results of the evaluation of eight rice genotypes showed that SUAKOKO-10 and NERICA-L19 were the most susceptible rice genotypes in comparison with the other genotypes; therefore, they were selected to be used as recurrent parents to improve their salinity tolerance using FL478 as the donor parent.

The study on farmer's perception revealed that farmers had a clear understanding of salinity problems occurring and affecting the crop production in Kilosa District. Farmers perceived four major factors contributing to the problem of salinity in their areas, namely, poor quality of irrigation water, poor drainage system, inadequate rainfall and inappropriate use of fertilizers. However, poor quality irrigation water and poor drainage infrastructure were the leading factors perceived to be contributing to the problem of salinity in the areas. As a result of these factors, farmers perceived poor harvest, poor production and poor yield of the rice crops. It was also established that farmers practiced crop diversification and increased farm size in response to the growing effects of salinity on their crop production and livelihoods.

Segregating populations along with the parent materials were evaluated in a saline environment using a randomized complete block design with four replications. Fresh leaf samples from young seedlings were collected for DNA extraction and marker-trait

association study as well as inheritance study. High heritability and high genetic advance were established in the new lines for all traits except grain yield per plant and 100-grain weight. Grain yield per plant was positively correlated with number of reproductive tillers and 100-grain weight. When the genotypes were scored for salinity injury, NLF3 population recorded the lowest score indicating that this population was highly tolerant to salinity as compared to other populations. The best performing genotypes among the introgression lines were NLF3 (F3 lines developed by crossing NERICA-L-19 and FL478) and SUF3 (F3 lines developed by crossing SUAKOKO-10 and FL478) for most of the traits studied. For inheritance and marker-trait association studies, the selected markers for the assessment of segregation and goodness of fit fitted well into the expected ratio of 1:2:1. The two markers loci (RM7075 and RM562) used were significantly associated with the number of filled grains per panicle and grain yield per plant in the studied rice materials.

DECLARATION

I, James Sulonkwiley Dolo do hereby declare to the Senate of Sokoine University of Agriculture, Morogoro, Tanzania, that this thesis is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

_____ _____

James Sulonkwiley Dolo Date

(Candidate)

The above declaration is confirmed by;

_____ _____

Professor Susan Nchimbi-Msolla Date

(Supervisor)

_____ _____

Professor John J. Msaky Date

(Co-Supervisor)

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DEDICATION

To the Almighty God, the Great “I AM THAT 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 Mrs. Matune J. C. Dolo; my children Joshua A. Dolo, Janice Y. Dolo and Frances Quiqui.

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LIST OF ABREVIATIONS AND SYMBOLS

ANOVA	Analysis of Variance
AOAC	Ammonium Acetate
BLKw	Walkley Black
CEC	Cation Exchange Capacity
Cl	Chlorine
DNA	Deoxyribonucleic Acid
ECe	Electrical Conductivity
ESP	Exchangeable Sodium Percentage
FAO	Food and Agricultural Organization
GA	Genetic Advance
GCV	Genotypic Coefficient of Variation
HSD	Honestly Significant Difference
IRRI	International Rice research Institute
KCL	Potassium Chloride
KDC	Kilosa District Council
LDCF	Least Developed Countries Fund
LNRDS	Liberian National Rice Development Strategies
MAS	Marker Assisted Selection
MSE	Mean Squared Errors
MSG	Mean Squares of Genotypes
NaCl	Sodium Chloride
NERICA	New Rice in Africa
NLF2	Second Filial Generation for “NERICA-L-19 x FL478”

NLF3	Third Filial Generation for “NERICA-L-19 x FL478”
PCI	Problem Confrontation Index
PCR	Polymerase Chain Reaction
PCV	Phenotypic Coefficient of Variation
QTL	Quantitative Trait loci
RDW	Root Dry Weight
RM	Rice Microsatellite
RR	Relative Reduction
SAR	Sodium Adsorption Ratio
SDW	Shoot Dry Weight
SES	Standard Evaluation Score
SII	Salinity Intensity Index
SPSS	Statistical Package for Social Sciences
SSI	Salinity Susceptible Index
SSR	Simple Sequence Repeat
SUA	Sokoine University of Agriculture
SUF2	Second Filial Generation for “SUAKOKO-10 x FL478”
SUF3	Third Filial Generation for “SUAKOKO-10 x FL478”
TDW	Total Dry Weight
TE	Tris- Ethylenediamine-tetraacetic Acid
TN	Total Nitrogen
TNBS	Tanzania National Bureau of Standards
TWAS	The World Academy of Sciences
UNESCO	United Nation education, Scientific and Cultural Organization
USDA	United State Development Agency

Vg	Genotypic Variance
Vp	Phenotypic Variance
WAAPP	West Africa Agricultural Productivity Program

CHAPTER ONE

1.0 GENERAL INTRODUCTION

One of the major challenges in relation to the world agriculture is producing more food to address the food insecurity problem facing the current population and an additional 2.3 billion people by 2 050 worldwide (FAO, 2009). Salinity (excess amount of soluble salts in the soil solution) is a major stress limiting the production of food crops. Salinity induces water deficit even in well watered soils by decreasing the osmotic potential of soil solutes thus making it difficult for roots to extract water from their surrounding media (Sairam *et al*., 2002). The effect of high salinity on plant can be observed at the whole plant level in terms of decrease in productivity and plant death (Parida *et al.*, 2004). The global projection show that salt-affected soils are increasing particularly in irrigated areas. In the last decades salt-affected areas have been reported to increase from 20 % (45 million hectares) to 33 % (74.25 million hectares) (Metternich and Zinck, 2003; Kumar and Shrivastava, 2015). These figures suggest that at a global scale, every day an area of about 2000 ha of irrigated cropland is affected by varying levels of salinity (Qadir *et al*., 2014). Therefore, it has been estimated that more than 50 % of the arable land would be salinized by the year 2050 (Jamil *et al.*, 2011).

Most soils within the arid and semiarid environments are already experiencing increasing levels of salinity. The main sources of salts include rainfall (Rengasamy and Olsson, 1993), mineral weathering (Macumber, 1991), irrigation and various saline water bodies (SPORE, 1995), undergroundwater which redistributes accumulated salts during evaporation (Macumber, 1991), chemical fertilizers applications (Rengasamy and Olsson, 1993) and man activities (Dregne, 1976). Rainwater contains 5 to 50 milligrams/kilograms of salt and 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; the accumulation of this salt in the soil would be considerable over millennia (Ghassemi *et al*., 1995; Munns, 2002). These sources lead to three different classes of salinization and sodification namely: saline ECe>4dSm-1, ESP<15%, pH<8.5); saline sodic (ECe>4dSm-1, ESP>15%, pH<8.5) and sodic soils (ECe<4dSm-1, ESP>15%, pH>8.5) (Richards, 1954).

In Tanzania, agriculture is the stronghold of the national economy, with rice being one of the major food and cash crops (Agritrade, 2012). The country is one of the largest rice producers accounting for about 80% of total production in Eastern Africa. Consequently, the country ranks second within East Africa in terms of rice production and consumption after Madagascar (Kafiriti *et al*., 2003 and Agritrade, 2012). Despite this promising trend, it is further noted that rice production is heavily dependent on rainfall, and under good weather conditions and improved husbandry, the country is potentially capable of achieving food self-sufficiency as measured by levels of grain production (Concern Worldwide, 2008). Rice is grown under three major ecosystems, namely rain-fed lowland, irrigation (or flooded conditions) and upland rice (non-flooded conditions), with each type characterized by a relatively low yet differing production potential depending on varying soil and climatic conditions (Kato, 2007).

Kilosa District is one of the areas in Tanzania with high potential for agricultural; this is partly attributed to relatively favourable climate and potentially fertile soils in the major part of the district (Kimaro, 2014). One of the principal crops grown in the district is rice (Concern worldwide, 2008). Despite the fact that Kilosa District has high potential for agricultural production, land productivity has remained low (Kimaro *et al*., 2001). One of

the reasons for the low productivity of land in the District could be attributed to the presence of soluble salts in the irrigated fields in the District.

In Tanzania, salt-affected soils are a major constraint to rice production that contributes to low yields in most rice producing irrigation schemes, (Kashenge-Killenga *et al.*, 2012a; Makoi and Ndakidemi, 2007), semi-arid irrigated, and non-irrigated, and in lowland areas characterised by high water tables (Kanyeka *et al.*, 1995; FAO, 2001). Breeding for salt tolerant rice genotypes is one of the, most effective and long term solution for resource poor farmers in salt-prone areas (Gregorio and Senadhira, 1993). For effective breeding, farmers' knowledge, and preferences for cultivars should be clearly identified through research –farmer interaction and collaboration. Farmers can provide vital information on the existing problem, type of cultivar, desired traits and insight on trade –offs they are willing to make in the design of the cultivar (Sperling *et al.*, 2001).

Salinity tolerance at different growth stages of crop appears to be controlled by independent genes (Shahbaz and Ashraf (2013). For example, in salt-tolerant cultivar Pokkali, a major *QTL* (*Saltol*) was identified on chromosome 1 and involved in salinity tolerance at the seedling stage (Bonilla *et al.*, 2002; Ghomi *et al*., 2013). This *QTL*, according to Bonilla *et al.* (2002), explains for 64% to 80% of the phenotypic variance in crop tolerance. Several studies have reported that this *QTL* has also been detected in some other wild rice cultivars (Ren *et al*., 2005; Takehisa *et al.*, 2004).

The following basic genetic approaches have been used to enhance salt tolerance of the rice crop: (i) exploitation of natural bio-diversity in rice gene pool through direct selection and mapping of quantitative trait loci (QTL) under stress environment (Foolad, 2004; Flowers, 2004) for traits associated with salinity tolerance, followed by marker assisted

selection (MAS) and (ii) development of transgenic plants to introduce novel genes as well as the alteration in expression levels of the existing genes to affect the degree of salt stress tolerance (Munns, 2005).

Breeder use the strategy of exploiting the genetic variability of the available germplasms for the identification of tolerant genotypes that may sustain a reasonable yield on salt affected soils (Ashraf *et al.*, 2006). The microsatellite marker or Simple Sequence Repeat (SSR) has been proved to be ideal for making genetic maps (Islam, 2004; Niones, 2004), assisting selection (Bhuiyan, 2005) and studying genetic diversity in rice germplasm. The microsatellite marker analysis is promising for the identification of major gene locus for salt tolerance that can be helpful for plant breeders to develop new rice cultivars.

The development of new rice cultivars for salinity tolerance has been achieved by the use of several breeding methods including; mutation breeding which has a significant contribution toward production of high yielding and salt stress tolerant rice varieties (Cassells and Doyle, 2003; Parry *et al*., 2009; Das *et al*., 2014). There are many reports where mutation breeding has resulted in enhanced salinity tolerance in various rice cultivars. For example, rice seeds irradiated with carbon (C) or neon (Ne) ions have generated mutant variety with high salt tolerance (Hayashi *et al*., 2007). Similarly, genetically engineered rice has also been developed and found to have high salt tolerance (Mohanty *et al.*, 2002). Salt tolerant varieties can also be developed through conventional breeding. According to Singh *et al.* (2004), in India six rice varieties developed through conventional breeding have been released as salt tolerant rice varieties.

However, the progress in breeding rice for salt tolerance is slow due to the complexity of the inheritance pattern of salt tolerance and difficulties in screening for salt tolerance, but

with the advancements in molecular biology a major QTL, '*Saltol*' in the rice variety Pokkali has been identified which accounts for 39.2, 43.9 and 43.2 per cent of phenotypic variation for three important salt-tolerance associated traits namely, Na^+, K^+ and Na/K absorption ratio respectively (Naresh-Babu *et al*., 2014; Vasuki and Geetha, 2016). The use of the microsatellite markers to selectively transfer this *QTL* into a desired genetic base could help to overcome the difficulties in screening for salinity tolerance in rice.

Rice, generally, is considered as a salt sensitive crop, but the extent of its sensitivity fluctuates during different growth and developmental stages. It is said that rice is tolerant to salinity stress during germination and active tillering, whereas it displays more sensitivity during early vegetative and reproductive stages (Lutts *et al*., 1995; Zhu *et al*., 2001).

Sodium ion (Na^+) and chloride ion (Cl^-) received from NaCl salts contaminate the soil, because these ions are well known as the toxic ions which damage plant cells; as a result, plant growth and development are directly restrained, leading to low yield and/or plant death (Lauchli and Grattan, 2007).

However, irrigation waters containing Na^+ and Cl^- have undesirable effects on the physical properties of soils because it is associated with the accumulation of sodium ion on the soil exchange complex. This eventually impacts the instability of the soil aggregates that results into the dispersion of soil particles and clogging of soil pores and the destruction of crops. Underground water containing soluble salts is mainly drawn to the surface by evapo-transpiration, where the soluble salts are deposited on the soil surface thereby forming white crusts which are clear indicators of the presence of salinity. Farming in such an area is severely impacted by this process (Tully *et al*., 2015), and mostly, the farmers

are the ones who suffer the adverse effects of this process on the productivity of their crops.

Therefore, understanding the farmers' perceptions on soil salinity and its effects on crop productivity is important in the development of the best cultivars that address the needs of the farmers. Additionally, it helps extension officers to promote soil and water conservation practices or decide on the best measures that safe guide the farmer production within a given location. Kruger (2006); and Wickham *et al.* (2006) reported that farmers' perceptions could be a good entry point for any intervention either by changing their perception through demonstrations or building on what they already know.

1.1 Problem Statement and Justification

1.1.2 Problem statement

There are a few scattered research reports on salt-affected soils in Tanzania (Mnkeni, 1996; Makoi and Ndakidemi, 2007; Kashenge-Killenga *et al.,* 2012a), but the extent of the problem is not well established. Mnkeni (1996) estimated that there were more than 2.9 million ha affected by soil salinity while, 700 000 ha had high sodicity. In FAO (2000) report, it was estimated that over 1.7 million ha are saline and 300 000 ha are sodic; while FAO (2003) further reported that salt affected soils were over 3.5 million ha with proportions of 16% and 84 % for sodicity and salinity, respectively. Studies conducted in Tanzania by Kashenge-Killenga *et al*. (2012 and 2014) reported a growing effects of salt affected soils in most of the rice irrigation schemes in Tanzania, such as Kilosa irrigation scheme. There was also an increasing concern from farmers, but the fell short of estimating the total areas affected by salinity in the country.

The disparity in the figures on the extent of salt-affected soil Tanzania, suggests that the total area affected by salt in the country is not well known. In reality, soil salinity is a serious problem that is turning agricultural land to barren, salty lands in Tanzania. In addition, several small scale rice irrigation schemes (classified as traditional irrigation schemes) are experiencing low yields due to salt (salinity) problems (Kashenge-Killenga *et al*., 2012b). Apart from the fact that the problem of salt-affected soils in rice production systems is increasing, information of the type of salts and their extent is scanty or lacking (Kashenge-Killenga *et al.*, 2012a).

1.1.3 Justification

The production of rice in Tanzania is dominated by small-scale farmers (Tanzania Agribusiness Report Q3, 2016). Kanyeka (2001) and Msomba *et al*. (2002) reported that more than 90% of rice in the country is cultivated by smallholder peasant farmers mainly using traditional irrigation systems on small holdings. According to Alam (2006) and Singh (2001), the traditional irrigation schemes (such as Kilosa irrigation schemes) are experiencing increasing levels of salinity because of mismanagement of the soils, irrigation, poorly designed and managed irrigation infrastructures, excessive and irrational use of irrigation water and also due to global climatic change (FAO, 2000; FAO, 2003).

Given the highly variable conditions, salinity-stressed environments and limited resources under which the crop is grown, yield below 1.5 t ha^{-1} have been reported in most irrigation schemes globally (Kanyeka, 2001; Msomba *et al.*, 2002; FAO, 2003; Kafiriti, 2004). According to Efisue *et al*. (2008) the production deficit can be overcome by the use of improved, high-yielding varieties adaptable to the salt-affected environments.

1.3 Study Objectives

1.3.1 Overall objective

The overall objective of this study was to develop salt tolerant rice genotypes for increased rice productivity in Kilosa District, Tanzania.

1.3.2 Specific objectives

i) To evaluate the responses of eight rice genotypes at various levels of salinity for the identification of parents for breeding program.

ii) To assess farmer's perceptions about salinity problems occurring in their fields in the study area.

iii) To estimate heritability and genetic advance of *saltol* in the new lines.

iv) To assess marker-traits association and segregation ratio of new rice (*Oryza sativa*, L) lines.

1.4 Research Hypothesis

i. Majority of the rice irrigation schemes of Kilosa District are saline.

ii. There are no differences in genotypes growth in response to soil salinity under field condition.

iii. Salinity tolerance is a polygenic trait; therefore there is no paternal effect on heritability of salt tolerance.

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CHAPTER TWO

SALINITY STRESS EFFECTS ON SOME MORPHO-PHYSIOLOGICAL TRAITS OF SELECTED RICE (*Oryza sativa* L.) GENOTYPES

Abstract

A study was conducted to evaluate the responses of selected rice genotypes, from IRRI and the AfricaRice Center, at various levels of salinity to identify parental materials for breeding purposes. The evaluation was done at the seedling stage in the Department of Crop Science and Horticulture of the Sokoine University of Agriculture, during November and December 2015. Eight rice genotypes were evaluated at three levels of NaCl concentrations (0 mM NaCL, 50 mM NaCl and 100 mM NaCl). Salt injury was scored on a 1-9 scale based on seedling growth characteristics following the modified Standard Evaluation Score (SES) of the International Rice Research Institute. The percent relative reduction (% RR), salinity tolerance index (STI) and salinity susceptibility index (SSI) were used to rank genotypes as tolerant or susceptible. On the basis of SES, phenotypic observation, three indices (SSI, STI and %RR), dry matter (DM) reduction, three rice genotypes (FL 478, IRRI 128, IR65192-4B-20-3,) were identified as salt tolerant; IRRI 113 and IRRI 112 were moderately tolerant while SUAKOKO-10, NERICA-L-19 and IRRI 124 were highly susceptible to salinity. Fl 478 and IR65192-4B-10-3 showed higher tolerance to salinity than other tolerant parent and was therefore selected as donor parent; similarly, SUAKOKO-10 and NERICA-L-19 were selected as recurrent parents to be used in a breeding program because of their high susceptibility and diverse genetic bases.

2.0 INTRODUCTION

There is wide range of variations among cereals for salt tolerance, and rice has shown to be the most sensitive cereal to salinity and barley the most tolerant cereal (Munns and Tester, 2008; and Karan *et al*., 2012). Globally, rice is one of the most important crops, but it is seriously affected by soil salinity. Rice responds to salt stress by minimizing influx, maintaining efflux, and translocation and compartmentalizing potentially toxic ions such as Na^+ and Cl^- (Tester and Davenport, 2003; Kader *et al*., 2006; Anil *et al.*, 2007).

Soil salinization has become one of the major environmental problems affecting plant growth and productivity worldwide (Allakhverdiev *et al.*, 2000). Salinity affects plants by inducing water deficit in plants even in well watered soils by decreasing the osmotic potential of soil solutes which makes it difficult for roots to take up water from the soil (Sairam *et al*., 2002). Salinity can affect crop by either causing plant death or decreasing the productivity of the crop (Parida *et al.*, 2004).

Salt stress can lead to a considerable decrease in the fresh and dry weights of leaves, stems, tillers, fertile tillers and roots of susceptible genotypes (Chartzoulakis and Klapaki, 2000). In susceptible plants, high sodium and chlorine ions concentration in soil compete with the uptake of essential nutrients, especially K^+, leading to K^+ deficiency. Inducing salinity environment in soil by using NaCl, increases the concentration of Na^+ and Cl^- level in soil and subsequently increases their absorption by susceptible plants. Thus, high concentration of Na^+ and Cl^- in plant affects the absorption of Ca^{2+}, Mg^{2+}, and K^+ by the plants (Khan *et al*., 1999). Ali *et al*. (2014) and Mohammad *et al*. (2015) conducted similar experiments using the hydroponic system and cultural medium respectively. Salinity stress limits the caloric and the nutritional potential of agricultural production

(Keshtehgar *et al*., 2013). Therefore, the objective of this study was to identify parental materials to be used in breeding programs.

2.1 Materials and Methods

The soil used for the experiment was analyzed before and after the experiment to establish the extent of NaCl concentration in soil as result of irrigation. Four soil samples were randomly collected. The samples were about ¼ leg of soil sample at a depth of 0-30 cm, composited and a sub-sample was collected through a quartering system. The final sample was air dried, ground and sieved through 2 mm mesh and then the pH, ECe, Na, K, Ca and Mg contents were determined. Soil pH was determined using the pH reader (Hanna Instrument pH Meter, Model Hi 9032) in a 1: 2.5 soil water ratio. Electrical conductivity was determined by the portable electrical conductivity meter (Hanna Instrument Conductivity Meter, Model Hi 9032) in 1:2.5 soil water ratios (Jackson, 1973). Available potassium and sodium were determined using the ammonium acetate extraction method. Soil Ca and Mg availability were determined using the saturated paste extraction method. The exchangeable sodium percentage (ESP) was determined using the following equation:

ESP = 0.94+1.119SAR

While SAR was determined using the following equation:

$$SAR = \frac{Na^{+}}{\sqrt{Ca^{2+} + Mg^{2+}/2}} \quad \text{.....(1)}$$

Organic carbon (OC) was determined using the Walkley-Black wet digestion method and was expressed in percentage (Allison, 1965).

Eight parent rice varieties with diverse genetic background were evaluated in the screen house to validate their tolerance to soil salinity and select varieties for a breeding program. Six of the parent materials were ordered from IRRI two from the AfricaRice Centre. The

materials from IRRI were recombinant inbred lines developed for salinity tolerance and the materials from AfricaRice Center had no prior information on their responses to salinity (Table 2.1). Suakoko-10 is an improved variety which was released for cultivation in newly developed swamps in Liberia during the wet season by IITA in 1979 at the Liberia's Central Experiment Station at Suakoko (Dana, 1986; Chaudhary *et al,*. 1998). Additionally, NERICA-L-19 is a high yielding adapted lowland rice variety in most of West African countries; and in the lowlands ecosystem, NERICA-L-19 has been reported to tolerate iron toxicity (AfricaRice, 2010), (Table 2.1).

Table 2.1: Genotypes classification and origin

Genotypes	Institution of origin	Classification of genotypes
FL478	IRRI	Salinity
IR65192-4B-10-3	IRRI	Salinity
IRRI 112	IRRI	Salinity
IRRI 113	IRRI	Salinity
IRRI 124	IRRI	Salinity
IRRI 128	IRRI	Salinity
SUAKOKO-10	AfricaRice	-
NERICA-L-19	AfricaRice	-

These materials were tested at different NaCl concentrations at the seedling stage under controlled conditions in a screen house at the Sokoine University of Agriculture (SUA) in 2015. The IRRI standard protocol (Gregorio *et al*., 1997) was used to evaluate salt tolerance of rice genotypes (Table 2.2).

Table 2.2: Modified standard evaluation score (SES) of visual salt injury at seedling stage

Scores	Observation	Tolerance
1	Normal growth on leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant
5	Growth severely retarded; most leaves rolled; only a few are elongating	Moderately tolerant
7	Complete cessation of growth; most leaves dry; some plants dying	Susceptible
9	Almost all plants dead or dying	Highly susceptible

Source: Gregorio *et al.,* 1997, IRRI

The rice genotypes were grown under three concentrations of salinity stress namely 100 mM NaCl, 50 mM NaCl and 0 mM NaCl using a randomized complete block design arranged in factorial with 3 replications. Prior to planting, seeds were germinated in glass petri-dishes and three seedlings transplanted per pot (with dimension, 18 x 19 cm) containing 1.7 kg of homogeneous mixture of planting medium including soil, farm yard manure and rice husk in the ratio of 6:2:10. Seedlings were watered with distilled water for 21 days after transplanting and once every week up to 43 days after transplanting for data collection. Salinity treatments were applied 21 days after transplanting. The control pots were irrigated with distilled water once weekly until 22 days after the salinity-induced treatments were applied.

Plant height was measured from the base of the plant (top of the soil) to the tip of the tallest leaf after 22 days of salinity application. Plants were then removed from pots and the roots of each plant were washed with tap water, rinsed with distilled water, blotted and dried using blotting paper and the roots and shoots were separated. All the plant samples (whole plant) were dried at 70 ^{o}C for 48 hours in an oven to a constant weight and dry weight (g plant^{-1}) was determined. Dried shoots and roots were weighed and ground to powder, where Na, Mg^{2+}, Ca^{2}, K^{+} and K^{+}/Na^{+} were determined using the ashing method at a temperature of 550 ^{o}C to 600 ^{o}C. Sodium (Na^{+}) and K^{+} contents (cmolg^{-1} dry weight) of shoots and roots were determined from a 0.5g dried digested sample using a flame photometer.

The percent relative reduction (RR %) of morphological traits was calculated as:

[RR% = 1–(BMs/BMcl), (Mohammad *et al*., 2014)]…..……...……(2)

Where: BMs=biomas under salinity; BMc=biomassunder control

The Salinity susceptibility index (SSI) was determined as,

$$SSI = \frac{Ys - Yns}{SII(Ys)} \quad \text{……………………………………………………………………………………… (3)}$$

Where Ys and Yns are the mean biomass of a given genotypes in saline and non-saline conditions respectively, and SII was the salinity intensity index, calculated as

$$SII = \frac{1 - X_S}{X_N} \quad \text{……………………………………………………………………………..(4)}$$

Where: X_S and X_N, are the means of all genotypes under salinity stressed and non - stressed environments respectively (Farid and Ali, 2012). The SSI as an index provides an assessment of the relative performance of a given entry with regard to the mean performance of all the genotypes Fischer and Maurer (1978). Salinity tolerance index (STI) was calculated as total dry weight of plant obtained from different salt treatments concentrations compared to total plant dry weight obtained from control.

$$STI = \frac{TDWsx}{TDWsi} X100 \quad \text{………………………………………………..…………………(5)}$$

Where; TDW=total dry weight, Si =control treatment, Sx= salt level treatment (Seydi, 2003).

Data obtained were subjected to analysis of variance using the Genstat Statistical Package 14^{th} edition (Goedhart and Thissen, 2011). Treatment means were compared using Tukey Honestly Significant Test (HSD).

2.2 Results and Discussion

2.2.1 Soil characteristics as determined during the experiment

The chemical and physical properties of the soil were analyzed before and after the experiment and the results are presented in Tables 2.3 and 2.4. The initial electrical conductivity (ECe) of the soil was 0.4 dsm^{-1} and the pH was 7.5. All exchangeable cations recorded low values. Similarly, the sodium adsorption ratio (SAR) and exchangeable

sodium percentage (ESP) of the soil were low (0.9 and 0.1 % respectively) in the initial soil sample. Increased NaCl concentration influenced the properties of the initial samples as well as the SAR and ESP values. The final ECe, SAR, ESP and exchangeable cations were all influenced by increased NaCl concentration at the end of the experiment, but soil pH decreased with increase in NaCl concentration (Table 2.4a). The soil Na^+ increased with increase in NaCl concentration. This was the result of accumulated effect over time (Khajeh-Hosseini *et al.,* 2003; Farhoudi *et al.,* 2007). Potassium, Magnesium and Calcium also increased (Table 2.4a). Soil texture did not change in the final analysis. Farmyard manure is a valuable source of organic matter, nitrogen, phosphorus, potassium, and some micronutrients (Berry *et al.,* 2003) by its addition to soil, it also gradually improves the soil macronutrient status (Rezig *et al*., 2013).

The use of irrigating water containing Na^+ and Cl^- for crop production creates long-term changes on the soil properties that eventually lead to the serious modification in the soil fertility. Alobaidy *et al.* (2010) reported that the use of irrigation water with a high Na^+ concentration causes high accumulation of exchangeable Na^+ around soil particles". Excess sodium on adsorption site is hazardous to plant health which affects the growth and yield of crops. Darwish *et al.* (2009) also stated that "almost every aspect of the plant's physiology and morphology is affected by soil salinity".

Table 2.3a: Initial chemical properties of soil

			Exchangeable cations:cmolkg^{-1}							
Soil sample	**Ece**	**pH**	**Ca^{2+}**	**Mg^{2+}**	**K^+**	**Na^+**	**OC (%)**	**SAR**	**ESP (%)**	**Na^+/K^+ ratio**
Original soil sample	0.4	7.5	11.5	9.5	10.2	9.8	5.1	3.02	4.3	0.9

Table 2.3b: Initial physical properties of soil

	% Particle size distribution			
Soil sample	**Silt**	**Sand**	**Clay**	**Textural class**
Original soil sample	10.9	54.2	34.8	Sand clay loam

Table 2.4a: Final chemical properties of soil

	Exchangeable cations:cmolkg^{-1}									
Treatments	**Ece**	**pH**	**Ca^{2+}**	**Mg^{2+}**	**K^{+}**	**Na^{+}**	**OC (%)**	**SAR**	**ESP (%)**	**Na^{+}/K^{+} ratio**
100mM NaCl	5.7	7.1	21.5	11.2	12.48	31.1	3.3	7.7	9.6	2.5
50mM NaCl	3.6	7.1	19.3	10.7	12.63	24.8	3.2	6.4	8.1	1.9
0mM NaCl	0.7	7.1	21.9	10.8	12.69	10.9	3.4	2.7	4	0.9

Table 2.4b: Final physical properties of soil

	% Particle size distribution			
Treatments	**Silt**	**Sand**	**Clay**	**Textural Class**
100mM NaCl	16.9	66.9	16.1	Sandy clay loam
50mM NaCl	12.7	67.9	19.3	Sandy clay loam
0mM NaCl	14.7	69.2	16.1	Sandy clay loam

2.3 Ranking of Rice Genotypes on the Basis Of Salt Injury at the Seedling Stage

The salinity tolerance scores calculated for the eight rice genotypes are shown in Fig. 2.1. All the eight rice genotypes grew healthily in the non-salinized condition. In salinized condition, the genotypes showed nearly normal growth at lower NaCl concentration (50 mM NaCl) from score 3 to 4.5. However, at higher NaCl concentration (100 mM NaCl) there was a wide range of phenotypic variations from score 3 (Nearly normal growth) to 7 (Complete cessation of growth) as shown in Fig. 2.1. The most salinity tolerant genotypes based on the SES scores were FL478, IRRI128, IR65192-4B-10-3 and IRRI 112; while the salinity susceptible genotypes based on SES scores were Suakoko-10, NERICA-L19, IRRI 124 and IRRI 113. Islam *et al.* (2007) made a similar observation on a wide variation in phenotypes from tolerant (score 3) to highly susceptible (score 9) rice lines using modified

SES of IRRI standard protocol. The susceptible genotypes were more stressed under saline condition than tolerant genotypes.

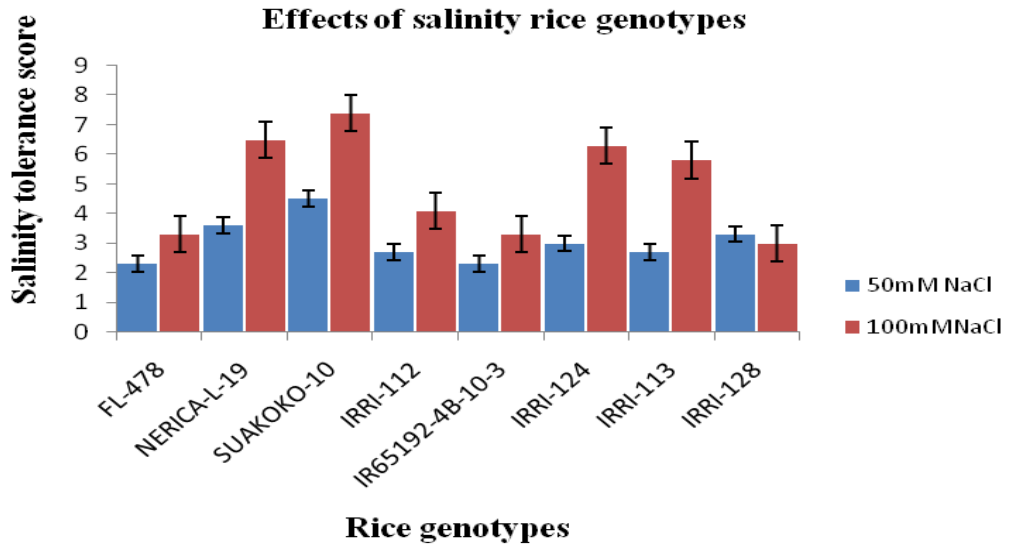

Figure 2.1: Modified standard evaluation score (SES) of visual salt injury at seedling stage

Note: 1= normal growth (highly tolerant) and 9 = all palnts completely dead (highly susceptible).

2.4 Relationship among Various Morpho-physiological Traits of Rice Genotypes

The salinity tolerance scores had significant negative correlation with the entire morpho-physiological traits investigated which includes plant height, SDW, RDW and root shoot ratio) as shown in Table 2.5. The traits shoot dry weight (SDW) and root dry weight (RDW), plant height and root/shoot ratio showed highly significant positive correlation with other traits except SES score, which showed negative correlation with all other traits investigated. The inverse correlation between scores and the other traits might have been the result of the inhibiting effects of salinity on roots and shoots elongations which probably led to the reduction in water uptake by the plant and subsequently reduces plant height and dry matter accumulation (Werner and Finkelstein, 1995). Marcum *et al.* (2005)

reported that the adverse effects of salinity stress on two grasses studied were more obvious on shoot than the root growth. Jamil and Rha (2007) observed a decrease in shoot length, root lengths and dry weights with increasing salt stress.

Table 2.5: **The Pearson's correlation coefficients for morpho- physiological traits of rice genotypes**

	Plant height	**RDW**	**SDW**	**root /shoot ratio**
Plant height				
RDW	0.89**			
SDW	0.56**	0.81**		
Root/Shoot Ratio	0.98**	0.88**	0.54**	
SES scores	-0.81**	-0.82**	-0.64**	-0.81**

Note: RDW— roots dry weights; SDW— shoots dry weights
** Correlation was significant at the $p < 0.01$.

2.5 Ranking of Rice Genotypes Based Salinity Indices

Rice genotypes were ranked on the basis of their tolerance, susceptibility and the percent reduction in morpho-physiological traits observed under salt stress. The relationships of the percent relative reduction in root dry weights (RDW), shoot dry weight (SDW), root-shoot ratio and plant height under saline condition (100 mM NaCl) to the salinity index (SI) are shown in Fig. 2.2 (a-d). A strong relationship was observed between the mean root dry weight and SSI as shown in Fig. 2.2 (a); the shoot dry weight and SSI in Fig. 2.2 (b) also showed a strong relationship. There were also strong relationships between root-shoot ratio and SSI as well as mean plant height and SSI (Fig. 2.2 c and d).

The co-efficient of determination shows that 88.7 % of variation in relative root dry weight can be attributed to salinity index and 85.3 % of variation relative shoot dry weight can also be attributed to salinity index. In the case of relative root- shoot ratio and relative plant height, shows that 69.9% and 68.6% of the variation in root-shoot ratio and plant

height can be explained by SSI respectively. In this study, the differences among the genotypes with increase in salinity level were much obvious as indicated by the reduction in morpho-physiological traits and the results of the various salinity indices (Tables 2.5 and 2.6, and Fig. 2.2). Reduction in dry matter accumulation is directly proportional to increased salinity levels (Tsuda and Hirai, 2007). The results of this study are similar to those reported by Majkowska *et al.* (2008). These results are also in line with the report of Masood *et al.* (2005) who suggested that "salt stress reduced the biomass of rice".

On the basis of tolerance and susceptibility indices (Tables 2.6 and 2.7), four genotypes were selected as tolerant (FL-478, IRRI-128, IR65192-4B-10-3 and IRRI-112), while the remaining four were considered susceptible to salinity stress (NERICA-L-19, Suakoko-10, IRRI113 and IRRI 124). The roots of plants were in direct contact with the growth media containing toxic salts that might have retarded the root development, shoot elongation and dry matter accumulation. The results of this study agree with Syvertsen *et al.* (2000) and Kasukabe *et al.* (2006) who reported that under salinity condition, CO_2 assimilation of plant which is a major energy source for growth and development becomes decreased. In addition, Vasquez *et al.* (2006) reported that reduction in root length was caused by the decrease in biomass which was observed under salt stress. There was a decrease in root length and root dry weight with increase in salinity in the present study.

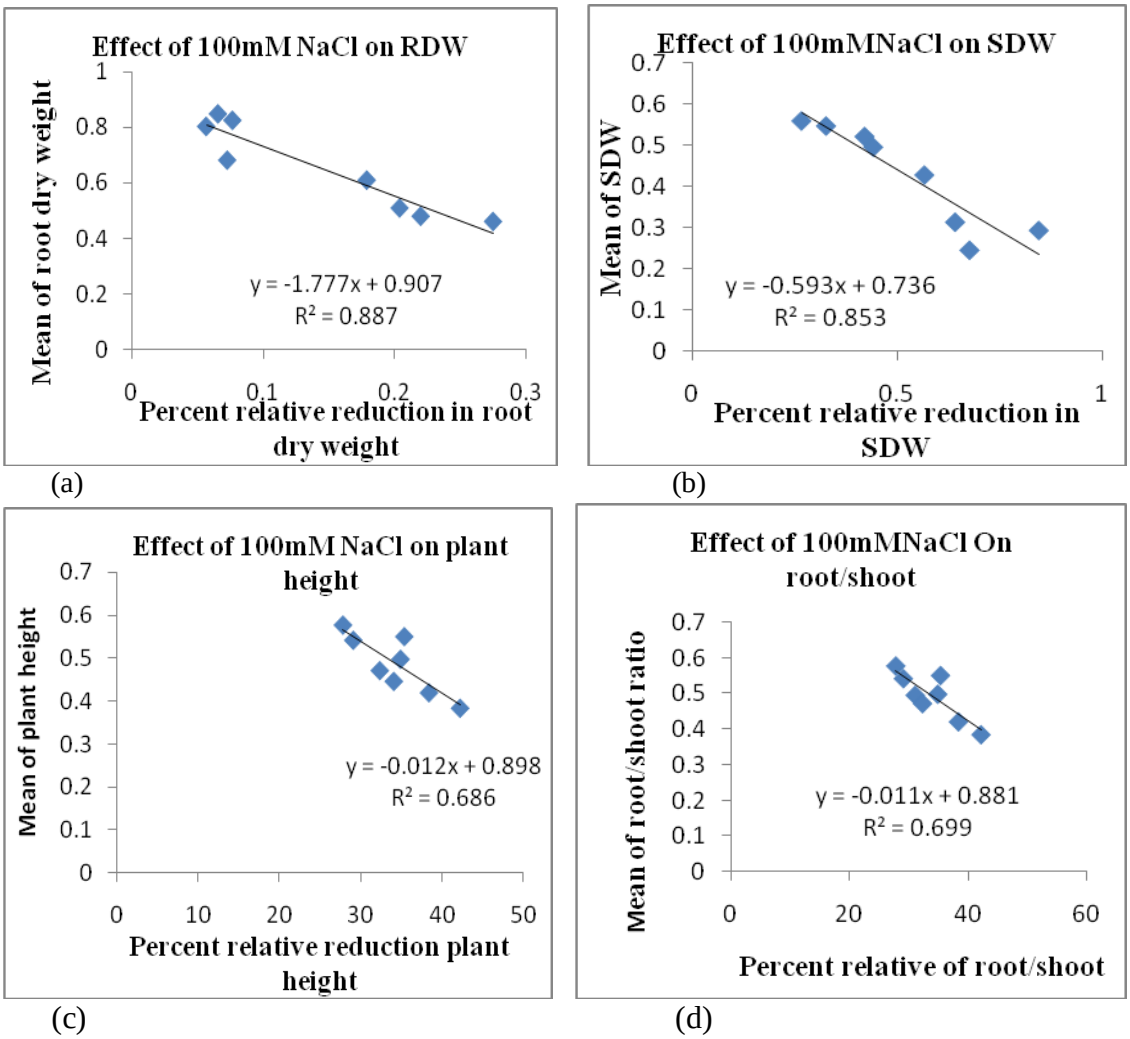

Figure 2.2: (a-d). Effects of salinity on growth and plant characteristics

Table 2.6: Salinity Tolerance Index at 100mMNaCl concentration

Genotypes	plant height	SDW	RDW	R/S Ratio	Mean of tolerance index value	Tolerance
FL-478	61.55	0.65	0.46	0.61	15.82	T
IRRI-128	57.93	0.66	0.33	0.57	14.87	T
IR65192-4B-10-3	55.29	0.75	0.37	0.50	14.23	T
IRRI-112	52.78	0.68	0.36	0.52	13.59	MT
IRRI-113	50.13	0.50	0.25	0.50	12.84	MS
IRRI-124	45.70	0.45	0.21	0.45	11.70	S
NERICA-l-19	44.85	0.59	0.26	0.44	11.53	S
SUAKOKO-10	42.16	0.44	0.26	0.42	10.82	HS

Note: RDW=Root dry weight; SDW = Shoot dry weight; R/S = Root-Shoot ratio; higher means indicate tolerance and lower means indicate susceptibility. Mean of tolerance index value for genotype was calculated as the average of all indices calculated for the morphological traits of each rice genotype.

Table 2.7: Salinity susceptibility index for physiological parameters (SSI)

Genotypes	Plant height	RDW	SDW	Root/shoot ratio	Mean	Tolerance
FL-478	0.79	0.79	0.80	0.78	0.79	**T**
IR65192-4B-10-3	0.92	0.92	0.57	1.00	0.85	**T**
IRRI-112	0.97	0.93	0.73	0.96	0.90	**T**
IRRI-113	1.02	1.11	1.15	1.01	1.07	**S**
IRRI-124	1.11	1.17	1.27	1.10	1.16	**S**
IRRI-128	0.86	0.98	0.79	0.85	0.87	**T**
NERICA-L-19	1.13	1.09	0.95	1.12	1.07	**S**
SUAKOKO-10	1.18	1.08	1.30	1.17	1.18	**S**

Note: The higher the mean the susceptible the genotype at 100 mMNaCl; genotypes which scored below 1.0 were considered tolerant and those which scored above 1.0 were considered susceptible.

2.6 Conclusions and Recommendations

2.6.1 Conclusions

The results of this study show that there were variations in the performance of rice genotypes at the different levels of NaCl concentration used during the study. Salinity stress affected all Morpho-physiological traits of rice genotypes whereby three of the rice genotypes from IRRI (FL 478, IRRI 128 and IR6592-4B-10-3) were salinity tolerant and one (IRRI 112) was moderately tolerant to salinity stress. At the same time, NERICA-L-19 and SUAKOKO-10 from AfricaRice and two of the genotypes from IRRI namely, IRRI 113 and IRRI124) were susceptible to salinity stress respectively. With these results, therefore, NERICA-L-19 and SUAKOKO-10 were selected as susceptible parents and two of the rice genotypes from IRRI (FL-478 and IR65192-4b-10-3) were selected as tolerant parents to be used in a breeding program.

2.6.2 Recommendations

1. The selected rice genotypes need to be further assessed by using molecular means to confirm the presence of the gene for tolerance (*saltol*).

2. The improvement of the selected susceptible cultivars should be done by the aid of a marker assisted selection in order to select the best genotypes.

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CHAPTER THREE

EVALUATION OF THE RESPONSES OF EIGHT RICE (*Oryza sativa*, L.) GENOTYPES TO VARIOUS CONCENTRATIONS OF *NaCl* IN A CONTROLLED ENVIRONMENT

Abstract

Salinity is an ever increasing problem that reduces yield in many rice fields around the world. Developing a salt tolerant genotype is one of the solutions to the problem of salinity. This experiment was carried out in the Department of Crop Science and Horticulture at SUA to assess the salinity tolerance of 8 rice (*Oryza sativa L.*) genotypes at the seedling stage. Ion accumulation in plants and dry matter content along with Molecular markers were used to evaluate the tolerance of each rice genotype. The genotypes were IRRI 112**,** IRRI 124, FL 478, IRRI 113, IR65912-4B-10-3, IRRI 128, NERICA-L-19 and SUAKOKO-10. In this experiment, the genotypes were exposed to three salinity levels in a randomized complete block design arranged in factorial with three replications. The salinity levels were 100 mM NaCl, 50 mM NaCl and 0 mM NaCl. A homogenous mixture of sand, farm yard manure and rice husk (ratio of 6:2:10 respectively) as the planting medium for all rice genotypes. The soil texture was sandy clay-loam. The growth of the genotypes, ion accumulation and dry matter contents were significantly ($p \leq 0.05$) affected by increase in NaCl concentration. Two *Saltol* SSR markers (RM7075 and RM562) were used to determine the presence of salinity tolerance (*saltol*) gene in rice genotypes. Based on the SSR markers, ion accumulation and dry weight of plants, two genotypes (IR65192-4B-10-3, and IRRI112) along with FL478 were selected as salt tolerant while two (IRRI-113 and IRRI-128) were moderately tolerant, and three (NERICA-19, SUAKOKO-10 and IRRI-124) were the most susceptible genotypes. Therefore, two susceptible and two tolerant parents were selected.

3.0 INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important crops used as a source of food in the world, and it accounts for more than 21% of the calorific intakes of the world's population (Ma *et al.*, 2007 and Melissa *et al*., 2009). Most of the people in rice producing areas of Asia, Africa and South America still depend on rice for their daily caloric intake (Surridge, 2004; Joseph *et al.,* 2010). Rice has being characterized as a salt sensitive crop, but there is variation in the extent of its sensitivity. It is known that rice is tolerant to salinity stress during germination and active tillering, whereas it displays more sensitivity during early vegetative and reproductive stages (Lutts *et al*., 1995; Zhu *et al*., 2001).

Screening of rice genotypes for salt tolerance at seedling stage is readily acceptable as it is based on a simple criterion of selection, and it also provides rapid screening which is difficult at the vegetative and reproductive stages (Gregorio *et al*., 1997). Screening of rice genotypes using the conventional method is very difficult because of the large effects of the environment and low narrow sense heritability of salt tolerance (Gregorio, 1997), but the introduction of DNA markers seems to be the best technique for efficient evaluation and selection of plant material (Bhowmik *et al*., 2009). Recent progress and technical advances in DNA marker technology permit reduction of time and accuracy of the breeding program where pronounced effects of environment lead to poor selection efficiency (Sultana *et al*., 2009).

When NaCl is used for screening for salt tolerance, the sodium (Na^+) and chloride ions (Cl^-) dissociate from NaCl salts and contaminate the soil medium, because these ions are well known as the toxic ions which damage plant cells in both ionic and osmotic effects. Plant growth and development are directly restrained by theses ions which lead to growth reduction and plant death (Lauchli and Grattan, 2007). Sodium and chlorine ions have the

ability to restrict the uptake of other essential plant nutrients such as potassium, magnesium and calcium.

Potassium is an essential nutrient that plays a very important role in growth and development of plants. It is actively involved in different cellular and physiological processes including osmotic adjustment, stomata regulation, and cation-anion balance (Marschner, 2012). Regulation of K^+/Na^+ homoeostasis within cells is an important indicator of salt tolerance in plants (Zhu, 2003; Siddiqui *et al*., 2008, 2009). Calcium is another essential element that helps in maintaining structural and functional integrity of membranes, stabilization of cell wall and regulation of ion homeostasis (Arshi *et al.,* 2010; Morgan *et al*., 2014). These two elements (Potassium and calcium) seem to be readily displaced from binding sites by sodium and chloride ions, therefore affecting plant growth. Maintaining sufficient concentrations of K and Ca in saline soil helps plants in overcoming specific ion toxicities, particularly in susceptible plants, which are more prone to salt damage (Grattan and Grieve, 1999). Also, K^+ and Mg^{2+} have been reported to play an important role in enzyme activation (Barker and Pilbeam, 2007). The role of Mg^{2+} under salt stress has been variable. For instance, Mg^{2+} increases in rice callus (Ahmad *et al*., 2009) and decreases in soybean callus (Liu and Staden, 2001).

In order to be able to adequately address the problem of soil salinization, the genetic variability of the available genotype needs to be exploited for the identification of tolerant genotype that may sustain a reasonable yield on salt affected soil (Ashraf *et al.,* 2006). The microsatellite marker or Simple Sequence Repeat (SSR) has been proved to be ideal for making genetic maps (Islam, 2004; Niones, 2004) and assisting in genotype selection (Bhuiyan, 2005). The SSR marker analysis is a promising mean of identifying genes loci for salt tolerance that can be helpful for plant breeders in the development of

new cultivars. Therefore the objective of this study was to assess the salinity tolerance of 8 rice (*Oryza sativa L.*) genotypes at the seedling stage using molecular markers, physiological traits and ion accumulation.

3.1 Materials and Methods

Eight rice genotypes (six from IRRI and two from AfricaRice) were tested at different levels of NaCl concentrations at the seedling stage in the screen house at the Sokoine University of Agriculture in 2015 (Table 4.1). IRRI standard protocol (Gregorio *et al.*, 1997) was used to assess the tolerance of the rice genotypes to salinity conditions Table 2.2. Two SSR markers, RM7075 and RM562, which have also been used for salinity tolerance screening, were used to assess the tolerance of the rice genotypes.

The rice genotypes were grown under three conditions of salinity stress using a randomized complete block design with factorial arrangement. The concentrations of NaCl in the irrigation water used were 100 mM NaCl, 50 mM NaCl and 0 mM NaCl respectively. Prior to planting of the genotypes, seeds were germinated and three seedlings sown per pot containing 1.7 kg of homogeneous mixture of planting medium including soil, farm yard manure and rice husk in the ratio 6:2:10. The seedlings were well watered with distilled water for a period of 21 days after sowing and then the salinity treatments were applied. The control pots were irrigated with distilled water up to the end of data collection which was done 43 days.

Plants were removed from pots 22 days after the application of salinity stress; each plant roots were washed with tap water and rinsed with distilled water. The roots were then blotted dry using blotting paper and then were separated from the shoot using scissors. Data were collected on roots and shoots dry weight. All the plant samples were dried at

70 ^{o}C for 48 hours in an oven to a constant weight and dry weight (g plant^{-1}) was determined. After drying, shoots and roots were weighed on an electronic beam balance and then ground to powder. The Na^{+}, Ca^{2+}, Mg^{2+}, K^{+} and K^{+}/Na^{+} were determined. Sodium (Na^{+}) Ca^{2+} Mg^{2+} and K^{+} contents (cmol g^{-1} dry weight) of shoot and root were determined from a 0.5g dried digested sample using a flame photometer.

3.2 Statistical Analysis

Data collected on dry weight and ion accumulation were subjected to two-way analysis of variance for a factorial arrangement in randomized complete block design using the genstat statistical package 14th edition (Goedhart and Thissen, 2011). Treatment (induced salinity) means were compared using Tukey honestly significant test (HSD).

3.3 DNA Extraction and Amplification of Microsatellite Markers

Genomic DNA was isolated from leaves of two-week old plants based on the DNA isolation protocol of Egnin *et al*. (1998). Two selected DNA primers [RM7075 (Bhowmik *et al*., 2009); and RM562 (Rajendran *et al.*, 2012)] were used for this study. Amplified microsatellite loci were analyzed for polymorphism using 1.5 % Agarose Gel. Electrophoresis and the result revealed that the two primers (RM7075 and RM562) detected clear polymorphism among the rice genotypes analyzed.

Each PCR reaction was carried out with 21.0 µl reaction mixtures containing DNA premix, 20 µl of primer master mix and 1.0 µl of each template DNA sample. PCR profile was maintained as initial denaturation at 94 ^{o}C for 3 minutes, followed by 33 cycles of denaturation at 94 ^{o}C for 30 seconds, annealing at 55-62 ^{o}C for 30 seconds, and polymerization at 72 ^{o}C for 1 minute; and final extension by 5 minutes at 72 ^{o}C. A 100 bp DNA ladder was used to determine the band location of the DNA sample.

3.4 Results and Discussion

3.4.1 Screening of salt tolerance by SSR markers

The banding pattern of the genotypes was scored by comparing the banding pattern of FL-478. The genotype that showed similar banding pattern like FL 478, were considered as tolerant and those with different banding pattern were considered susceptible. The selected recurrent parents (NERICA-L-19 and SUAKOKO-10) showed banding patterns different from that of FL-478 and two of the genotypes from IRRI showed similar banding patterns like FL-478 and were therefore tolerant. Two of the tolerant genotypes IR65912-4B-10-3 and FL478 which also had lower percent reduction in dry weight and lower Na^+ accumulation under saline conditions were selected as the donor parents to be used in the breeding program.

The RM7075 marker identified five tolerant genotypes namely IRR-112, IRRI-113, IRRI-128, IR65192-4B-10-3, FL-478, while three genotypes, IRRI-124, NERICA-L-19 and SUAKOKO-10 were found to be susceptible (Fig. 3.1). These results were followed by a breeding scheme as shown in (Fig. 5.1). A field survey in chapter four aided in identifying an ideal site for the field evaluation of the new genotypes developed in chapter five.

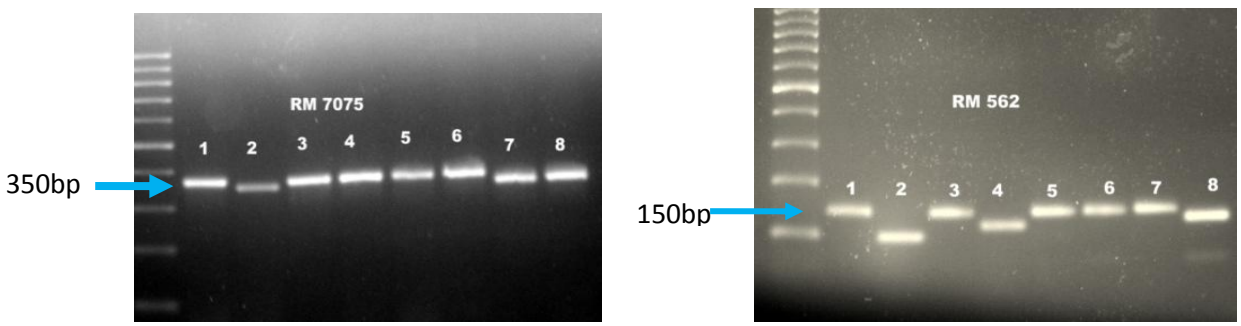

Figure 3.1: **Gel images of the foreground selection of donor and recurrent parents using salt tolerance markers RM 7075(A), and RM 562 (B).** 1. **IRRI 112**; 2. **IRRI 124**; 3. **FL 478**; 4. **IRRI 113**; 5**. IR65912-4B-10-3**; 6. **IRRI 128**; 7. **NERICA-L-19**; 8. **SUAKOKO-10**.

Note: The bands of interest are indicated by the arrows.

The marker RM 562 identified five tolerant genotypes namely IRRI 112, IR65192-4B-10-3, FL478, IRRI 128 and NERICA-L-19, and three susceptible genotypes; SUAKOKO-10, IRRI 113 and IRRI 124 (Fig. 3.1). The markers showed a clear relationship with the salt tolerance alleles in the rice genotypes. This shows that molecular markers are capable of identifying alleles that are associated with key phenotypic traits (Xu *et al*., 2004). For example, Nguyen *et al*. (2001) found that microsatellite marker was associated with NaCl tolerant alleles at seedling stage in a crop population and similar results were also reported by Lang *et al*. (2000).

3.4.2 Evaluating the effects of salinity on dry weight of rice genotypes

Table 3.1 presents the mean square values of dry matter weight of 8 rice genotypes. Significant differences were observed among genotypes after the application of salinity treatments. NaCl concentration effects were significant at ($p<0.01$). The variety x NaCl concentration interaction was highly significant ($p < 0.05$) for root – shoot ratio. In terms of the root dry weight and the shoot dry weight, there were no significant differences. There were decrease in shoot and root dry weights with increase in NaCl concentration as shown in Appendix 15. The decrease of shoot and root dry weight might have been due to a reduction in turgor which resulted in lower water potential in plant or a disturbance in mineral supply to root and shoot. These results are similar to the findings of Alam *et al*. (2004); and Mahmood *et al*. (2009).

Table 3.1: Mean square (ANOVA) of rice genotypes under salt stress

Sources of variation	df	SDW	Root/Shoot Ratio	RDW
Salinity levels	2	0.857**	746**	0.874**
Variety	7	0.220**	189.3**	0.044**
Salinity levels x Variety	14	0.013^{ns}	28.22**	0.003^{ns}

****** = significant at (1%)
ns = no significant difference
Note: SDW-shoot dry weight; RDW-root dry weight; plant height (cm) and dry weight (grams).

3.6 Ion Accumulation in Rice Genotypes

The mean square effects of NaCl on the ion accumulation in the 8 rice genotypes are presented in Table 3.2 and Appendix 14. There were significant ($P < 0.01$) differences observed in ion accumulation in all the genotypes after the application of NaCl. Sodium chloride concentration had significant ($P < 0.01$) effects on ion accumulation. The variety x NaCl concentrations interactions were highly significant ($p < 0.05$) for accumulated ions in all the genotypes. One of the mechanisms of salt tolerant plant under high salinity conditions is to accrue and partition Na^+ in the older leaves, but sensitive rice genotypes are not able to do this successfully (Munns and Tester, 2008). As a result of high salt concentration, the dry weights of the susceptible genotypes were severely affected in the saline environments. The effects on dry weights of rice genotypes were probably due to high salinity caused by increased sodium concentration and subsequent increase in the absorption of sodium in plants. The current study observed a high potassium-sodium ratio in the shoots of tolerant rice genotypes. This might have been due to the ability of the tolerant rice genotypes to absorb more potassium than sodium in shoots or compartmentalize the sodium ion in the leaves as opposed to the susceptible genotypes. Potassium is considered an essential element in plant growth under saline conditions, because of its role in osmo-regulation and stress mitigation in saline environments (Cakmak, 2010).

Table 3.2: **Means square values of NaCl effect on nutrients uptake in rice genotypes**

Sources of variation	df	SK^+	S Na^+	$SK^+/S\ Na^+$	RCa^{2+}	RK^+	RMg^{2+}	RNa^+	SCa^{2+}	SMg^{2+}
Salinity levels	2	0.06**	1.7**	0.96**	0.36**	1.5**	0.001**	1.1**	0.03**	0.04**
Variety	7	0.20**	0.07**	0.09**	0.04**	0.12**	0.01**	0.04**	0.02**	0.04**
Salinity level x Variety	1 4	0.16**	0.04**	0.07**	0.11**	0.05**	0.004**	0.04**	0.02**	0.01**

** = significant at (1%)

Note: SK-patassium on shoot; SNa^+-sodium in shoot; SCa^{2+}-calcium in shoot; SMg^{2+}-magnesium in shoot; RCa^{2+}-calcium in root; RK^+-Potassium in root; RMg^{2+}-magnesium in root; RNa^+-sodium in root; SK^+/SNa^+-sodium potassium ratio in shoot.

3.7 Ranking of Rice Genotypes Based on Salinity Tolerance

Results show that the differences in performance among the genotypes at high salinity level were much obvious (Tables 3.3 and 3.4). High salinity level adversely influenced the performances of the rice genotypes. It was clear that increasing salinity level from 0mM NaCl to 100 mM NaCl resulted in significant reductions in plant dry weight (root dry weight, shoot dry weight and root and shoot ratio). However, the maximum reduction was obtained at 100 mM, the highest NaCl concentration applied. Reduction in dry matter accumulation was direct by proportion to increased salinity levels. The result of this study agrees with results reported by Majkowska *et al.* (2008). High salinity might have inhibited the root and shoot elongation due to the slowing down of the water absorption by the plant as reported by (Jeannette *et al.,* 2002). Sagi *et al*. (1997) observed that salinity stress adversely affected shoot more than the root growth and Jamil and Rha (2007) reported that the shoot length, root lengths and the dry matter weight of radish plants were decreased with increase in salinity stress. This result is also in agreement with previous reports by Masood *et al.* (2005) which suggested that salt stress reduced the biomass of rice. Essa (2002) reported that shoot dry weight was more sensitive to salinity than root dry weight. The effects of NaCl on ion accumulation in rice root and shoot under saline condition are presented in Appendixes 11 & 12.

Table 3.3: Percent reduction in physiological traits of rice genotypes

Genotypes	Plant height		RDW		SDW		Root/shoot ratio			
	50mM NaCl	100mM NaCl	50Mm Na	100mM NaCl	50mM NaCl	100mM NaCl	50mM NaCl	100mM NaCl	Mean of tralts	Tolerance
FL-478	31.2	38.4	42.9	53.6	25.1	41	31.2	38.4	42.9	T
IR65192-4B-10103	38.1	44.7	51.4	62.5	20.8	24.5	38.1	49.5	45.3	T
IRR-112	40	47.2	42.2	63.2	4.1	31.3	40	47.2	47.2	T
IRRI-113	39.6	49.8	49.7	74.9	16.8	49.6	39.6	49.8	56.0	MT
IRRI-124	47.7	54.2	60.6	79	25.7	54.7	47.7	54.2	60.5	S
IRRI-128	39.2	42.1	47.5	66.2	30.3	53.2	39.2	42.1	50.9	MT
NERICAL-L-19	40.7	55.2	47.4	73.4	11.2	40.8	40.7	55.1	56.1	S
SU AKOKO-10	46	57.8	58.9	73.1	23.1	55.9	46.6	57.8	61.2	S

Note: RDW = root dry weight; SDW = shoot dry weight; plant height (cm) and dry weight (grams).

Table 3.4: Effects of NaCl concentrations on dry matter weight of eight rice genotypes

	RDW			SDW			Root/Shoot Ratio		
Genotypes	**0mM NaCl**	**50 mM NaCl**	**100mM NaCl**	**0mM NaCl**	**50 mM NaCl**	**100mM NaCl**	**0mM NaCl**	**50 mM NaCl**	**100mM NaCl**
FL478	0.65J	0.37d-h	0.30a-f	1.19d	0.90b-d	0.71a-c	68.60o	47.20k	42.2j
IR65192-4B-10-3	0.56h-j	0.27a-f	0.21a-e	0.90b-d	0.71a-d	0.68a-d	61.60l	38.10h	31.10c
IRRI-112	0.57h-j	0.33c-g	0.21a-e	0.94cd	0.90b-d	0.64a-d	61.30l	36.70g	32.30d
IRRI-113	0.61ij	0.31a-f	0.15a-c	0.88b-d	0.73a-d	0.44a-c	69.60p	42.00j	34.90f
IRRI-124	0.45f-j	0.18a-f	0.09a	0.72a-d	0.54a-c	0.33ab	63.60m	33.20e	29.10b
IRRI-128	0.52g-j	0.27a-f	0.18a-d	0.99cd	0.69a-d	0.46a-c	66.30n	40.20i	38.40h
NERICA-L-19	0.61ij	0.32b-g	0.16a-d	0.78a-d	0.69a-d	0.46a-c	78.90q	46.70k	35.40f
SUAKOKO-10	0.39e-i	0.16a-d	0.11ab	0.61a-c	0.47a-c	0.27a	66.00n	35.20a	27.80f
Salinity level (s.e.)		0.02			0.05			0.06	
Genotpe (s.e.d)		0.03			0.09			0.1	
Salinity x Genotype (s.e.d)		0.06			0.15			0.17	

Tukey ($p \leq 0.05$);
Note: RDW = root dry weight; SDW = shoot dry weight; plant height (cm) and dry weight (grams).

3.8 Conclusions and Recommendations

3.8.1 Conclusion

Salt stress induced changes in ion accumulation in rice and dry matter at the seedling stage. The variations in ion accumulation and dry matter weight of rice genotypes, clearly distinguished the tolerant from susceptible genotypes. The maximum variation was realized when NaCl concentration was increased to 100 mM.

Additionally, there were differences among the genotypes in terms of sodium and potassium absorption, and the molecular markers used showed clear polymorphism among the genotypes. The SSR markers used in this study were able to clearly distinguish tolerant genotypes from susceptible. Further, on the basis of SSR marker analysis, the K^+/Na^+ ratio in the shoots and the interaction among the genotypes at the different levels of salinity, NERICA-L-19 and SUAKOKO-10 were selected as the recurrent parents to be improved for salt tolerance.

3.8.2 Recommendations

1. The molecular markers used for this study should be used to evaluate the subsequent breeding materials, because the markers are polymorphic for salt tolerance.
2. The selected rice genotypes should be further used to develop new rice lines for salt tolerance.

3.9 References

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CHAPTER FOUR

FARMERS' PERCEPTIONS ON SALINITY PROBLEMS IN IRRIGATED FIELDS IN KILOSA DISTRICT

Abstract

Soil salinity contributes to one of the most serious ecological and environmental problems in most of the irrigation schemes in Tanzania, including Kilosa District, where the small-scale farmers cultivate rice for their livelihood. Plant breeders can help these farmers find a lasting solution to the problem. In this regards, understanding the farmers' perceptions of the problem of salinity and its effects on crop productivity is important in the development of the best cultivars that address the needs of the farmers. Additionally, it helps extension officers to promote the best cultivar and water conservation practices or decide on the best measures that safe guide the farmer production within a given location. A study was conducted in Chanzuru and Ilonga villages in Kilosa District in 2016 to determine farmers' perceptions on soil salinity problems in the villages. A socio-economic survey was carried out on 60 respondents within the two villages. Data were collected using the semi-structured questionnaires, and analyzed the Statistical Package for Social Science (SPSS) software. The results showed that farmers perceived salinity more on the basis of location than they did on the basis of socio-demographics. The main causes of soil salinity as perceived by farmers were poor quality of irrigation water and poor drainage systems. Some socioeconomic and demographic characteristics that significantly influenced the farmers' perceptions were sex and household size. The perceptions of farmers in the study area varied significantly from village to village, with their socio-demographic determinants. Farmers employed the strategy of crop diversification and increase in farm size in response to the problem of salinity occurring in their fields. Farmer perception on

salinity should therefore be used as entry point by stakeholders to develop intervention programs that help to solve the problems occurring in the farmers' fields.

4.0 INTRODUCTION

Salinization of Soil is one of the serious environmental factors that limit crops productivity worldwide. Because of this adverse factor, most of the agricultural crops are now considered salt-sensitive due to the high concentration of salts in the soil (Munns and Tester, 2008). Salinity is a serious problem and, it affects about 800 million ha of arable lands worldwide. Approximately 33 % of irrigated areas (about 74.25 million ha) are currently considered threatened by soil salinization at various degrees (Kumar and Shrivastava, 2015). It has been projected that by the year 2050, there will be more than 50 % of the farm land worldwide, which would become salt-affected (Jamil *et al.*, 2011).

Rice has shown to be the most sensitive cereal crop while barley the most tolerant cereal crop (Munns and Tester, 2008; and Karan *et al*., 2012). Salinity has serious effects on percentage of filled spikelets, grain weight, and can also hinder the absorption of essential nutrients in rice (Clermont-Dauphin *et al*., 2010).

Most irrigation schemes, which are especially within the arid and semiarid environments, are already experiencing increasing levels of salt-affected soil, due to the mismanagement of the soils, the use of poor quality irrigation water, poor drainage system, poorly designed and managed irrigation infrastructures, excessive use of irrigation water and climate change (Kashenge-Killenga, 2010).

Irrigation water containing Na^+ and Cl^- has adverse effects on the physical properties of soil, because it is mostly connected with the buildup of sodium ion on the soil exchange complex. The quality of water used for irrigation can impact the volatility of the soil aggregates which eventually leads to the dispersion of the clay particles and the clogging of soil pores. When underground water moves to the soil surface by evapo-transpiration,

the soluble salts condense on the soil particles on the surface and form a white crust (Plate 4.1). Irrigation practices affect the land by increasing the rates of leakage and groundwater recharge which results into the rise in water table. The water tables when it rises it brings salts into the plant root zone which affects both plant growth and soil structure; then the salts remain behind on the soil surface after the water has been taken up by plants or lost due to evaporation (Podmore, 2009). This process is what leads to the formation of saline soil in the farmers' fields and consequently affects the farm lands and the productivity of crops, such as rice.

Therefore, understanding the perception of farmers on the causes and effects of soil salinity makes room for stakeholders to decide on the best measures that safe guide the farmers' production within a given location. Kruger (2006); and Wickham *et al.* (2006) reported that farmers' perceptions could be a good entry point for any intervention on the environmental conservation either by changing their perception through practical demonstrations or by building on what they already know.

Plate 4.1: Saline soil in the study area (Chanzuru, Kilosa)

Farmers' perceptions on salinity are defined by their understanding of factors influencing salinity and the consequences for crop production. This perception is also defined by the way the farmers judge the severity of the soil salinity in the fulfillment of their farming objectives in the light of the possibilities and constraints of their farming system (Kielen, 1996). Farmers' perceptions on salinity result from their knowledge of soil salinity, their farming experiences and salinity constraints. On the basis of this perception, the farmer defines a strategy to cope with salinity (Kielen, 1996).

There is similarity between farmer's perceptions and farmers' response to a particular stress in the environment. The farmers' response to a particular stress depends in most cases on socio-demographic factors. The number of response strategies depends on how immediate or severe the problem is perceived to be by the farmer (Meze-Hausken, 2000).

In short, the perception of a farmer largely depends on the amount of information available to them and the extent to which they are able to correctly interpret the information they have acquired in order to respond to a given situation (Nelson and Quick, 1997).

For instance, most farmers' who perceive salinity as a problem may employ local adaptation options in response to salinity symptoms such as, planting of tolerant varieties, crop diversification and water management. Furthermore, farmers' perception of stress condition and weather variability might influence their investment decision and consequently, the farmer's production and food security (Mamba *et al.*, 2015). Therefore, the objectives of the study were:

i. To determine farmers' perception on salinity problems affecting rice production.
ii. To examine farmers perception on factors contributing to salinity.
iii. To assess the strategies employed by farmers to cope with salinity problem in their areas.

4.1 Materials and Methods

4.1.1 Location of study

The study was conducted in two villages (Chanzuru and Ilonga) in Kilosa District, Morogoro Region (Fig. 4.1). The location was selected based on the reported potential of the District with respect to rice production and the presence of salt affected soils in the various irrigation schemes within the District. Morogoro region contains six administrative districts namely Morogoro Urban, Morogoro Rural, Mvomero, Kilosa, Kilombero and Ulanga. Kilosa District is located about 300 km inland from the coast of Dar es Salaam (Benjaminsen *et al.*, 2009). Kilosa District is 14 245 km^2 in size making up about 20 per cent of the region (KDC, 2010); and has a population of 438 175 people (TNBS, 2013).

The study area has a semi-humid climate with an average rainfall of 800 mm annually. The early rain starts in November and ends in January followed by heavy rainfall between March and May. The district experiences a long dry season from June to October and the average annual temperature is 24.6^{o}C. Rice farmers in Chanzuru ward begin rice cultivation activities in November and end in May, depending on the availability of water. Rice is rotated with other crops during the dry season. The water from the Ilonga irrigation scheme is the main source of irrigation water for both Chaanzuru and Ilonga irrigation schemes.

The district lies between 6°S and 8°S, and 36°30'E and 38°E. It borders the Tanga Region to the north and Morogoro District to the east. In the south, it is bordered by the Kilombero District and part of Iringa Region (KDC, 2000). Kilosa District comprises mostly flat lowland that covers the whole of the eastern part called Mkata Plains. The district has several big permanent rivers that together account for 32 000 ha of irrigable land of which only 11 000 ha are being exploited. One of the principal crops grown in the district is rice (Concern worldwide, 2008).

Rice is a major crop grown in Ilonga and Chanzuru irrigated schemes, but there are other crops also grown by farmers such as maize, vegetables, beans and sunflower in upland plots between June and November. The irrigation schemes in Ilonga and Chanzuru are located about 15 km from Kilosa Town, and share similar source of irrigation. The irrigation scheme in Ilonga is positioned in the upstream area of Chanzuru, and it's in more favourable condition in terms of the availability of irrigation water and the access to good drainage system. Unlike the Chanzuru scheme, the Ilonga's has better irrigation infrastructure where most of the canals are cemented and well maintained.

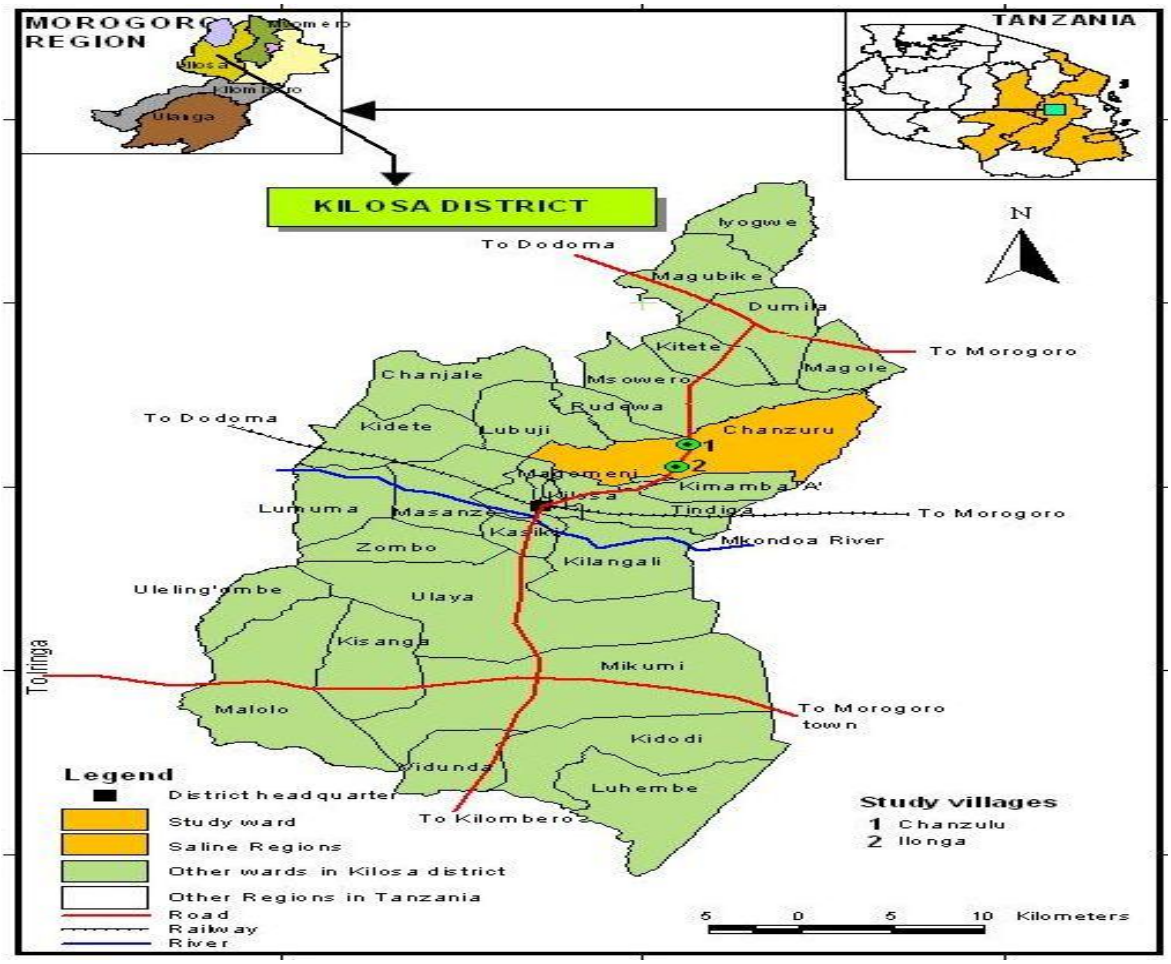

Figure 4.2: Map showing the study areas

The two villages (Ilonga and Chanzuru) represent one of the major rice producing areas in Kilosa District. Farmers in these villages, produce rice mainly as irrigated crop and a small portion in the lowland for rain-fed rice production.

4.1.2 Research design

4.1.2.1 Sampling design

The target population comprised rice farmers in Ilonga and Chanzuru villages in Kilosa District, Morogoro Region. A simple random sample of 30 farmers was selected from each village making a total sample of 60 farmers representing farmers of both villages.

4.1.3 Data collection and analysis

4.1.3.1 Data collection

Semi-structured questionnaire was administered to 60 randomly selected smallholder rice farmers from the two sample villages. The enumerators were trained for two days prior to administering the questionnaire. Data were collected on socio-demographic characteristics and variables relating to farmers' perceptions on soil salinity. Additionally, each respondent was asked to grade their perceived constraints based on a 0–3 point's scale (*i.e.*, ranging from "no problem" to "very serious problem").

4.1.3.2 Data processing and analysis

The perceived factors contributing to salinity constraints were rated and grouped into categories as "*no problem", "a problem", "a serious problem" and "a very serious problem*" as it relates to impacts on rice productivity in the study areas, and a ranking was conducted using the Problem Confrontation Index (PCI) as suggested by Ndamani and Watanabe (2015). The values of PCI were estimated using the following formula:

$$\mathbf{PCI} = \boldsymbol{P_{np}} \times \mathbf{0} + \boldsymbol{P_{p}} \times \mathbf{1} + \boldsymbol{P_{sp}} \times \mathbf{2} + \boldsymbol{P_{vsp}} \times \mathbf{3}$$

PCI = Problem Confrontation Index

P_{np} = Number of respondents who said "no problem"

P_{p} = Number of respondents who said "a problem"

P_{sp} = Number of respondents who said "a serious problem"

P_{vsp} = Number of respondents who said "a very serious problem"

Farmers' perception on factors contributing to salinity was analyzed using descriptive statistics in SPSS version 20. The Chi square test was conducted to verify the significant level of association between farmer's perceptions and their determinants.

4.2 Results and Discussion

4.2.1 Farmer's Perceived effects of salinity on rice production in the study areas

There were variations in the ranking of perceived problem by locations (Table 4.1 and 4.2). The PCI values obtained from farmers in Ilonga on the problem posed by salinity ranged from 43 to 47, indicating low perception of the problem. Farmers from Ilonga ranked "*salinity reduces yield*" (the product produced by a farm) as the most serious problem and "*salinity reduces harvest*" (the particular amount of produce that the farmers gathered in) was ranked as the least problem and "*salinity affects crop production*, was ranked second.

In Chanzuru village, famers perceived salinity problem differently than farmers in Ilonga village as indicated by the higher PCI values for each problem. The PCI values obtained from Chanzuru village were quite higher than those from Ilonga village; they ranged from 61 to 68, which meant that those farmers perceived the problems as more serious than their colleague farmers in Ilonga. "*Salinity affects crop production*", "*salinity reduces rice yield"*, and "*salinity reduces harvest"* were ranked first second and thirds serious problems in the village.

A combined analysis of the problem differed in rank (Table 4.3). The PCI values for the problems ranged from 104 to 113 and the first serious common problem to both villages was that "*salinity affects crop production*" with a PCI value of 113. The least perceived problem was "*salinity reduces harvest*". Farmers in both villages perceived the severity of salinity problems in completely different ways; however, they all had some knowledge of each problem and its effects on their crops and yields.

Farmers' perceptions of salinity issues are critical in the development of solutions to soil management problems. The challenge is to listen and to learn from the knowledge of farmers, because the knowledge of farmers on soils problems offers a completely different set of scales with regard to land use, which has important implications for sustainable agriculture. Similar observation was made by (Nederlof and Dangbegnon, 2007; Kassa *et al.*, 2013).

Table 4.1: Farmers' perception of salinity problems affecting rice farming in Ilonga village (n = 30)

Constraints	Very serious problem	Serious problem	Problem	No problem	PCI	Rank
Salinity reduces rice yield	7	10	6	7	47	1
Salinity affects crop production	6	9	9	6	45	2
Salinity reduces harvest	6	9	7	8	43	3

Table 4.2: Farmers' perceptions of Salinity problems affecting rice farming in Chanzuru village (n = 30)

Constraints	Very serious problem	Serious problem	Problem	No problem	PCI	Rank
Salinity affects crop production	9	20	1	0	68	1
Salinity reduces rice yield	8	17	5	0	63	2
Salinity reduces harvest	10	11	9	0	61	3

Table 4.3: Combined analysis of salinity problems affecting rice farmers in Chanzuru ward, Kilosa District (n = 60)

CONSTRAINTS	Very serious problem	Serious problem	Problem	No problem	PCI	Rank
Salinity affects crop production	15	19	17	9	113	1
Salinity reduces rice yield	14	26	12	8	110	2
Salinity reduces harvest	18	29	6	7	104	3

4.2.2 Farmers' perceptions on factors contributing to salinity

Table 4.4 presents the perception of farmers on factors contributing to salinity by location. During the study, farmers identified four factors which they perceived were responsible for salinity problems in their fields/schemes. Those factors were poor quality irrigation water (saline water), inadequate rainfall, poor drainage system, and inappropriate use of fertilizer in the schemes; however, the perception of these factors varied from village to village. Descriptive statistics and chi square test was conducted to describe the perceptions of the farmers in each village and to verify the significant level of association between farmer's perceptions and locations (villages) respectively. The differences in farmers' perception per location for the various contributing factors to salinity were significant for poor drainage system (x^2= 8.149, p<00.05) and poor quality of irrigation water (X^2=10.36, p<.01). This indicates that there were significant association between the two villages in terms of the drainage infrastructure and the quality of water used for irrigation and factors contributing to salinity occurrence. Poor drainage system and poor quality irrigation water were highly associated with farmer's perception of factors contributing to salinity in the two villages. Farmers perceived these factors as being the ultimate factors responsible for the problem of salinity in their fields. The inappropriate use of fertilizer and the inadequate rainfall were not significantly associated with the problem of salinity as perceived by farmers in both villages (X^2=.42, p >0.05; and X^2=0.089, P= 0.76) respectively. More farmers in Chanzuru village considered poor quality irrigation water and poor drainage system as major factors contributing to soil salinity, than farmers in Ilonga village.

Farmer's perception can be influenced by socio-demographic characteristics (Ngigi, 2009). Furthermore, perception on salinity is shaped by individuals' background and nature and degree of engagement with the environment (Rahman, 2009). Therefore, these factors were also analysed to determine the level of association between socio-

demographic characteristics and the farmer's perception on factors contributing to salinity (Tables 4.5). The study revealed that farmer's perceptions were more associated with location than socio-demographic characters, except for sex and household size. Gardebroek *et al*. (2010) reported that perceptions are context and location specific due to heterogeneity in factors that influence them such as, education, gender, age, resource endowments and institutional factors. In tables 4.5a and 4.5e, the perception on poor quality irrigation water was significantly associated with sex of respondent and household size (X^2=11.25, $p < 0.01$ and X^2=4.05, $p < 0.05$) respectively. This implies that perception of few factors contributing to salinity was more influenced by the sex (females rather than males) of respondent and the size (below 5 members) of the household to which a farmer belonged. This might be that salinity constraint is likely to deepen gender inequality as women depend more on the natural environment for their livelihoods (Falaki *et al*., 2013). Large household size may provide the needed labour requirement for farming than a small household size (Omoregbee *et al*., 2013).

Table 4.4: Perception of farmers on factor contributing to salinity by location

	Ilonga village		Chanzuru village			
Actors contributing to salinity	**Problem**	**No problem**	**Problem**	**No problem**	**X^2**	**P-value**
Poor drainage system	8 (13.3%)	22 (36.7%)	19 (31.7)	11(18.3)	8.148	0.004
Inadequate rainfall	7 (11.7%)	23 (38.3%)	8 (13.3)	22 (36.7%)	0.089	0.76
Poor quality of irrigation water	12 (20%)	18 (30%)	24 (40%)	6 (10%)	10.36	0.001
Wrong use of fertilizer	5 (8.3%)	25 (41.6%)	6 (10%)	24 (40%)	0.42	0.52

Note: Frequency and percentage on bracket.

Table 4.5: Perceptions of farmers on factors contributing to salinity using socio-demographic characteristics

Table 4.5a.	**Sex**					
	Male		**Female**			
Perceived constraints	**Problem**	**No problem**	**Problem**	**No problem**	**X^2**	**P-value**
Poor drainage system	15 (25%)	25 (41.7%)	12 (20%)	8 (13.3%)	2.73	0.09
Inadequate rainfall	8 (13.3%)	32 (53.3%)	7 (11.7%)	13 (21.7%)	1.6	0.21
Poor quality of irrigation water	18 (30%)	22 (436.7%)	18 (30%)	2 (3.3%)	11.25	0.001
Wrong use of fertilizer	7 (11.7%)	33 (55%)	5 (8.3%)	15 (25%)	0.47	0.49

Table 4.5b.	**Marital status**					
	Single		**Married**			
Perceived constraints	**Problem**	**No problem**	**Problem**	**No problem**	**X^2**	**P-value**
Poor drainage system	8 (13.3%)	9 (15%)	19 (31.7%)	24 (40%)	0.04	0.84
Inadequate rainfall	4 (6.7%)	13(21.7%)	11 (18.3)	32 (53.3%)	0.027	0.87
Poor quality of irrigation water	10 (16.7%)	7 (11.7%	26 (43.3%)	17 (28.3%)	0.014	0.907
Wrong use of fertilizer	2 (3.3%)	15 (25%)	10 (16.7%)	33 (55%)	1.003	0.314

Table 4.5c.	**Education**					
	Below primary		**Above primary**			
Perceived constraints	**Problem**	**No problem**	**Problem**	**No problem**	**X^2**	**P-value**
Poor drainage system	20 (33.3%)	23 (38.3%)	7 (11.7%)	10 (16.7%)	0.14	0.708
Inadequate rainfall	10 (16.7%)	33 (55%)	5 (8.3%)	12 (20%)	0.246	0.62
Poor quality of irrigation water	28 (46.7%)	15 (25%)	8 (13.3%)	9 (15%)	1.66	0.198
Wrong use of fertilizer	9 (15%)	34 (56.7%)	3 (5%)	14 (23.3%)	0.082	0.774

Note: Frequency and percentage on bracket.

	Age					
Table 4.5d.	**20 to 40 years**		**Above 40 years**			
Problem		**No problem**	**Problem**	**No problem**	**X^2**	**P-value**
Poor drainage system	14 (23.3%)	23 (38.3%)	13 (21.7%)	10 (16.7%)	2	0.157
Inadequate rainfall	9 (15%)	28 (46.7%)	6 (10%)	17 (28.3%)	0.024	0.878
Poor quality of irrigation water	19 (31.7%)	18 (30%)	17 (28.3%)	6 (10%)	3	0.083
Wrong use of fertilizer	8 (10%)	27 (45%)	6 (610%)	19 (31.7%)	0.159	0.69

Table 4.5e..	**Household size**					
	1-5 members		**Above 5 members**			
Perceived constraints	**Problem**	**No problem**	**Problem**	**No problem**	**X^2**	**P-value**
Poor drainage system	14 (23.3%)	19 (31.7%)	13 (21.7%)	14 (23.3%)	0.197	0.657
Inadequate rainfall	7(11.7%)	26 (43.3%)	8 (13.3%)	19 (31.7%)	0.561	0.454
Poor quality of irrigation water	16 (26.7%)	17 (28.3%)	7 (11.7%)	20 (33.3%)	4.05	0.044
Wrong use of fertilizer	7 (11.7%)	26 (43.3%)	5 (8.3%)	22 (36.7%)	0.067	0.795

Table 4.5f.	Years of experience					
	1-15 years		Above 15 years			
Perceived constraints	**Problem**	**No problem**	**Problem**	**No problem**	**X^2**	**P-value**
Poor drainage system	18 (30%)	23 (38.3%)	9 (15%)	10 (16.7%)	0.063	0.802
Inadequate rainfall	10 (16.7%)	31 (51.7%)	5 (8.3%)	14 (23.3%)	0.026	0.872
Poor quality of irrigation water	24 (40%)	17 (28.3%)	12 (20%)	7 (11.7%)	0.116	0.734
Wrong use of fertilizer	7 (11.7%)	34 (56.7%)	5 (8.3%)	14 (23.3%)	0.693	0.405

4.2.3 Farmer's coping strategies for sustainable livelihood

Tables 4.6 to 4.8 present farmer coping strategies in relation to perceived salinity problem in their fields. In Chanzuru ward, farmers cultivated maize, beans and sunflower in addition to rice as alternative crops for food and income, but the amount of land area cultivated per crop also varied between the two villages. Farmers who perceived salinity as a serious problem allotted more land for each crop production. In Chanzuru, farmers cultivated rice on 1 to 7 acres of land with an average of 2.3 acres. In Ilonga, farmers planted 1 to 4 acres of rice with an average of 1.61 acres. In terms of maize cultivation, farmers in Chanzuru farmed 0 to 3 acres of land (average land area of 1.04 acres) while Ilonga farmers 0 to 9 acres was farmed for maize with an average area of 2.48 acres. The average area allocated for beans cultivation was slightly more for Chanzuru farmers than Ilonga farmers, but area cultivated for sunflower was similar for both villages.

In addition to the cultivation of other crops as alternative sources of food and income, most rice farmers also cultivated some selected salinity tolerant rice genotypes in areas perceived to have high salinity in their fields, so as to minimize yield reduction in those areas. Five percent (5%) of farmers in Ilonga cultivated Saro-5 (TXD-306) and 6.7% cultivated Kisegese as salinity tolerant rice cultivars. In Chanzuru, 18 % of farmers cultivated Saro-5(TXD-306) and 10% percent cultivated Kisegese as salinity tolerant rice cultivars. Crop diversification strategies have been incorporated in several development

programs worldwide to improve household income in less-developed areas (Papadimitriou and Dent, 2001). Besides, crop diversification also helps for proper utilization of agricultural resources including land, water and other resources through providing farmers with viable options to grow different crops on their land (Fetien *et al*., 2009; Wondimagegn *et al*., 2011; Degye *et al*., 2012; Rehima *et al*., 2013).

Table 4.6: Farmers' crop diversification in Ilonga and Chanzuru villages

	Chanzuru			
	Rice	Maize	Beans	Sunflower
Mean (acre)	2.32	1.04	2.3	2
Min(acre)	1	0	1	1
Max(acre)	7	3	3	3
Std. deviation	1.73	2.47	0.76	0.69
	Ilonga			
	Rice	Maize	Beans	Sunflower
Mean (acre)	1.61	2.48	2	1.9
Min (acre)	1	0	1	1
Max (acre)	4	9	3	3
Std. deviation	0.97	0.53	0.82	0.76

Area cultivated per crop (acres)

Table 4.7: Farmers perceived tolerant rice varieties in Ilonga village

Tolerant variety	Response	Frequency	percentage
Saro-5 (TXD-306)	Yes	3	5%
	No	57	95%
Kisegese	Yes	4	6.7%
	No	56	93.3%

Table 4.8: Farmers perceived tolerant rice varieties in Chanzuru village

Tolerant variety	Response	Frequency	Percentage
Saro-5 (TXD-306)	Yes	11	18.3
	No	49	81.7
Kisegese	Yes	6	10.0
	No	54	90.0

4.2.4 Farmers' sources of information on salinity problems

The source and quality of information received by farmers influence their perception. Velandia *et al*. (2010) reported that the uses of information sources are complementary to extension, but farmers prioritize some sources over and above others based on the importance these sources play in decision making processes.

The study found three sources of information on the causes of salinity (Fig. 4.2) in the two villages. Farmers mentioned self-discovery as the main source of information on salinity problem. The second source of information was extension services followed by information from fellow farmers. Extension was expected to be best source of information, but was ineffective; this could probably be due to the shortage of extension officers in the areas. This is in agreement to the previous study which found that inadequate number of Agricultural Extension officers is a barrier to farmer accessing quality information (Isinika and Mdoe, 2001). According to Aina (2006), farmers hardly obtain new information on problem associated with crop production in the areas because of the low number of agricultural extension officers.

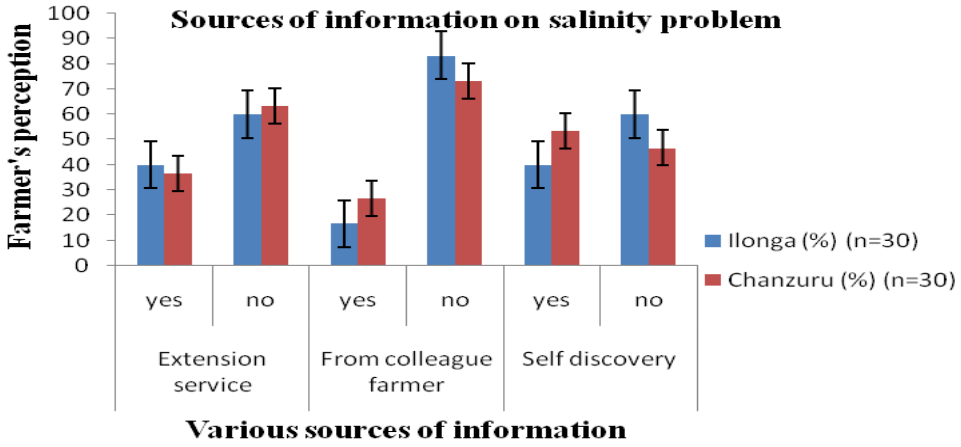

Figure 4.1: Farmers' sources of information on salinity in study areas in Kilosa district, 2016.

4.3 Conclusion and Recommendations

4.3.1 Conclusion

The results indicated that farmers in the two villages clearly perceived soil salinity problem differently as well as the factors contributing to the salinity problem. Moreover, the Farmers in Chanzuru village perceived salinity as a serious problem to crop production than farmers in Ilonga village. The poor quality of irrigation water and poor drainage

system were the main contributing factors to the occurrence of salinity, in the two villages. Farmers employed a strategy of crop diversification which probably served as alternative sources of food and income. The extension services in the villages were ineffective.

4.3.2 Recommendations

1. An effective extension program for the dissemination of useful agricultural information and farmer education on problems in the field is required for better handling of the problem in the field.
2. The farmers in the study area would need to cultivate improved salinity tolerant rice varieties to widen the genetic diversity in those saline environments.

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CHAPTER FIVE

ESTIMATION OF HERITABILITY AND GENETIC ADVANCE OF SALTOL IN SEGREGATING RICE (*Oryza Sativa* L.) GENOTYPES USING PHENOTYPIC ATTRIBUTES

Abstract

Soil salinity is a serious threat to crop yields, and sometimes considered a silent killer especially in countries where irrigation is an essential practice in the agricultural system. Two rice (*Oryza saliva* L.) genotypes susceptible to salinity (NERICA-L-19 and SUAKOKO-10) were crossed to tolerant genotype (FL478) at Sokoine University of Agriculture, Morogoro, Tanzania to produce F2 and F3 generations for the study of heritability and genetic advance for eight quantitative traits. The marker assisted selection method was applied in the selection and development of these populations. One hundred-ninety two (96) F2 and (96) F3 populations respectively along with three parents and a known susceptible check were evaluated using a randomized complete block design with four replications. There were considerable differences for genotypic coefficient of variation (GCV) from 4.5 % for days to 50% flowering to 22.23% for SES score; meanwhile, the estimates of phenotypic coefficient of variation (PCV) varied from 4.56 % for days to 50% flowering to 25.01 for standard evaluation score (SES). The estimate of heritability ranged from 0.82 for SES score to 0. 98 for days to 50 % flowering. Genetic advance ranged from 1.14 g for grain yield per plant to 13.87 cm for plant height. The high estimate of genetic advance was observed for plant height and days to 50% flowering; reproductive tillers and spikelet sterility were moderate. The GA values recorded for 100-grain weight and grain yield per plant were the lowest. There were high heritability estimates associated with high expected genetic advance for plant height and

days to 50% flowering; therefore, phenotypic selection of these traits will be more effective for their improvement. There were positive correlation coefficients among yield and yield components. Grain yield (g/plant) showed significant positive correlation with 100-grain weight, number of reproductive tillers which indicates that yield can be improved by using these traits as selection criteria for subsequent generations. It can be concluded that heritability, genetic advance and positive association of traits should be used as selection criteria for the improvement of rice genotypes.

5.0 INTRODUCTION

Rice; *Oryza sativa* (Asian rice) or *Oryza glaberrima* (African rice) is the most important food crop and a major food grain for most of the world's population (Zhao *et al,* 2011). Rice provides about 21% of global human per capita energy and 15% of per capital protein and Calories. Rice is particularly important in Asia, especially among the poor, where it accounts for 50-80% of daily caloric intake (IRRI, 2001). However, rice is considered to be very sensitive to saline conditions (Naheed *et al.*, 2008; Shahbaz and Zia, 2011), that constraints production of the crop.

The development of improved salt tolerant rice varieties is an effective mean of alleviating some of these constraints (i.e. salinity stress). Genetic variability in rice is important for the development of an effective rice breeding program (Abebe *et al*., 2017). Genetic variation is the occurrence of differences among the individuals due to the differences in their genetic composition and the environment in which they were raised (Falconer and Mackay, 1996).

The effectiveness of selection depends on the amount of heritability for the traits being selected. Heritability helps the breeder to predict the genetic gain under selection which assists the breeder to formulate the suitable breeding methodology (Tiwari, 2015). Heritability estimates along with genetic advance are normally useful in predicting the gain under selection (Johnson *et al*., 1955). Heritability is a good index of the transmission of character from parents to their offspring (Falconer, 1981). The estimates of heritability help the plant breeder during selection of elite genotypes from diverse genetic populations, and the genetic advance is the measure of genetic gain under selection (Tiwari, 2015). The estimation of genetic advance under selection depends on genetic variability, heritability and selection intensity.

The development of rice cultivars which have potential for salt tolerance is an important aim in the breeding programs (Peng *et al.*, 2008). The genetic variation for the traits under selection process and a high heritability and genetic advance help to predict the best cultivars in breeding programs (Ulloa, 2006; Ramanjinappa *et al.*, 2011). Breeding programs associated with understanding of key traits could positively impact the breeding process (Flowers and Flowers, 2005). Ray and Islam (2008) reported that mitigation of saline soil through various methods, such as reclamation, irrigation and drainage are not always economical or practical, but breeding for salt tolerance offers more promising, energy efficient, economical, and socially acceptable approach to solving the problem. Many scientists have developed salinity tolerant rice genotypes by the use of molecular breeding (molecular markers). Therefore, the present investigation was undertaken to estimate the heritability and genetic advance for F2 and F3 segregating populations along with the parental lines.

5.1 Materials and Methods

5.1.1 Study location

The study was conducted at Chanzuru village in Kilosa District, Morogoro Region, but water samples were collected from Chanzuru and Ilonga villages to compare the quality of irrigation water. Kilosa District is located approximately 300 km inland from the coast and Dar es Salaam. Kilosa is also one of six districts within Morogoro region, it is 14 245 km^2 in size making up about 20 per cent of the region (KDC, 2010). The district lies between 6°S and 8°S, and 36°30'E and 38°E. It borders Tanga Region to the north and Morogoro District to the East. In the South, it is bordered by Kilombero District and part of Iringa Region (KDC, 2000). Kilosa District comprises mostly of flat lowland that covers the whole of the eastern part called Mkata Plains.

5.1.2 Experimental design

Forty-eight seeds each for the parents, each F3 and F2 families from a cross NERICA-L-19 x FL 478 and SUAKOKO-10 x FL 478 along with a susceptible check were planted in the field at Chanzuru irrigation scheme, Kilosa in April 2016. The design of the experiment was the randomized complete block (Kalton, 1948; Mishra *et al.*, 2003; Zdravković *et al.*, 2011; Nakhaei *et al.*, 2014; Menezes-Júnior *et al.*, 2016; Liu *et al.*, 2016) with four replications. Each replicate consisted of one row of 3.25 m with a plant spacing of 25 cm x 25 cm and 50 cm between replicates. The border lines were planted with FL478.

Breeding scheme for the development of rice (*Oryza sativa*, L) genotypes

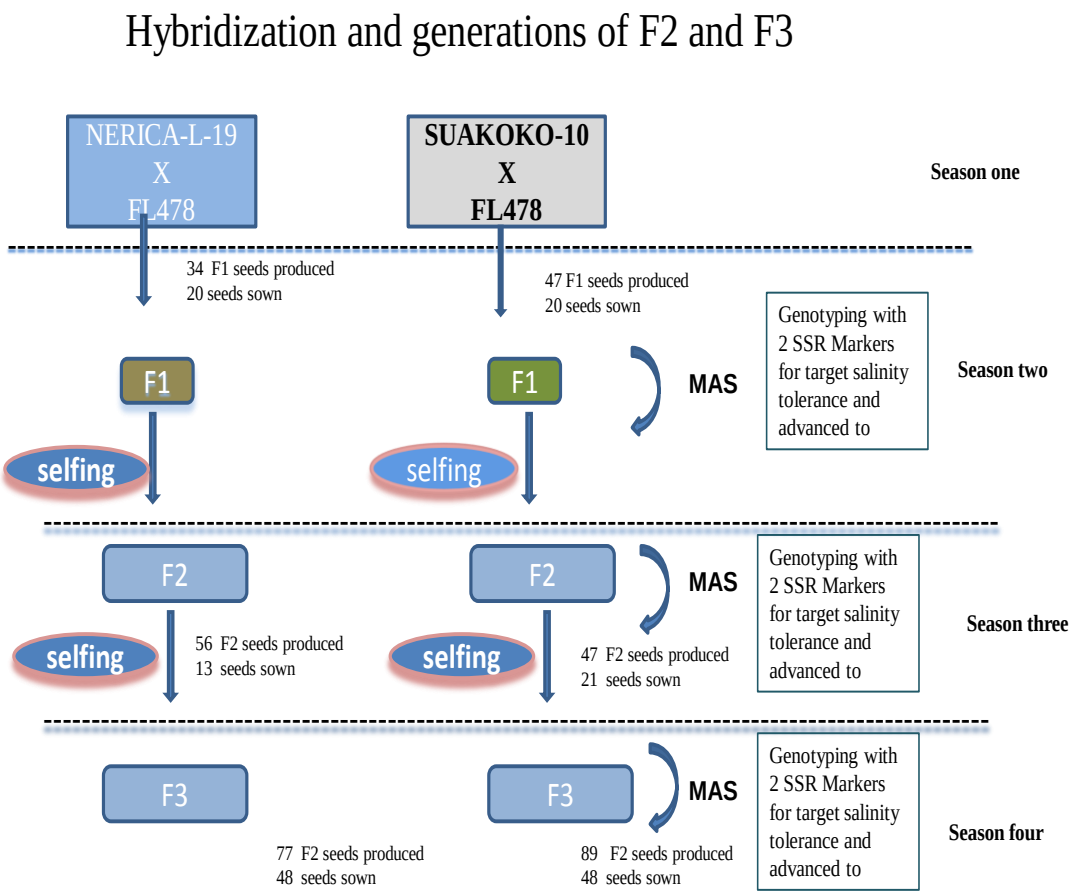

Figure 5.1: Marker assisted selection breeding adapted for the introgression of salt tolerance markers into improved genetic bases

5.2 Soil and Water Sampling and Analyses

Four composite soil samples were taken from the site of the experiment (30-cm top soil) at the corners and center of each plot prior to the experiment. The soil samples were air dried, from which soil solutions were made by mixing soil and water at the ratio of 1:2.5 (soils: water). The solutions were analyzed for Na^{+}, Mg^{2+}, K^{+}, Ca^{2+}, OC, TN, P, $pH_{1:2.5}$ and $EC_{1:2.5}$. Total nitrogen (TN) was determined by Kjeldahl method, organic carbon was determined by Walkley black (BLKw) method, exchangeable phosphorous (P) was determined by Olsen method. Available potassium and sodium, Magnesium and Calcium were determined using the ammonium acetate extraction method. Soil pH was determined using the pH reader (Hanna Instrument pH Meter, Model Hi 9032) in a 1: 2.5 soil water ratio and the electrical conductivity of soil was determined by the portable electrical conductivity meter (Hanna Instrument Conductivity Meter, Model Hi 9032) in 1:2.5 soil water ratios (Jackson, 1973). Irrigation and discharged waters were sampled from Chanzuru and Ilonga irrigation schemes prior to the study. The water samples were collected from the Main River, experimental site, irrigation pond and adjacent wells. Water samples were analyzed for three bases namely calcium, magnesium, and sodium. Sodium was determined using the flame photometer, while calcium and magnesium were determined using the atomic adsorption spectro-photometer.

5.2.1 Data collection

Evaluation of the rice genotypes was carried out for eight different quantitative variables representing the reproductive and ripening growth stages of rice. The standard evaluation score was according Gregorio *et al.* (1977). Traits selection and measurement techniques were based on IRRI standard evaluation system of rice (IRRI, 1980). Panicle length was measured in centimeters from the base to the tip of the panicle. Plant height was recorded in centimeters from the base of the shoot to the tip of the tallest leaf blade. Spikelet

sterility readings were obtained from a count of well-developed spikelets in proportion to total number of spikelets of five panicles. 100-grain weight was determined from a random sample of 100 well developed, whole grains within a population, dried to 13% moisture content and weighed on precision balance to obtain the 100-grain weight. Reproductive tillers were considered as those tillers that produced spikelets with or without filled grains. Reproductive tillers were counted for all genotypes. Numbers of tiller were considered the number of shoots that grew after the initial parent shoot. Days to 50% flowering was determined as the number of days from sowing to when 50 % of the rice population had flowered and grain yield was considered as the average weight of filled grains on five panicles per plant. Plants were scored based on the IRRI standard evaluation of salinity injury. Data were collected on five plants per hill and averaged for data analysis.

In order to assess and quantify the genetic variability among the genotypes, the variance components and values of heritability and genetic advance were estimated using the formula given by Burton and De Vane (1953) and applied by many researchers such as Johnson *et al.* (1955), Fehr *et al.* (1987), Allard (1999); Baye (2002), Tuhina-Khatun *et al.* (2015) among others. Vg = (MSG-MSE/r); Vp = (MSG/r); Ve = MSE/r);
Where; MSG is mean square of genotypes, MSE is mean squares of error and *r* is replication.

Genotypic coefficient of variability (GCV) and phenotypic coefficient of variation were calculated using the following formulae: PCV= (σp/M) x 100; GCV= (σg/M) x 100.
Where; σg and σp are the genotypic, phenotypic standard deviation respectively and M= grand mean of the trait respectively. Heritability in the broad sense (h^2), was estimated on genotypic means as $h^2 = Vg/Vp$. Where h^2 is heritability; V_g is genotypic variance and V_P

is phenotypic variance. The genetic gain (GA) was estimated by the following method: $GA=k\sigma p.h^2_b$.

Where; *k* is selection intensity in standard unit. Five percent (5%) of F2 and F3 were selected to produce the populations of the next generations, and k=2.06, h^2_b= broad sense heritability; σp = the phenotypic standard deviation estimated as the square root of the variance.

5.2.2 Data analysis

All recorded morphological data for the traits were analyzed using Genstat statistical package version 14 and means of traits were separated using Tukey Honestly Significant Test (HSD).

5.3 Results and Discussion

5.3.1 Weather data, soil and water properties

The weather data collected during the period of the experiment is shown in Appendix 1. The minimum and maximum temperatures were 18.27^{o}C and 29.64^{o}C respectively. Results from the soil analysis in Appendix 2 show that the site selected for the experiment was a completely saline environment with electrical conductivity (ECe) ranging from 4.98 to 6.78 dsm^{-1} and pH ranging from 7.43 to 7.74. The texture of the soil was sandy-clay and the clay percentage was high enough to prevent percolation of water in the soil. The exchangeable sodium percentage (ESP) ranged between 5.4 to 12 % indicating that the soil was saline and non-sodic. For saline soils the ESP value must not exceed 15 %; and an ESP value higher than 15 % indicates sodicity. Saline soil has ESP less 15 % and electrical conductivity is more than 4mS/cm, while for sodic soil, ESP is more than 15% and the electrical conductivity is less than 4 dsm^{-1} at 25° C (U.S. Salinity Laboratory Staff, 1954).

The water sample (Appendices 3 and 4) collected from the major dam used as main source of irrigation for both schemes was non-saline (0.18dsm^{-1}), however, water samples collected from the project site and other alternative sources (wells) of water in Chanzuru village, recorded high salinity. The application of such highly saline water as irrigation water would increase salt-affected areas in a short period. Patel *et al*. (2011) reported that area under salt- affected soils will continue to increase each year due to the introduction of irrigation in new areas.

The average rainfall during the time of the experiment (March to September 2016) was 7.86 mm (Appendix 1). High temperature and low average rainfall are factors which contribute significantly to the buildup of salinity in the fields. The appearance of the soil during the experiment is shown in Appendix 7 and the results of water analysis for Chanzuru irrigation scheme is shown in Appendix 8. The results obtained from the water samples collected from the study area are shown in Table 1. The pH of the samples ranged from 7.36 for a pond to 7.57 for the main irrigation dam. The electric conductivity of irrigation water samples (ECiw) varied significantly among the different sources from which the samples were collected. The lowest ECiw (0.18 dsm^{-1}) was recorded for sample taken from the main dam and the highest ECiw (8.9 dsm^{-1}) was recorded for sample taken from an underground source/well. The sample collected from the experimental site recorded an ECiw of 6.23 dsm^{-1}. Sodium Adsorption Ratio (SAR) was used to predict the sodium (Na) hazard in the soil. Results from the soil analysis showed variation in the values of the SAR.. The SAR values for the underground sources were higher (values ranged from 17.77 to 18.24) indicating sodicity (James *et al.*, 1982; Texas AgriLife Extension Service, 2017). The SAR values for samples collected from the experimental site, the main dam and pond ranged between 4.52 and 12.68, thus indicating salinity rather

than sodicity (James *et al.*, 1982; Texas AgriLife Extension Service, 2017). Appendices 9 and 10 give tables of interpreting results.

5.4 Genetic Parameters for Agronomic Characters of Rice Generations

The phenotypic variance (Vp), genotypic variance (Vg), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (h^2b) and genetic advance (GA) are shown in Table 5.1. The PCV was higher than the corresponding GCV for all traits thus indicating that there were to some degree interaction of all traits with the environment. Components of phenotypic variance (σ^2p) were found to be greater than the components genotypic variance (σ^2g) for all the characters indicating that the expressions of these characters were influenced by environmental factors, but the environmental influence was low because of the low differences between PCV and GCV. Breeding programs depend on genetic variation of traits, genetic systems controlling inheritance and genetic and environmental factors that influence their expression (Rameeh, 2015). Knowledge of genetic variation assists the plant breeders in choosing which agronomic traits should be used in their breeding programmes. High heritability was observed for days to 50% flowering (98.0 %), plant height (96 %), panicle length (88 %), spikelet sterility (853%), reproductive tillers (85 %), salinity injury (82 %), and number of tillers (56 %). According to Johnson *et al.* (1955), heritability and genetic advance of a trait are important to make sufficient improvement through selection. Therefore, the genetic advance for each trait was determined. There was high genetic advance for plant height (13.87 cm), and days to 50% flowering (8.97 days). High genetic advance and high heritability were observed for plant height and days to 50% flowering. Other traits recorded high heritability and moderate to low GA. High heritability with high genetic suggest that effective progress in improvement through selection could be achieved for yield (Akbar *et al.*, 2003). The high heritability with moderate to low genetic advance

values were recorded for spikelet sterility (4.7 %), reproductive tillers (3.54), SES score (2.08), panicle length (2.35 cm), 100-grain weight (1.15 g) and grain yield/plant (1.14 g) respectively. This indicates that selection for these traits is less effective compared to those traits with high heritability and high genetic advance (Eid, 2009). Number tillers had low heritability and low genetic advance indicating that this trait was highly influenced by the environment.

Table 5.1: Genetic parameters for agronomic characters of four rice generations

Traits	Vg	Vp	GCV%	PCV%	h^2_B	GA
Days to 50% Flow. (days)	19.1	19.82	4.5	4.56	0.98	8.97
100-grain weight (g)	0.33	0.35	13.1	23.4	0.94	1.15
Grain yield (g/plant)	0.37	0.44	11.12	12.19	0.83	1.14
Panicle length (cm)	1.49	1.7	6.16	6.56	0.88	2.35
Plant height (cm)	47.3	49.35	10.43	10.65	0.96	13.87
Reproductive tillers (#)	3.5	4.14	13.05	14.19	0.85	3.54
SES Scores (scale)	1.25	1.53	22.23	25.01	0.82	2.08
Spikelet sterility (%)	6.27	7.54	15.11	13.81	0.83	4.70
No. of Tillers	1.6	2.88	6.74	9.05	0.56	2.00

Selection intensity (5%) = 2.06; Ve = Environmental variance, GCV= Genotypic coefficient of variation, PCV= Phenotypic coefficient of variation, Vp = phenotypic variance, Vg=genotypic variance, GA= Genetic advance/Genetic gain.

5.5 Analysis of Variance and Mean Performance of Rice Genotypes under Saline Conditions

The analysis of variance (ANOVA) showed that mean square due to genotypes was highly significant; indicating that considerable amount of genetic variability existed among the genotypes (Table 5.2). The genotypic variability was high for plant height (197.34 cm) followed by days to 50% flowering (79.28), spikelet sterility (31.11%), and reproductive tillers (25.95) and plant height (20.0 cm) respectively. High amount of genetic variability for many of these traits had also been reported earlier by various scientists (Pandey *et al.*, 2012; and Sharma *et al.*, 2014). However, there were low genetic variability recorded for

panicle length (6.79 cm) SES scores (6.64), Grain yield/plant (1.85g) and 100-grain weight (1.38g) respectively. The results are similar to those reported by Jahan *et al*. (2013) who found significant variations among all the genotypes (*Brassica rapa*) for ten characters studied.

Table 5.2: Mean square, range, means, SES and coefficient of variation for yield and morphological traits

	Mean square			
Traits	**Genotypic variation**	**Range**	**Mean**	**CV%**
Days to 50% Flowering	79.28***	87.0-101	97.66	1.7
100-grain weight	1.38***	1.4-2.41	2.45	11.4
Grain yield (g/plant)	1.85** *	1.33-2.31	2.01	12.2
Panicle length	6.79***	18.1-21.53	19.8	4.6
Plant height	197.34***	56.82-76.3	65.95	4.3
Reproductive tillers	25.95***	11.58-16.32	14.87	10.2
SES Scores	6.64***	3.63-6.88	4.89	20.7
Spikelet sterility	31.11***	15.12-22.06	19.12	10.2

***Significant at $p \leq 0.001$

The mean performance varied among different rice genotypes used in the experiment as shown in Table 5.3 and Appendix 13. The longest days to 50% flowering was recorded for Suakoko-10 (101.0 days) while the minimum was recorded for FL478 (87 days). Among the segregating lines, days to 50% flowering were similar among the genotypes; however, NL F3 and SUF3 flowered earlier than NLF2 and SUF2 (Table 5.3). As for 100-grain weight, the maximum weight (2.61 g) was recorded for FL478 and minimum (1.40 g) was recorded for NERICA-L-19. For the segregating lines, grain weights recorded varied, and, SUF3 recorded a higher weight (2.43 g) while NLF3 recorded the lowest (2.25 g). Grain yield per plant was also determined and the maximum grain yield/plant (1.92 g) was recorded for NLF3 and the minimum grain yield/plant (1.33 g) was recorded for IR 29. In light of the segregating lines, SUF2 recorded lowest grainyield/plant (1.66 g) and NLF3 recorded the highest grain yield/plant (1.92 g). Panicle length was measured and the result

showed that SUF3 had the longest (21.03 cm) length, while FL478 recorded the shortest (18.1 cm) length. Plant height for all the genotypes ranged from 76.30 cm for NERICA-L-19 to 56.82 cm for FL478. Among the segregating lines, NLF3 recorded the shortest height (59.95 cm). The maximum number of reproductive tillers (19.92) was recorded for SUF3 and the lowest number of reproductive tillers (11.58) was recorded for IR 29. The results on sterile spikelets, show Suakoko-10 recording the highest number (22.95 %) of sterile spikelets, while NLF2 recorded the lowest number of sterile spikelets (13.76 %). When the genotypes were scored for salinity injury, NLF3 recorded the lowest score (3.63) indicating that this population was highly tolerant to salinity as compared to other genotypes. The most susceptible genotype was SUAKOKO-10 which recorded a score of 6.88. The best performing genotypes among the segregating generations were NLF3 and SUF3 for most of the traits studied. The SES scores varied significantly among all the rice genotypes. Appendices 5 and 6 show the seed and grain colors of parental materials and progenies used during the experiment.

Table 5.3: Mean performance of rice genotypes under saline condition

Genotypes	Days to 50% Flowering	100-grain weight(g)	Grain yield (g/plant)	Panicle length (cm)	Plant height (cm)	Reproductive tillers (No. of tillers)	SES Scores	Spikelet sterility (%)
NLF2 (NERICA-L-19 X FL 478)	97.2	2.33	1.75	19.42	75.24	15.41	4.76	13.76
NLF3 (NERICA-L-19 X FL478)	96.7	2.25	1.92	18.31	59.95	16.25	3.63	13.79
SUF2 (SUAKOKO-10 X FL 478)	97.2	2.31	1.66	20.4	67.51	19.78	4.45	18.93
SUF3 (SUAKOKO-10 X FL 478)	96.8	2,43	1.84	21.03	64.33	19.92	4.18	17.49
NERICA-L-19 (Recurrent)	98.5	1.4	1.63	20.45	76.3	13.95	5.91	21.72
SUAKOKO-10 (Recurrent)	101	1.42	1.42	20.13	66.79	12.35	6.88	22.95
FL 478 (Donor)	87	2.61	1.84	18.1	56.82	15.54	3.77	15.12
IR 29 (Susceptible check)	100.3	1.59	1.33	19.06	60.7	11.58	6.38	22.06
S.E	1.7	0.23	0.21	0.91	2.87	1.63	1.04	2.4
CV %	1.7	11.4	12.2	4.6	4.2	11.3	20.6	11.3

5.6 Correlation Coefficients of Grain Yield and Yield Components

The correlation coefficients of yield and agro-physiological traits of rice genotypes are presented in Table 5.4. Correlation coefficients estimated for yield and its components showed that grain yield had positive and highly significant correlation with 100-grain weight ($r=0.58$; $p<0.01$) and positive significant correlation with number of reproductive tillers ($r=0.38$; $p<0.05$). These results indicate that 100-grain weight and number of reproductive tiller were found to be the principle yield components. This result is similar to the results that have been reported by Fiyaz *et al.* (2011) and Babu *et al.* (2012) for reproductive tillers and Hassan *et al.* (2016) for 1000-grain weight.

Grain yield/plant had negative and significant correlation with salinity injury scores and spikelet sterility, while days to 50% flowering had negative but non-significant correlation with grain yield/plant. Spikelet sterility had highly significant negative correlation with grain weight, grain yield, and number of reproductive tillers, but positive correlation with panicle length and salinity injury score. This result agrees with Hassan *et al.* 2016) for 100-grain weight and grain yield/plant, but not for the other traits; this was probably due to the different sets of genotypes used and the environment. Plant height had a non-significant positive correlation with spikelet sterility. Plant height was significantly and positively correlated with panicle length. This is an indication that increased plant height would result in an increase in panicle length of plant. This result agrees with the report of Nayak *et al.* (2001). Soil salinity in the field adversely affected the grain filling process, and increased the numbers of unfilled grains as a result of spikelet sterility (Fig. 5.1). The results of spikelet sterility show that about 55% of the variability in spikelet sterility can be attributed to soil salinity.

Table 5.4: Correlation coefficients of yield and agro-physiological traits of four rice generations

	Days to Flowering (days)	Grain weight (g)	Grain yield (g/plant)	Panicle length (cm)	Plant height (cm)	No. of Reprod. Tillers	SES scores	Spikelet sterility (%)
Days to Flowering (days)								
Grain weight(g)	-0.52**							
Grain yield (g/plant)	-0.33ns	0.58***						
Panicle length (cm)	0.37*	-0.07	0.02					
Plant height (cm)	0.29ns	-0.29	-0.01	0.36*				
No. of Reprod. Tillers	-0.12ns	0.54**	0.38*	0.26	-0.22			
SES scores	0.42**	-0.65***	-0.42*	0.11	-0.02	-0.60***		
Spikelet sterility (%)	0.65***	-0.73***	-0.47**	0.19	0.28	-0.43*	0.68***	
Number of tillers	0.17ns	0	0.01	0.23	-0.08	0.65***	-0.19	-0.11

Note: *, ** and ******* are the levels of significance at P<0.05, P<0.01 and P<0.001 respectively.

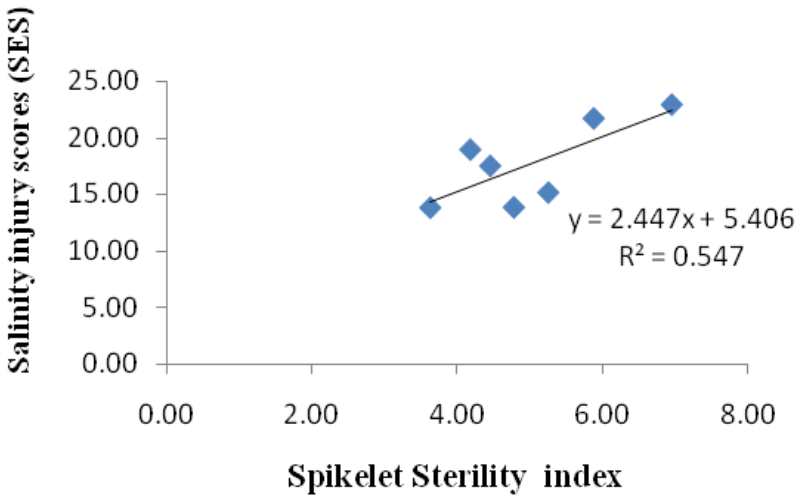

Figure 5.2: **Relationship of salinity injury scores and spikelet sterility index of seven rice genotypes**

5.7 Conclusion

The results of this study indicate that there was adequate genetic variability present in the materials studied. The NLF3 and SUF3 populations recorded the best performances for all traits studied as compared to NF2, SUF2 and the parental populations. High heritability and high genetic advance were recorded for days to 50% flowering and plant height, while grain yield of the genotypes correlated positively with grain weight and number of productive tillers. The NF3 populations recorded the lowest SES value followed by SUF3 population, indicating their improved tolerance to salinity.

5.8 Recommendations to Work on

1. The selection of the next parental genotypes should be based on the traits (grain weight and number of reproductive tillers) which positively correlated with grain yield as well as those which had high heritability and genetic advance for the effective improvement of yield of rice genotypes; and
2. The salinity tolerant populations (NLF3 and SUF3) are to be used in further breeding programs to test for agronomic performance.

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CHAPTER SIX

ASSESSMENT OF MARKER-TRAIT ASSOCIATION AND SEGREGATION RATIO OF NEW RICE (*Oryza sativa, L*) LINES

Abstract

Marker Assisted Selection technique is very useful and reliable and can help in the selection of several traits associated with abiotic stresses such as salinity tolerance and can also accelerate the breeding process and increase selection efficiency. The study was conducted to assess the association of markers for grain related traits and determine the genetic segregation ratios. Four population of the segregating lines (F2 and F3) of two different crosses (NERICA-L-19 /FL478 and SUAKOKO-1/FL478) along with three parental lines were used for the study. The genotypic segregation patterns of F_2 and F_3 individuals of these crosses, were studied using chi-square test. The Marker trait association was also performed using linear regression to identify the association of tolerance component traits with linked polymorphic markers. The selected markers for the assessment of segregation and goodness of fit fitted well the expected ratio of 1:2:1. The two marker loci (RM7075 and RM562) were significantly associated with the number of filled grains and grain yield per panicle in the studied rice materials.

6.0 INTRODUCTION

Rice (*Oryza sativa* L.) is a one of the most important cereal crops and serves as the staple food for over one-third of the world's population (Mohammadi-Nejad *et al*., 2010). However the productivity of rice is greatly affected due to soil salinity which is the second most widespread soil problem after drought in rice growing areas of the world (Sabouri and Sabouri, 2008; Islam *et al*., 2011). Several salt tolerant rice lines have been developed by incorporating *Saltol* QTL into modern high yielding, but salt-sensitive rice varieties through a targeted marker assisted selection approach (Ali *et al*., 2013; Huyen *et al*., 2013).

Application of molecular markers has played a growing role in the rice breeding and genetics programmes during the last few decades. Among the different types of molecular markers, microsatellites have been utilized most extensively because they can be readily amplified by PCR and have large amount of allelic variation at each locus. These markers are generally categorised as hybridization and PCR based markers (Collard *et al*., 2005).

Conventional breeding programs have been used to develop rice varieties tolerant to salt in an effort to incorporate salt tolerance into elite rice genotypes from their wild relatives. Conventional method of breeding for salt tolerance has met with very limited success, due to complex nature of the traits. Salt tolerance is a complex trait genetically and physiologically (Flowers, 2004). A number of genomic tools have been developed by breeders, to improve the efficiency of breeding programs. These include microsatellite markers that have been used effectively to map *QTLs* associated with salt tolerance in chromosome 1 of a rice genome (Singh *et al*., 2007).

Marker-traits association is an alternative approach, to identifying DNA-markers which are located in or in the neighborhood of the genes of interest. The strategies to identify marker-trait association could be used for natural (unknown ancestry) or breeding population (known ancestry) (Thomson, 2014).

The Simple Sequence Repeat (SSR) markers were used to sort salt tolerant progeny from segregating generations (Bhuiyan, 2005); thus, microsatellite marker analysis is important for developing marker-assisted selection programs (Gregorio *et al*., 2002). Therefore, the present investigation was carried out to find association of markers for yield related traits and determine the genetic segregation ratios among new lines.

6.1 Materials and Methods

6.1.1 Plant materials

On the basis of previous phenotypic and genotypic evaluation for salt tolerance, three rice genotypes NERICA-L-19, SUAKOKO-10 (high yielding varieties) and FL-478 (exotic salt tolerant) were selected as parents for transferring of salt tolerant genes from tolerant to high yielding rice varieties. Crosses were made between NERICA-L-19 and FL-378; and SUAKOKO-10 and FL-478 during September 2014 to February 2016, where NERICA-L-19 and SUAKOKO-10 were the recipient parents and FL-478 was the donor parent (Fig. 5.1). F2 and F3 generations were produced from these crosses for field evaluation along with parental materials and a susceptible check variety in Kilosa District during April to August 2016. Leaf samples were collected from 200 plants of introgression lines and four parents and subjected to genotyping through marker analysis (using SSR markers). The genotypes used were obtained from crosses between two recurrent and one donor parents; and two markers were used namely RM7075 and RM562).

6.1.2 Collection of leaf samples for molecular analysis

Fresh leaf samples were collected from 21-day old seedlings to extract genomic DNA. Initially, healthy portion of the youngest leaves of the tiller were cut apart with sterilized scissors and washed in distilled water and ethanol (70%) and dried on fresh tissue paper to remove spore of microorganisms and any other source of foreign DNA. The collected leaf samples were then kept in white polythene bags containing silica gel. The leaf samples were then taken to the Laboratory at SUA and stored for a week and then DNA extraction was performed.

6.2 Isolation of Genomic DNA

Genomic DNA was isolated from leaves of 21-day old plant based on the DNA isolation protocol of Egnin *et al.* (1998). Samples were ground to powder using the geno-grinder. A 1000 µl ice cold extraction buffer was added, and then incubated in boiling water bath for 7 minutes and then incubated on ice for 5 minutes. Six microliters (6 µl) Rnase "A" was added and incubated at 37^{o}C for 40 minutes then spun at maximum speed for 10 minutes and supernatants transferred to new tubes. One tenth (1/10) volume of 7.5M AOAC and equal volume of ice cold isopropanol was added and then incubated at -20^{o}C for 30 minutes. Samples were then centrifuged at regular speed and supernatant discarded and pellets dissolved in 600µl of 2M AOAC for 30 minutes then centrifuged and supernatant transferred into new tubes. Equal volume of isopropanol was added, mixed by inversion and incubated at -20^{o}C for 45 minutes; and afterward centrifuged then supernatant discarded and pellets washed in 70% ethanol, centrifuged and ethanol discarded and DNA pellet air-dry. The DNA pellets were resuspended in 60µl of 1x T.E buffer for use at a later date.

6.3 Amplification and Agarose gel Electrophoresis

Two selected DNA primers [RM7075 (Bhowmik *et al*., 2009); and RM562 (Rajendran *et al*., 2012)] were used for this study. Amplified microsatellite loci were analyzed for polymorphism using 2 % Agarose Gel Electrophoresis and the result revealed that the two primers detected clear polymorphism among the rice genotypes analyzed. RM7075 and RM562 were polymorphic and showed clear bands for each rice genotype. The PCR primix was obtained from Biolab inc., England. Each PCR reaction was carried out with 26.0 µl reaction mixtures containing DNA premix; 25 µl of primer master mix and 1.0 µl of each template DNA samples. PCR profile was maintained as initial denaturation at 94 ^{o}C for 3 minutes, followed by 33 cycles of denaturation at 94 oC for 30 seconds, annealing at 55-57 ^{o}C for 30 seconds, and polymerization at 72 ^{o}C for 1 minute; and final extension by 5 minutes at 72 ^{o}C. A 100 bp DNA ladder was used to compare the size of the molecule and position of the bands for the samples loaded. The Gel was post-stained in ethidium bromide. Banding patterns were visualized with ultraviolet trans-illuminator. The banding patterns were scored compared with tolerant control and susceptible control varieties and similar banding pattern to FL-478 were considered as salinity tolerant and NERICA-L-19 & SUAKOKO-10 were considered as salinity susceptible genotypes.

6.4 Data Collection

Data were collected on days to 50% flowering, grain yield, grain weight, plant height, spikelet sterility , number of productive tillers, panicle length Informative bands were scored as present (1) or absent (0) Present bands were further classified as heterozygous (H), tolerant (T) or susceptible (S). Standard evaluation scores (SES) were recorded 25 days after transplant, 50 days after transplant and at reproductive stage.

6.5 Data Analysis

6.5.1 Chi-square Test

For the F_2 and F_3, generations the genotypic segregation patterns were studied using chi-square test. The SSR are co-dominant markers, therefore the goodness of fit was tested for 1:2:1 segregation (Gomez and Gomez, 1984).

6.5.2 Single-marker Analysis

The sequences of the markers are presented in Table 6.1. Marker-trait association was performed using SPSS version 20.0 software to identify the association of tolerance component traits with linked polymorphic markers of the salinity tolerance gene by using linear regression model. The P-value determines whether a marker was associated with the phenotype, and R^2 for a marker, evaluates the magnitude of quantitative trait loci effect to phenotypes.

Linear model used:

$Yi = \mu+\beta Xi+\epsilon i$

Where Yi is the respondent trait; Xi is the marker allele of the F3 lines; β is corresponding regression coefficient and ϵi is the random error.

Table 6.1: The sequence and size of the microsatellite markers used for screening salt tolerant rice genotypes

	Sequence		
PRIMER	**FORWARD**	**REVERSE**	**ANN. TEMP. ^{O}C**
RM7075	GCGTTGCAGCGGAATTTGTAGG	CCCTGCTTCTCTCGTGCAGTCG	55
RM562	GGAAAGGAAGAATCAGACACAGAGC	GTACCGTTCCTTTCGTCACTTCC	55

6.6 Results and Discussion

6.6.1 Markers segregations for salinity tolerance

The Gel Electrophoresis result revealed that the two primers detected clear polymorphism among the rice genotypes analyzed (Fig. 6.1 and 6.2). The selected markers were assessed for segregation and goodness of fit was tested for 1:2:1 ratio using chi-square test in all the segregating populations and the results for each population are given in Table 6.2. The segregation of two markers RM7075 and RM562 (chromosome 1) was assessed and it fitted well into expected 1:2:1 ratio. The probability values were varied and indicated non-significant difference between the expected and observed numbers (Table 6.2). The markers used in this study were co-dominant markers and showed clear polymorphism for the rice genotypes.

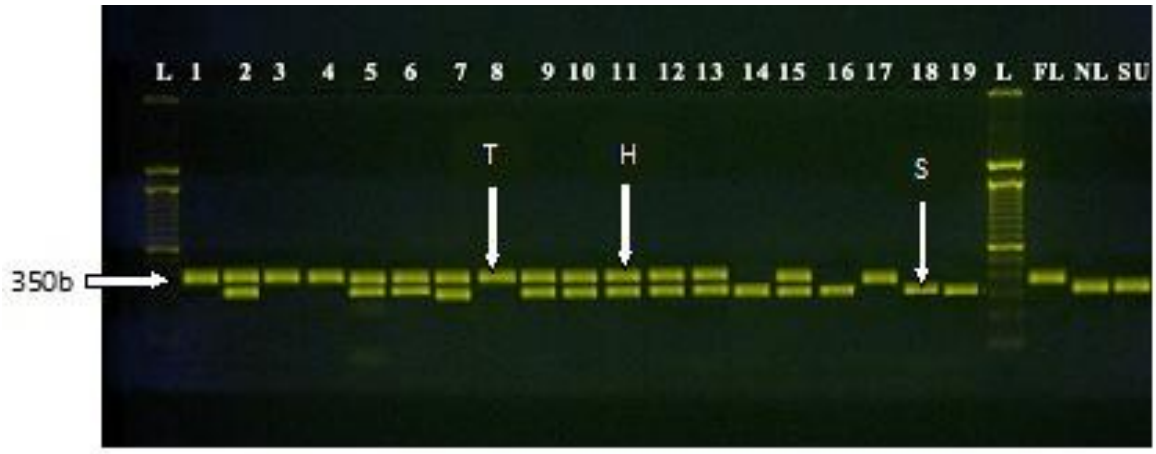

Figure 6.1: DNA bands amplified from leaves of NLF3 rice genotypes using microsatellite RM 7075 DNA marker. Lanes 1-19 are F3 individuals while Fl, NL and SU are referred to as Fl-478, NERICA-L-19 and SUAKOKO-10 respectively. Lanes L are DNA ladders.

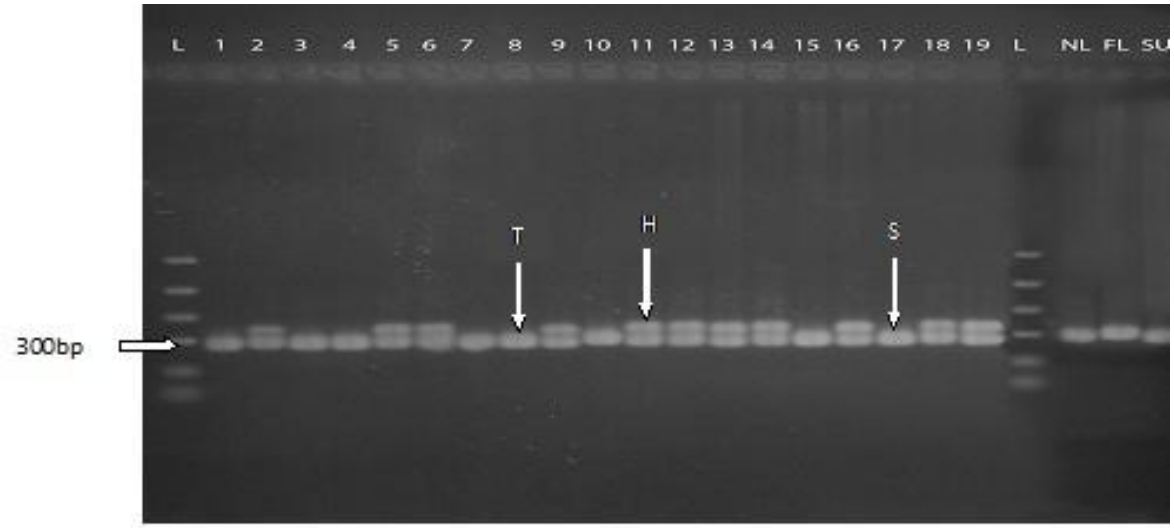

Figure 6.2: **DNA bands amplified from leaves of NLF2 rice genotypes using microsatellite RM 7075 DNA marker. Lanes 1-19 are F3 individuals while Fl, NL and SU are referred to as Fl-478, NERICA-L-19 and SUAKOKO-10 respectively. Lanes L are DNA ladders.**

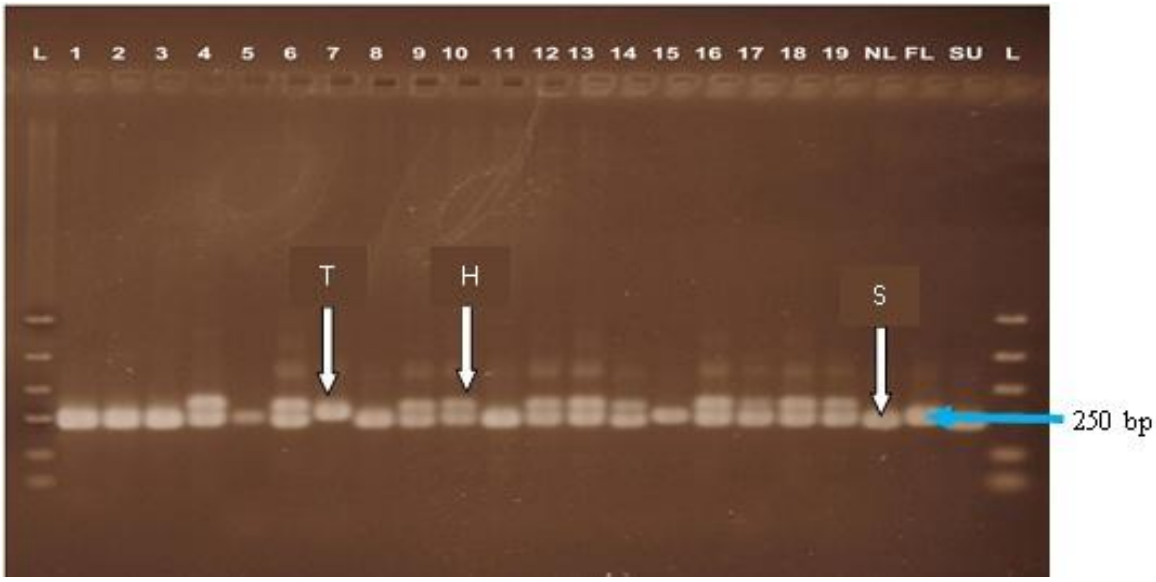

Figure 6.3: DNA bands amplified from leaves of SUF3 rice genotypes using microsatellite RM562 DNA marker. Lanes 1-19 are F3 individuals while Fl, NL and SU are referred to as Fl-478, NERICA-L-19 and SUAKOKO-10 respectively. Lanes L are DNA ladders.

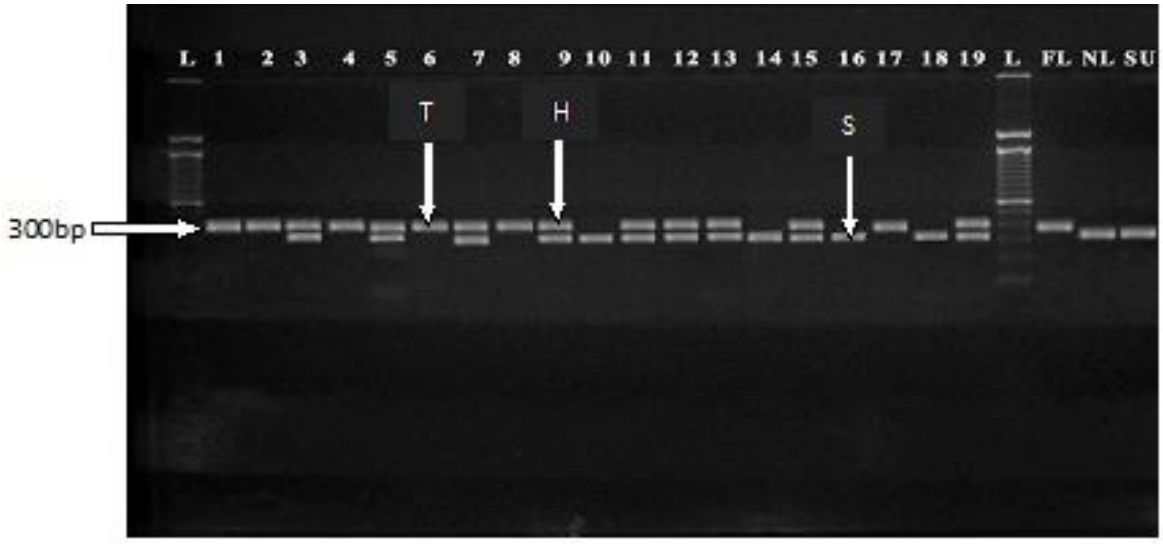

Figure 6.4: DNA bands amplified from leaves of SUF2 rice genotypes using microsatellite RM562 DNA marker. Lanes 1-19 are F3 individuals while Fl, NL and SU are referred to as Fl-478, NERICA-L-19 and SUAKOKO-10 respectively. Lanes L are DNA ladders.

Table 6.2: Observed and expected segregation ratios of tolerant and susceptible plants in the F2& F3 generation for the genetic cross between the rice genotypes NERICA-L-19 × FL478 and SUAKOKO-10 x FL478.

MARKER	Generation	Reaction (Allele favored in segregation)	Observed No.	Expected No.	X^2 (1:2:1)	P-value
RM7075	NLF2	Tolerant	15	12	0.33ns	0.54
		Heterozygous	22	24	0.17ns	
		Susceptible	12	12	0.00ns	
	SUF2	Tolerant	12	12	0.00ns	0.78
		Heterozygous	21	24	0.17ns	
		Susceptible	15	12	0.33ns	
	NLF3	Tolerant	14	12	0.33ns	0.79
		Heterozygous	23	24	0.04ns	
		Susceptible	11	12	0.08ns	
	SUF3	Tolerant	17	12	0.95ns	0.47
		Heterozygous	20	24	0..67ns	
		Susceptible	11	12	0.08ns	
RM562	NLF2	Tolerant	14	12	0.33ns	0.35
		Heterozygous	19	24	1.04ns	
		Susceptible	15	12	0.75ns	
	SUF2	Tolerant	16	12	1.33ns	0.40
		Heterozygous	22	24	0.14ns	
		Susceptible	10	12	0.33ns	
	NLF3	Tolerant	14	12	0.33ns	0.57
		Heterozygous	25	25	0.00ns	
		Susceptible	9	12	0.75ns	
	SUF3	Tolerant	15	12	0.75ns	0.35
		Heterozygous	25	24	0.04ns	
		Susceptible	8	12	1.33ns	

NOTE: **NLF2, 3** = NERRICA-L-19 X FL478 and SUF2**, 3** =SUAKOKO-10 X FL478; ^{ns} = Not significant.

6.8 SSR Marker- trait Association

Two agronomic traits which were significantly associated with the marker used were number of filled grains and grain yield per panicle. The two significant SSR loci identified for the agronomic traits had R^2 of total variation explained ranging from 9.2 % to 42.9 % for the entire segregating individual studied. For number of filled grains, the two loci in NLF2 individuals had a significant association ($p<0.001$); and RM7075 explained 39.0 % of the total agronomic variation in number of filled grains. RM7075 had the largest effect, explaining 11.7 % of total variation in grain yield/plant. In the SUF2 individuals, the marker loci were significantly associated with number of filled grains, and RM7075

explained 42.9 % of the total variation in agronomic traits. Within the same individuals, the marker loci were significantly associated with grain yield per plant; RM562 explained 10.7 % of the total variation. Within the F3 individuals, the marker loci were also found associated with filled grains and number of grain per plant. With NLF3 individuals; the marker locus RM7075 had the largest effect, explaining 23.5 % of the total variation in number of filled grains; and RM 7075 explained 18.9 % of the total variation in grain yield indicating that this locus had the largest effect. With SUF3 individuals, the loci exhibited significant association with the traits studied. For number of filled grains, the marker locus RM7075 showed the largest genetic association, explaining 29.0 % of the total variation. Marker locus with the largest effect on grain yield was RM7075 which explained 13.2 % of the total variation in grain yield. These results are similar to the findings of Agrama *et al.* (2007) and Borba *et al*. (2010) who found significant associations of SSR markers with grain yield and yield components.

The marker locus RM7075 on chromosome 1 was significantly associated with grain yield/plant and number of filled grains/panicle simultaneously, thus demonstrating its strong effect on agronomic variations. These pleiotropic effects associated with markers locus RM7075 could be used in marker-assisted selection to improve breeding efficiency.

Table 6.3: Association relationship of the markers with grain weight and grain yield of F2 and F3 populations

Genotype	Traits	Marker	R^2	df	Regressions coefficient	F-value	P-value
NLF_2	No. of filled grains	RM7075	39	42	0.62	26.24	<0.001
		RM562	16	42	0.40	8.12	0.007
	Grain yield/plant	RM7075	11.7	42	0.34	5.42	0.025
		RM562	11.3	42	0.33	5.21	0.028
SUF_2	No. of filled grains	RM7075	42.9	42	0.65	30.75	<0.001
		RM562	12.2	42	0.35	6.87	0.002
	Grain yield/plant	RM7075	9.2	42	0.30	4.14	0.048
		RM562	10.7	42	0.32	4.88	0.033
NLF_3	No. of filled grains	RM7075	23.5	47	0.50	15.44	<0.001
		RM562	19.8	47	0.44	11.34	0.002
	Grain yield/plant	RM7075	18.9	47	0.43	10.73	0.002
		RM562	12.5	47	0.35	6.545	0.014
SUF_3	No. of filled grains	RM7075	29	47	0.53	18.79	<0.001
		RM562	21.9	47	0.46	12.93	0.001
	Grain yield/plant	RM7075	13.2	47	,363	6.52	0.014
		RM562	11.4	47	0.35	5.94	0.019

Note: df = degree of freedom, R^2 = coefficient of determination.

6.9 Conclusion

The selected markers assessed for segregation and goodness of fit, fitted well into the expected ratio of 1:2:1, and the marker loci (RM562 and RM7075) were significantly ($P<0.005$) associated with grain yield per panicle and number of filled grains per panicle. These two associated SSR markers are potential candidates for marker-assisted selection to improve salinity tolerance in rice. The detected salt tolerant rice genotypes could be considered as the potential sources to improve the salt susceptible genotype.

6.10 Recommendations

1. The yield related traits influenced the yield of rice, directly or indirectly; therefore, the identification of genic regions controlling these traits is valuable to the promotion of rice breeding programs.
2. These SSR markers may also be used to screen larger germplasm populations to identify additional donors to breed for salt tolerance in rice.

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CHAPTER SEVEN

7.0 CONCLUSION AND RECOMMENDATIONS

7.1 Conclusion

Farmers in the Chanzuru and Ilonga villages clearly perceived soil salinity as a problem which affects their crop yield, harvest and production. Irrigation water rich in salts and poor drainage system were perceived by farmers as the main contributing factors to salinity occurrence in the irrigation schemes. These factors were perceived more by farmers in Chanzuru village than the farmers in Ilonga village. To cope with the problem of salinity, farmer in the two villages diversified their crop production in response to the salinity problem and as a means of seeking alternative sources of food and income.

There were reductions in physiological traits, ion accumulation and dry matter contents of rice genotypes which clearly distinguished the tolerance from susceptible genotypes. The molecular markers used in the study were able to discriminate well tolerant genotypes from susceptible, therefore, the genotypes, NERICA-L-19 and SUAKOKO-10 were selected as the susceptible parents to be used for improvement of salt tolerance, and FL478 was used as the donor parents.

There were high heritability and genetic advance observed for all traits studied except 100-grain weight and grain yield per plant; and also there were highly significant correlations among the various yield components. The components which correlated well with grain yield were grain weight and number of reproductive tillers. Two of the segregating populations (NLF3 and SUF3) were more tolerant and performed better than NLF2 and SUF2 population under saline conditions.

The selected markers assessed for segregation and goodness of fit, fitted well into the expected 1:2:1 ratio and the marker loci were significantly ($P<0.005$) associated with grain yield per panicle and number of filled grains per panicle.

7.2 Recommendations

1. Further study needs to be conducted in Chanzuru village to investigations and identify the root cause(s) of poor quality irrigation water and recommend measures to save crop losses in farmers' fields.
2. The two rice genotypes from the AfricaRice Center have now been identified as salt sensitive rice genotypes; therefore, other researchers should be made aware of the use of these genotypes as recurrent parents in further salt tolerant breeding programs.
3. Furthermore, traits showing strong correlations with grain yield (100-grain weight and days to 50% flowering), high heritability estimates and high genetic advance (days to 50% flowering, panicle length, plant height and reproductive tiller) from selection in the field experiment should be used as selection criteria for salinity tolerance in the field.
4. The NLF3 and SUF3 populations should be used in further selection program to enhance the yield performance of succeeding generations.

APPENDICES

Appendix 1: **Weather data for Chanzuru ward for March to September, 2016**

Weather information			
	Temperature ^{O}C		
Month	**Minimum**	**Maximum**	**Rainfall (mm)**
March	22.8	34.1	7.24
April	21.4	29.9	21.6
May	18	28.8	14.1
June	16.2	27.8	2.2
July	15.3	28.3	2
August	16.6	28.9	5.3
September	17.6	29.7	2.6
Average	**18.27**	**29.64**	**7.86**

Appendix 2: Physical and chemical of soil from project site in Chanzuru

							TN	OC	Ex. P	CEC			Exchangeable bases				
		Ece	Particle size density			Texture	(Kjeld)	(BlkW)	(mg/kg)	(cmol/kg)	zinc	Copper	(cmol/kg)				ESP
Lab. Nos	pH	(dsm^{-1})	% cla y	% silt	% sand	Class	%	%	Pbry-1	CEC	mg/kg	mg/kg	Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}	(%)
BLOCK I	**7.74**	**4.98**	**38.32**	**3.28**	**58.4**	**Sandy clay**	**0.1**	**0.98**	**13.97**	**15**	**1.53**	**6.87**	**6.49**	**7.88**	**0.41**	**0.81**	**5.4**
BLOCK II	7.43	6.57	40.32	3.28	56.4	Sandy clay	0.11	1.19	16.14	16	1.14	7.34	12.8	9.94	0.38	1.82	11.4
BLOCK III	7.53	6.48	41.31	2.29	56.5	Sandy clay	0.12	0.87	19.79	18.2	1.64	7.45	5.2	10.42	0.58	2.18	12
BLOCK IV	7.61	6.78	38.35	5.38	56.3	Sandy clay	0.11	1.19	24.25	17.3	1.06	7.34	6.08	9.65	0.49	1.5	8.7

Appendix 3: Water samples collected from Ilonga irrigation scheme

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
pH	6.43	6.2	6.52	6.15	6.38
Ece ($dsm^{-1)}$	0.22	0.17	0.15	0.16	0.19

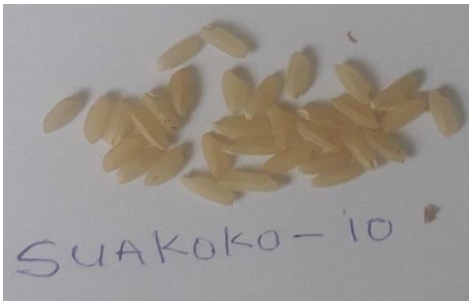
Appendix 4: Water samples collected from Chanzuru irrigation scheme

	Irrigation Dam	Project site	Pond	Well 1	Well 2
pH	7.57	7.74	7.36	7.4	7.59
Ece	0.18	6.23	0.34	4.7	8.9

Appendix 5: Seeds and grain colors of rice parental genotypes

A. Donor parent

B. Susceptible parent 1



C. Susceptible parent 2

Appendix 6: Seeds and grain colors of rice segregating genotypes

A. SUAKOKO-10 x FL478

B. SUAKOKO-10 x FL478

C. NERICA-L-19 x FL478

D. NERICA-L-19 x FL478

Appendix 7: Photos from site of experiment

A. Salinity condition at experiment site (Chanzuru, Kilosa District)

Appendix 8: Chemical properties of water samples collected from study area

Field Reference	Water pH (in H2O)	ECe ds/m	Na (mgL^{-1})	Ca (mgL^{-1})	Mg (mgL^{-1})	SAR
Project site	7.74	6.23	205.21	228.4	295.69	12.68
Well No. 1	7.4	4.7	413.83	485.87	598.98	17.77
Well No. 2	7.59	8.9	514.38	579.83	1010.88	18.24
Dam	7.57	0.18	33.71	101.04	10.31	4.52
Pond	7.36	0.34	94.23	69.88	33.29	11.98

Appendix 9: The sodium hazard of water based on SAR values

Classes of water	EC_{iw} (dsm^{-1})	Comments
Class 1, Ex cellent	0.25-0.75	
Class 2, Good	0.75-2	
Class 3, Permissible	2-3	Leaching needed if used
Class 4, Doubtful	3.0	Good drainage needed and sensitive plants will have difficulty obtaining stands
Class 5, Unsuitable	>3.0	

Source: Texas AgriLife Extension Service

Appendix 10: The sodium hazard of water based on SAR Values

SAR values	Sodium hazard of water	Comments
1-10	Low	Use on sodium sensitive crops must be caution
10-18	Medium	Amendments (such as Gypsum) and leaching needed
18-26	High	Generally unsuitable for continuous use
>26	Very high	Generally unsuitable for use

Source: Texas AgriLife Extension Service

Appendix 11: Effect of salinity on biochemical traits in rice root under saline condition

Genotypes	Calcium (root)			Potassium (root)			Magnesium (root)			Sodium (root)		
	0mM NaCl	50mM NaCl	100mMNaCl	0mM NaCl	50mM NaCl	100mMNaCl	0mM NaCl	50mM NaCl	100mMNaCl	0mM NaCl	50mM NaCl	100mMNaCl
IRRI 113	1.004g	1.285l	0.653a	1.0953o	0.6937i	0.5433f	0.8387k	0.8553l	0.737c	1.584c	1.928g	1.879f
IRRI 128	0.828b	1.144j	0.934e	1.1967p	0.7447j	0.644h	0.7987i	0.8427k	0.7613e	1.584c	1.879f	1.927g
FL 478	0.864c	1.601m	1.039h	0.946m	0.543f	0.745j	0.772gh	0.7717gh	0.753d	1.535b	1.879f	1.879f
IR65192-4B-10-3	1.249k	0.899d	1.004g	0.8953l	0.493e	0.3927c	0.7653ef	0.694a	0.741c	1.485a	1.879f	1.829e
NERICA-L-19	0.899c	1.144j	0.969f	1.0953o	0.5433f	0.594g	0.7763h	0.7487d	0.7723gh	1.485a	1.879f	2.076i
IRRI 124	1.039h	1.109i	0.969f	0.6943i	0.5943g	0.4433d	0.7987i	0.8267j	0.737c	1.485a	1.977h	1.879f
SUAKOKO-10	1.109i	1.144j	0.969f	1.0463n	0.3423b	0.1913a	0.7687fg	0.7253b	0.6887a	1.584c	1.977h	1.535b
IRRI 112	0.828b	1.109i	1.144j	0.795k	0.694i	0.3927c	0.7413c	0.761e	0.7763h	1.633d	2.125j	1.977h
Sal_levels (s.e.d)		0.001			0.001			0.001			0.0004	
Genotype (s.e.d)		0.001			0.001			0.001			0.001	
Sal_levels x genotype (s.e.d)		0.002			0.002			0.002			0.001	

Appendix 12: Effect of salinity on biochemical traits in rice root under saline condition

Genotypes	Calcium (shoot)			Potassium (shoot)			Magnesium (shoot)			Sodium (shoot)			K^+/Na^+		
	0mM NaCl	50mM NaCl	100mM NaCl	0mM NaCl	50mM NaCl	100mM NaCl	0mM NaCl	50mM NaCl	100mM NaCl	0mM NaCl	50mM NaCl	100mM NaCl	0mM NaCl	50mM NaCl	100mM NaCl
IRRI 113	0.617c	0.618c	0.618c	1.751b	2.153j	1.851d	0.838k	0.855l	0.737c	1.535d	1.928g	1.879f	1.140e	1.117de	0.985bcd
IRRI 128	0.548a	0.688e	0.583b	2.231k	1.801c	1.998g	0.798i	0.842k	0.761e	1.485c	1.879f	1.928g	1.502fg	0.959bc	1.037bcde
FL 478	0.581b	0.688e	0.618c	2.505m	2.047h	2.203k	0.772gh	0.771gh	0.753d	1.535d	1.879f	1.878f	1.631g	1.090cde	1.173e
IR65192-4B-10-3	0.653d	0.584b	0.688e	2.052h	1.549a	1.751b	0.765ef	0.694a	0.741c	1.485c	1.879f	1.829e	1.382f	0.955bc	0.986bcd
NERICA-L-19	0.583b	0.688e	0.866h	1.751b	1.801c	1.851d	0.776h	0.748d	0.772gh	1.535d	1.879f	2.076i	1.141e	0.958bc	0.892b
IRRI 124	0.583b	0.688e	0.618c	1.549a	1.901e	1.952f	0.798i	0.826j	0.737c	1.387a	1.977h	1.879f	1.117de	0.961bc	1.039bcde
SUAKOKO-10	0.653d	0.723f	0.864h	2.203k	2.103i	2.354l	0.768fg	0.725b	0.688a	1.436b	1.977h	1.535d	1.534g	0.940bc	0.652a
IRRI 112	0.757g	0.688e	0.653d	1.751b	2.103i	1.851d	0.741c	0.761e	0.776h	1.535d	2.125j	1.977h	1.141e	0.990bcd	0.936b
Sal_levels (s.e.d)		0.001			0.003			0.001			0.001			0.014	
Genotype (s.e.d)		0.002			0.005			0.001			0.001			0.023	
Sal_levels x genotype (s.e.d)		0.003			0.009			0.002			0.002			0.039	

Appendix 13: **Mean separation of rice genotypes under saline condition**

Genotypes	Days to 50% Flowering	100-grain weight(g)	Grain yield (g/plant)	Panicle length (cm)	Plant height (cm)	Reproductive tillers (No. of tillers)	SES Scores	Spikelet sterility (%)
NLF2 (NERICA-L-19 X FL 478)	97.20b	2.33b	1.75abc	19.42abcd	75.24e	15.41ab	4.76abc	13.76a
NLF3 (NERICA-L-19 X FL478)	96.70b	2.25b	1.92c	18.31ab	59.95ab	16.25ab	3.63a	13.79a
SUF2 (SUAKOKO-10 X FL 478)	97.20b	2.43b	1.66abc	20.40cd	67.51d	19.78b	4.45abc	18.93ab
SUF3 (SUAKOKO-10 X FL 478)	96.80b	2.31b	1.84bc	21.03cd	64.33bcd	19.92b	4.18ab	17.49ab
NERICA-L-19 (Recurrent)	98.50b	1.40a	1.63abc	20.45cd	76.30e	13.95a	5.91abc	21.72bc
SUAKOKO-10 (Recurrent)	101.00b	1.42a	1.42ab	20.13cd	66.79cd	12.35a	6.88c	22.95c
FL 478 (Donor)	87.00a	2.61b	1.84bc	18.10a	56.82a	15.54ab	3.77a	15.12ab
IR 29 (Susceptible check)	100.30b	1.59a	1.33a	19.06abc	60.70abc	11.58a	6.38bc	22.06c
Replication (s.e.d)	0.56	0.05	0.15	0.49	0.82	0.47	0.46	0.74
Genotype (s.e.d)	1.20	0.15	0.14	0.57	2.03	1.97	0.73	2.07
Salinity x genotype (s.e.d)	1.70	0.21	0.20	0.80	2.87	2.70	1.04	2.92

Appendix 14: The effects of salinity on ion accumulation in the rice genotypes

	% SCa^{2+}			%SMg^{2+}			% SK^{+}			% SNa^{+}			
VARIETY	**100mM NaCl**	**50mM NaCl**	**0 mM NaCl**	**100mM NaCl**	**50mM NaCl**	**0 mM NaCl**	**100mM NaCl**	**50mM NaCl**	**0 mM NaCl**	**100mM NaCl**	**50mM NaCl**	**0 mM NaCl**	**SK/Na**
IRRI-113	0.61c	0.61c	0.61c	0.79fgh	0.87k	0.86k	1.85d	2.15j	1.75b	1.97f	1.78g	1.53g	0.8
NERICA	0.86h	0.68e	0.58b	0.68c	0.88l	0.78fg	1.85d	1.80c	1.75b	2.07i	2.22f	1.53d	0.83
IRRI-124	0.61c	0.68e	0.58b	0.714d	0.76e	0.80h	1.95f	1.90e	1.54a	2.02f	1.87h	1.38a	0.95
IRRI-112	0.65d	0.68e	0.75g	0.76e	0.83j	0.84j	1.85d	2.10i	1.75b	2.17h	2.12j	1.53d	0.87
IRRI-128	0.58b	0.68e	0.54a	0.66a	0.76e	0.78f	2.00g	1.80c	2.20k	1.73g	1.78f	1.48c	0.93
IR65192	0.68e	0.58b	0.65d	0.70d	0.67bc	0.79gh	1.75b	1.54a	2.05h	1.92e	1.73f	1.48c	1.09
SUAKOKO	0.86h	0.72f	0.65d	0.93m	0.87k	0.89l	2.35l	2.10i	2.20k	2.56d	2.17h	1.43b	0.94
FL-478	0.61c	0.68e	0.58b	0.67ab	0.66a	0.82i	2.20k	2.05h	2.50m	1.73f	1.82f	1.53d	1.17
Salinity level (s.e.d)		0.001			0.001			0..3			0.001		0.01
Genotype (s.e.d)		0.002			0.001			0.005			0.001		0.02
Genotype x salinity level		0.003			0.002			0.009			0.002		0.03

Appendix 15: Effects NaCl on dry weights of rice genotypes

	RDW			SDW			Root/Shoot Ratio		
Genotypes	**0mM NaCl**	**50 mMNaCl**	**100mM NaCl**	**0mM NaCl**	**50 mMNaCl**	**100mM NaCl**	**0mM NaCl**	**50 mMNaCl**	**100mM NaCl**
FL478	0.65J	0.37d-h	0.30a-f	1.19d	0.90b-d	0.71a-c	68.60o	47.20k	42.2j
IR65192-4B-10-3	0.56h-j	0.27a-f	0.21a-e	0.90b-d	0.71a-d	0.68a-d	61.60l	38.10h	31.10c
IRRI-112	0.57h-j	0.33c-g	0.21a-e	0.94cd	0.90b-d	0.64a-d	61.30l	36.70g	32.30d
IRRI-113	0.61ij	0.31a-f	0.15a-c	0.88b-d	0.73a-d	0.44a-c	69.60p	42.00j	34.90f
IRRI-124	0.45f-j	0.18a-f	0.09a	0.72a-d	0.54a-c	0.33ab	63.60m	33.20e	29.10b
IRRI-128	0.52g-j	0.27a-f	0.18a-d	0.99cd	0.69a-d	0.46a-c	66.30n	40.20i	38.40h
NERICA-L-19	0.61ij	0.32b-g	0.16a-d	0.78a-d	0.69a-d	0.46a-c	78.90q	46.70k	35.40f
SUAKOKO-10	0.39e-i	0.16a-d	0.11ab	0.61a-c	0.47a-c	0.27a	66.00n	35.20a	27.80f
Salinity level (s.e.)		0.02			0.05			0.06	
Genotpe (s.e.d)		0.03			0.09			0.1	
Salinity x Genotype (s.e.d)		0.06			0.15			0.17	