KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

KUMASI

COLLEGE OF AGRICULTURE AND NATURAL RESOURCES

DEPARTMENT OF CROP SCIENCE

BREEDING FOR TOLERANCE TO SALT STRESS IN RICE USING A NEW TOLERANCE DONOR, MADINA KOYO.

BY

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THIS THESIS IS SUBMITTED TO THE KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY DEGREE IN PLANT BREEDING

NOVEMBER , 2019

DECLARATION

I hereby declare that except for references to works of other researchers, which have been duly cited, this work is my original research and that neither part nor whole has been presented elsewhere for the award of a degree.

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ABSTRACT

Salt stress is a major constraint that reduces productivity of many rice fields across the world. Susceptibility of the crop comes within the first few weeks of establishment and later during the reproductive stage. Many studies conducted to improve the adaptability of the crop to saline soils has focused on introgressing alleles from a few donor genetic background. To broaden the genetic base of tolerant alleles, a new salt stress donor cultivar, "Madina Koyo" was used in developing a set of introgression lines with a farmer preferred indica variety, „Sahel 317". To identify lines that are tolerant to the stress at the two most susceptible stages, F_2 derived F_3 lines ($F_{2:3}$) and $F_{3:4}$ lines were evaluated at the early seedling and reproductive stages respectively. To better understand the regions controlling tolerance in this new donor and to validate previously reported regions, QTL mapping was undertaken using

Single Nucleotide Polymorphism (SNP) analysis from Genotype by sequencing (GBS). Salt stress caused a drastic reduction of 72% in grain yield among the $F_{2:3}$ progenies. Altogether, an average reduction of 63.4% was observed for all fitnessrelated traits among selected $F_{3:4}$ progenies at the early seedling stage. A total of 45 progenies had better or comparable grain yield to the donor parent "Madina Koyo" and were adjudged to be tolerant at the reproductive stage. Subsequently, another 46 progenies, representing 5% of the evaluated progenies, recorded an average SES score 1-3 and were rated as tolerant to salt stress at the early seedling stage. Out of the combined 91 progenies, only one progeny, ARS1181-1-6-27, was observed to be tolerant to salt stress at both growth stages. Another four progenies (ARS1181-1-7-6, ARS1181-1-6-6, ARS1181-1-8-26 and ARS1181-1-10-1) combined tolerance at the seedling stage with better yield stability. These five promising progenies should be further evaluated with the aim of releasing as *per se* or be used as parents in future

forward breeding efforts. A high-density genetic linkage map was constructed for the 12 rice chromosomes using 3698 SNP markers. Composite interval mapping identified 46 QTLs on 10 chromosomes (1, 2, 3, 4, 7, 8, 9, 10, 11 and 12) for SES, shoot length, shoot dry weight and root dry weight. No QTL was identified for root length. Thirty-three out of the 46 QTLs were shoot-related QTLs while five were root related. Breeders should, therefore, focus on shoot related traits when evaluating rice germplasm for salt tolerance at the seedling stage. None of the QTLs identified was mapped in the region of previously reported major effect QTL *Saltol* on chromosome 1, suggesting that Madina Koyo controls tolerance from a different region by a different QTL(s). Six out of the 46 (*qSDW1.1, qSDW2, qSL1.1, qSL2.2, qSL2.3* and *qSL2.4*) were major effect QTLs with phenotypic contributions ranging from 11% - 99%. Comparison with literature suggests the novelty of these major effect QTLs. Fine mapping of these novel QTLs in a different genetic background is necessary to confirm their stability and use in breeding to enhance the level of tolerance through MAS for the pyramiding of different QTLs to one genetic background

DEDICATION

To my parents: Mr. Stephen Bentil Amoah (Late) and Madam Agnes Alice Osae.

Papa and Maa, your wish for me has materialized!

ACKNOWLEDGEMENTS

My profound gratitude to Africa Rice Center (AfricaRice), who, through the Stress Tolerant Rice for Africa and South Asia (STRASA) project funded by the Bill and Melinda Gates Foundation, generously offered me the scholarship to pursue the PhD study at the Kwame Nkrumah University of Science and Technology (KNUST), Ghana. My profound gratitude also goes to my supervisors Professor Richard Akromah, Dr. Alex Wireko Kena and Dr. Isaac Kofi Bimpong for their patience and invaluable guidance into research activities and writing of this thesis. Many thanks for the mentorship!

A very big thank you to the Head, Capacity Development Unit, Dr. Khady Nani Dramé and her assistant Dalie Lucie for their administrative support that ensured timely release of funds for fees and research purposes. I am grateful to the AfricaRice team in the Sahel station in Senegal notably Dr. Baboucarr Manneh, Mamadou Sock, Faty Diaw, Anta Gueye, Mor Diaw, Soulé Samba Ba and Coulibaly Tiemoko for their administrative and technical support, not forgetting all the field and laboratory support staff, without whose assistance, this thesis would not have seen the light of day. I appreciate the AfricaRice Team in Mbe Station, Cote d‟Ivoire especially, Nadege Loukou, Jean Kuoassi Konan, Rein Amon Kimou, Guillaume, Robert Koffi Kouakou, Adjoua Kouadio and Luc Sonon Konan for their technical support in experimental implementation and data collection.

Sincerest of thanks to the Excellence in Breeding (EiB) Platform, the Integrated Genotyping Service and Support (IGSS, Kenya) for the collaboration that provided subsidized charges on genotyping services and training on data analysis. My heartfelt gratitude to the Genomic Open-source Breeding Informatics Initiative (GOBii) and the High Through-put Genotyping (HTPG) project teams especially Drs. Elizabeth Jones,

Star Gao and Eng Hwa Ng, for their timely and holistic training in SNP data analysis. I am particularly grateful for the support I received from Milcah Kigoni on data analysis, many thanks Milcah.

I could not have written this thesis without the strong moral support and encouragement I received from my family. To my amazing wife Mrs. Dorcas Agyeman Amoah, my boys Michael Charles Kwabena Amoah and Nana Kofi Abaka Amoah Jnr., I say a big thanks for lifting me up during all those moments when I had a mind-block. To my mum, Agnes A. Osae and siblings DR. Michael Amoah, Mrs. Adwoa Edua Yeboah and Paa Kwesi Amoah, your diverse support and prayers are very much appreciated. Now, to Kofi Konadu Boateng, my demands were as though you were the one pursuing this degree. Not even once did you hesitate, Kofi, you are a good human being! I cannot thank you enough. To Mrs. Mercy Okyere-Larbi, Alfred Asamoah, Daniel Oppong-Sekyere, Grace Odoso Osei and Bright Boakye Preprah, you guys rock; I will forever be in your debt.

Through it all, glory be to God Almighty for making all this possible.

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LIST OF ABBREVIATIONS

ABA	Abscisic Acid
AC	Anther culture

APX	Ascorbate Peroxide
AsA	Ascorbic Acid
BecA	Biosciences eastern and central Africa
Ca^{2+}	Calcium ions
CAPS	Cleaved Amplified Polymorphic Sequence
CAR	Companion to Applied Regression
CAT	Catalase
Cl^-	Chlorine ions
CO_2	Carbon Dioxide
CSSRI	Central Soil Salt Stress Research Institute
DArT	Diversity Arrays Technology
DEGs	Differentially expressed genes
DH	Double haploids
DNA	Deoxyribonucleic Acid
EC	Electrical Conductivity
ECe	Electrical Conductivity of extracts
FAO	Food and Agriculture Organization of the United Nations
GA	Genetic Advance
GAm	Genetic Advance as percentage of mean
GBS	Genotype by Sequencing
GMO	Genetically Modified Organism
GO	Gene Ontology
GR	Glutathione Reductase
GSH	Reduced Glutathione
GWAS	Genome-wide Association Studies

GxE	Genotype by Environment Interaction
h	Heritability
H_2O_2	Hydrogen peroxides
HKT	High affinity Potassium Transporters
ICAR	Indian Council of Agricultural Research
IGSS	Integrated Genotyping Service and Support
ILRI	International Livestock Research Institute
InDel	Insertion/Deletion
IRRI	International Rice Research Institute
LG	Linkage group
LOD	Logarithm of Odds
LSD	Least Significant Difference
MAS	Marker Assisted Selection
Na^+	Sodium ions
Na^+/K^+	Sodium to Potassium ratio
NaCl	Sodium Chloride
NHX	Na^+/H^+ antiporters
O_2^-	Superoxides
^-OH	Hydroxyl radical
QTL	Quantitative Trait Loci
RFLP	Restriction Fragment Length Polymorphism
RIL	Recombinant Inbred Line
ROS	Reactive Oxygen Species
S	Selection Differential
SD	Standard Deviation

SES	Salt Evaluation Score
SIS	Salt Injury Score
SNP	Single Nucleotide Polymorphism
SOD	Superoxide dismutase
SOS1	Salt Overly-sensitive 1
SSR	Simple Sequence Repeats
TDS	Total Dissolved Solutes
USDA	United State Department of Agriculture

CHAPTER ONE

1.0 GENERAL INTRODUCTION

Rice (*Oryza sativa*) was domesticated some 8000 years ago and has since played diverse pivotal roles in civilization and most importantly, feeding the ever-growing populations worldwide. Second only to maize as food cereal, rice has become a daily necessity, nourishing million homes and providing an income source for several others in every step of the rice value chain. Estimates from over 20 years (19942016) shows Asia to be responsible for more than 90% of world rice (FAO, 2018) with China, India, Indonesia, Bangladesh, Vietnam and Thailand as the leading producers (USDA, 2017). With a global rice production projected at 486,730,00 metric tons in the 2016/2017 market year, China and India combined produce more than half of world‟s rice (52%). However, China consumes most of thier production and even requires additional imports making the country the biggest importer of rice. Though Asia is the highest consumer of rice, the region remains the biggest rice exporter, with India as the world‟s leader. Average per capita consumption of rice from 2000 to 2017 is estimated at 53.8 kg/person worldwide (Statista, 2018).

Egypt is the highest rice producer in Africa followed by Nigeria, Madagascar, Tanzania, Mali and Cote d‟Ivoire, in that order (USDA, 2017). In most parts of the continent, rice is arguably the fastest growing food source, replacing majority of traditional staples in most homes. In Ghana for example, rice was seen as a luxury food and eaten on special occasions but now, its consumption is regular, probably because rice is easier and quick to cook, readily available and affordable. This phenomenon is driving a massive expansion in the rice sector among several African countries with regards to imports since the continent is nowhere near selfsufficiency. With a total of 41.1 million tons of rice imports worldwide, Africa accounted for 13.9 million tons representing 34% of global imports in 2016 (FAO, 2018), placing a heavy drain on

scarce foreign reserves of already financially challenged countries in the region. The need to increase rice production in Africa to drive cuts in rice importation and preserve foreign exchange reserves is of great importance.

Despite rice being a semi-aquatic crop with the possibility to maintain lower concentrations of salts as a result of diluting effect from the water in its natural habitat, it remains one of the most susceptible cereals (Munns and Tester, 2008). Salt stress, as low as 3 dS/m is enough to cause significant yield loss in rice (Grattan *et al*., 2002). About 20% of the total arable lands around the world and 30% of irrigated rice ecologies are afflicted by high salt stress (Tanji, 1990; Wu and Garg, 2003; Shrivastava and Kumar 2015). The crop is particularly susceptible to salt stress during the early seedling and the reproductive growth stages (Ismail *et al*. 2007; Singh *et al*., 2010). More damaging is the fact that these figures are increasing by the day with an annual estimation of 10% for various climate change factors such as low precipitation, high surface evaporation, weathering of native rocks, irrigation with saline water, and poor cultural practices (Shrivastava and Kumar 2015). It has been predicted that more than 50% of arable land worldwide would be salinized by the year 2050 (Jamil *et al*., 2011). This massive reduction in agricultural lands comes in the era where population growth and urbanization are competing with agriculture for land space. To be able to feed the world during and beyond the said 2050, significant increase, approximately 50% or more, in yields of major food crops such as rice, wheat and maize should be achieved (Godfray *et al*., 2010) on a rather much-reduced land area. There is, therefore, an urgent need to improve the productivity of rice under saline conditions.

Water and soil management practices such as flushing fields with fresh non-saline water, application of gypsum, organic matter, phytoremediation and use of plant growth-promoting bacteria, have facilitated agricultural production on soils

marginalized by salt stress but an additional gain from these approaches seems problematic (Zahir *et al*., 2008). Improving the crop‟s ability to adapt well and be productive under saline conditions through breeding seems sustainable and economically viable option to ensure the attainment of food security. However, not much success has been made in developing varieties tolerant to salt stress due to the complex nature of the stress. In most fields, salt stress occurs heterogeneously, causing a high genotype x environment (GxE) interaction which greatly affects heritability of the trait measured under saline conditions. Salt stress also triggers several nutrient deficiencies and or toxicities, making accurate evaluation of test entries more difficult. Consequently, it is important to have in place protocols that efficiently eliminates environmental bias and at the same time discriminate among test entries at the susceptible stages (early seedling and reproductive stages). Gregorio *et al*. (1997) designed a screening technique based on salinized nutrient solution devoid of the heterogenous nature of field salt stress to evaluate rice at the early seedling stage for tolerance to salt stress. This system is easy to implement and can handle large sample numbers at a go. It allows for first level discrimination where only lines tolerant at the seedling stage are screened at the second susceptible growth phase (reproductive stage) so that, resulting promising lines will be productive at both critical growth stages as far as salt stress is concerned.

Another limitation in developing salt stress tolerant varieties is the mode of inheritance as a trait. Like other abiotic stresses, salt stress tolerance is quantitatively inherited, thus governed by several loci or regions acting together in the genome.

Identification of these genomic regions or Quantitative Trait Loci (QTLs) that dictate tolerance to salt stress is key in developing salt stress tolerant varieties. Example of one such QTL with large effect that has contributed tremendously to the development

of salt tolerant rice varieties is *Saltol*. This QTL was mapped onto the shorter arm of chromosome 1 (Gregorio 1997; Bonilla *et al*., 2002). After several decades of QTL mapping, it still remains one of the most common approaches for the genetic dissection of quantitative traits and provides the basis for map-based cloning of genes. Following fine mapping of identified QTLs, specific markers can be designed to complement conventional breeding by way of accurate selection and rapid advancement of desired progenies in marker-assisted selection (MAS).

Recent advances in molecular biology has produced technologies which so well complement conventional breeding that development of new plant varieties is now achieved with much accuracy, relatively faster and affordable. Sequencing plant genomes is now achieved with much ease and precision. Information that a sequenced genome provides is crucial in dissecting the genetic basis of salt tolerance, cloning of genes involved and development of molecular markers. Through genome sequencing, geneticists have developed by far, the most precise and abundant molecular markers called Single Nucleotide Polymorphism (SNPs) which allows exhaustive genotyping in QTL related studies anchored by Genotype by Sequencing (GBS; for bi-parental population) or genome-wide association studies (GWAS; for mostly unrelated individuals).

There is a wide genetic diversity in rice for salt tolerance making it possible to develop new cultivars that are tolerant to salt stress. Whiles most cultivars are susceptible, some have been found to possess better tolerance to salt stress, especially landraces such as „Pokkali" and „Nona Bokra" (Thomson *et al*., 2010; Platten *et al*., 2013; Ahmadi *et al*., 2011; Ul-Haq *et al*., 2010). However, these two have been used severally as donor sources in a number of breeding programs, making narrow, the resulting genepool. It is, therefore, important to identify new donor sources with similar or better adaptation

to ensure sustainable development of salt tolerant varieties. Bimpong *et al*. (2011) identified new sources of donors that have a different allele from that of „Pokkali" and „Nona Bokra" following their exploration to the Casamance region of Senegal; the center of origin for the African rice, *O. glaberrima*. Among such newly identified donor genotypes was a cultivar called „Madina Koyo". This new genetic resource or donor, should have potential in creating new tolerant varieties with different genetic base. It should also allow for the pyramiding of tolerance genes to create even far more tolerant varieties. However, to reliably and efficiently utilize these new donors in breeding programs, it is imperative to elucidate the genetic basis of its tolerance, through molecular profiling of its genome using GBS and QTL mapping.

The objectives of this study were, therefore to;

i. Develop mapping population between Madina Koyo and farmer preferred variety Sahel 317.
ii. Identify progenies with better adaptability to salt stress at the early seedling stage.
iii. Identify progenies with better adaptability to salt stress at the reproductive stage.
iv. Identify QTL controlling tolerance to salt stress among the population using high density SNP markers through GBS.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Rice; production ecology, utilization and importance

Rice belongs to the genus *Oryza* which comprises 25 species and 10 genomes (AA, BB, CC, BBCC, CCDD, EE, FF, GG, HHJJ and HHKK) from the tribe Oryzeae and family Gramineae (Poaceae) (Vaughan, 1994). Of these 25, only two (which are diploids with 12 gametic chromosomes (2n=24)) are domesticated leaving the rest as

wild relatives (Vaughan, 1994; Brar and Khush, 2003). Among the two domesticated is the widely cultivated *Oryza sativa* of Asian origin and the lesser cultivated *Oryza glaberrima* native and cultivated solely in West Africa. *Oryza rufipogon*, a perennial and *O. nivara*, an annual species have been identified to be the progenitors of *O. sativa* whiles *O. barthii* and *O. longistaminata* are designated as the progenitors of *O. glaberrima* (Vaughan and Morishima, 2003). *Oryza sativa* contains two major subspecies: *indica* and *japonica*. The most cultivated, *indica*, has a non-sticky long slender grain and is grown mostly under lowland submerged conditions. *Japonica* on the other hand, has a bold short-grain that is sticky when cooked. There are two variants of japonica; those usually cultivated in dry fields, in temperate East Asia, upland areas of Southeast Asia, and high elevations in South Asia (*temperate Japonica*) and those that thrive under tropical conditions called *tropical japonica* or *javanica*.

Based on the source of water for rice cultivation (either rainfall, irrigation, water table, uncontrolled flood water and/or sea/brackish water), rice cultures are classified into 6 ecologies; rainfed upland, rainfed lowland, irrigated upland, irrigated lowland, mangrove-swamp and deep-water (Diagne *et al*., 2013).

Upland ecology, as the name implies, is located at the high end of the toposequence, where rice depends solely on rainfall as the water table is out of the reach of rice roots for much of the growing season (Figure 2.1). Conversely, lowland ecology is situated at the lower end of the toposequence, where rice plants can reach the water table or profit directly from flood water. Along the toposequence, interactions exist between water and nutrient flow from upland to lowland. These interlinkages can blur the boundaries between rice environments, as in the hydromorphic zones, between the upland and lowland areas, which rather than being flooded for long periods have a water table close enough to the surface for rice roots to reach during the growing season (Diagne *et al*., 2013). Another unclear transition exists between rainfed and irrigated lowlands, where a continuum of water management exists ranging from the strictly rainfed (no water control or only drainage), which may evolve (via investments in water control measures) to the fully irrigated lowlands (Diagne *et al*., 2013).

Upland	Hydromorphic	Lowland	Intensified Lowland	Irrigated Lowland
Rainfall	Rainfall, water table	Rainfall, water table, flood water	Regulated flood water	Irrigation

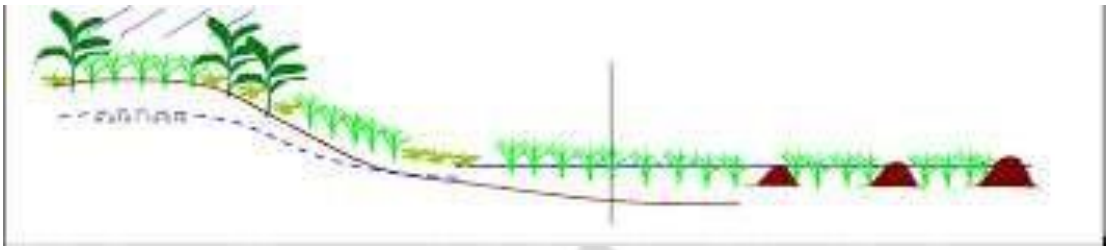


Figure 2.1. Major rice environments and their attributes in Africa. (Adapted from WARDA, 2004, reproduced with permission from Africa Rice Center).

Rice is the food source for almost half of the world population (GRiSP, 2013).Compared to other important cereals such a s wheat and maize, rice produces more food, energy and protein supply per hectare, hence, the crop supports more people per unit of land than the two other staples (Lu and Chang, 1980). Rice is a source of the B vitamins; thiamine, riboflavin and niacin with a high glutamic and aspartic acids, but low in lysine profiles (Grist, 1986). However, milling of brown rice into white rice reduces by half most of the vitamins and the iron contents though some amino acids, especially lysine, are less affected by the milling process (Kik, 1957; Mickus and Luh 1980; Juliano, 1985a). Rice is low in sodium and fat and is free of cholesterol hence contributes to diets targeted at preventing hypertension. It is also free from allergens and is widely used in baby foods (James and McCaskill, 1983). Flour made from rice is pure in starch and free from allergens, therefore, used as a main component of face powders and infant formulas. Its low fiber content has led to an increased use of rice powder in polishing camera lenses and expensive jeweler. Rice starch can also serve as a substitute for glucose in oral rehydration solution for infants suffering from diarrhea (Juliano, 1985b). The coarse and silicarich rice hull is used as

animal feed and as a construction material. Rice also serves as a raw material mainly in the making of beer, fermented products, and rice bran oil, and rice wine remains a major alcoholic beverage in East Asia.

2.2 Soil Salt stress

Soils are said to be saline when salts accumulate to such a level that they interfere with the normal physiology of plants (that thrive there-in). Thus, salt stress can be said to be a soil condition characterized by significant accumulation of soluble salts mainly those of calcium, sodium, potassium and magnesium. Shrivastava and Kumar (2015) defines soil salt stress as one in which the electrical conductivity (EC) of the saturation extract (EC_e) in the root zone exceeds 4 dS m^{-1} (approximately 40 mM NaCl) at 25 °C and has an exchangeable sodium of 15%. The ECe quantifies the degree of soil salt stress and takes the units of either deciSiemens per meter (dS/m), microSiemens per centimeter (µS/cm), milliSiemens per centimeter (mS/cm) or milli mhos per centimeter (Mmhos/cm). In general, ECe is equivalent to the concentration of salts in saturated soil or in a hydroponic solution. Soils are categorized as saline at ECe of 4 dS/m and above (USDA-ARS, 2008). Salt stress can also be measured either as total dissolved salts (TDS, is the sum of all ions present in a sample of water and represents the total salt content of the water) in part per million (ppm) or milligram per litre (mg/L) (Table 2.1). Additionally, soil salt stress levels could also be quantified using a 2:1 ($EC_{2:1}$) or 5:1 ($EC_{5:1}$) gram water to gram dry soil mixture than saturated paste EC_e. By conversion, $EC_e = 4\ EC_{2:1}$ (ILRI, 2003).

Table 2.1. Units for measuring salt stress and conversion factors

Measurement and Units	Application	1dS/m is equal to:	Equivalent Units
Conductivity (dS/m)	soils	1	1 dS/m = 1 mS/cm = 1 mmho/cm

Conductivity (µS/cm)	irrigation and river water	1000 µS/cm	1 µS/cm = 1 µmho/cm
Total dissolved salts (mg/L)	irrigation and river water	640 mg/L (approx.)	1 mg/L = 1 mg/kg = 1 ppm
Molarity of NaCl (mM)	Laboratory	10 mM	1 mM = 1 mmol/L

Worldwide, an estimated 230 million ha irrigated land is responsible for about one third of world‟s food (FAO, 2008) of which 40% is affected by salt stress (Xiong and Zhu, 2001). Approximately, 45 million ha (representing 20%) of the total cropping system is affected by salt stress (FAO, 2008). Wang *et al*. (2003) predicted salinity to affect 50% of current cultivated land by 2050. Soil salt stress occurs via two main channels. The first, being the primary cause, occurs naturally by weathering of the underlying parent material or bedrock through the release of soluble salts of various types, mainly sodium, calcium and magnesium and in minute quantities sulfates and carbonates (Rengasamy 2002). Other natural cause of soil salt stress is the deposition of oceanic salts in wind, aquifers, rain and cyclones (Howard and Mullings, 1996; Cyrus *et al*., 1997; Bond, 1998; Sultana *et al*., 2001). Secondary cause of salt stress is predominantly due to human interventions such as indiscriminate land clearing and improper irrigation/drainage practices both of which cause a rise in the groundwater table (Domries, 1991; Szaboles, 1994).

2.2.1 Salt stress and Rice

Rice is the most important food source for more than half of the world population. The crop also provides an income source for many farmers and others in the rice value chain, making the rice industry a major contributor to economic development in many rural farming communities around the world. Unfortunately, among cereals, rice has the least adaptation to saline conditions (Munns and Tester, 2008). A threshold above the range of 1.9 - 3 dS/m has been reported to cause significant yield loss in rice

(Grattan *et al*., 2002). Rice is relatively tolerant to salt stress during germination, becomes sensitive during the early seedling stage, gains tolerance during active tillering, becomes sensitive during the reproductive stage and then becomes relatively more tolerant at maturity (Figure 2.1; Pearson and Bernstein

1959; Akbar *et al*., 1972; Heenan *et al*., 1988; Lutts *et al*., 1995; Ismail *et al*., 2007, Singh *et al*., 2010). However, tolerance to salt stress at early seedling and reproductive stages have been found to be weakly correlated (Moradi *et al*., 2003) suggesting that different genes and biochemical pathways are responsible for the tolerance mechanisms at the two respective growth stages. Though tolerance at reproductive stage is very important, as it determines yield, tolerance at the seedling stage is of equal importance because good crop establishment is a pre-requisite for good crop yield. Salts of sodium, particularly, sodium chloride (NaCl) seems the most soluble hence has resulted in many works focusing on NaCl (Jamil *et al*., 2012,

Platten *et al*., 2013, Bimpong *et al*., 2014, Ali *et al*., 2014, Bimpong *et al*., 2016).

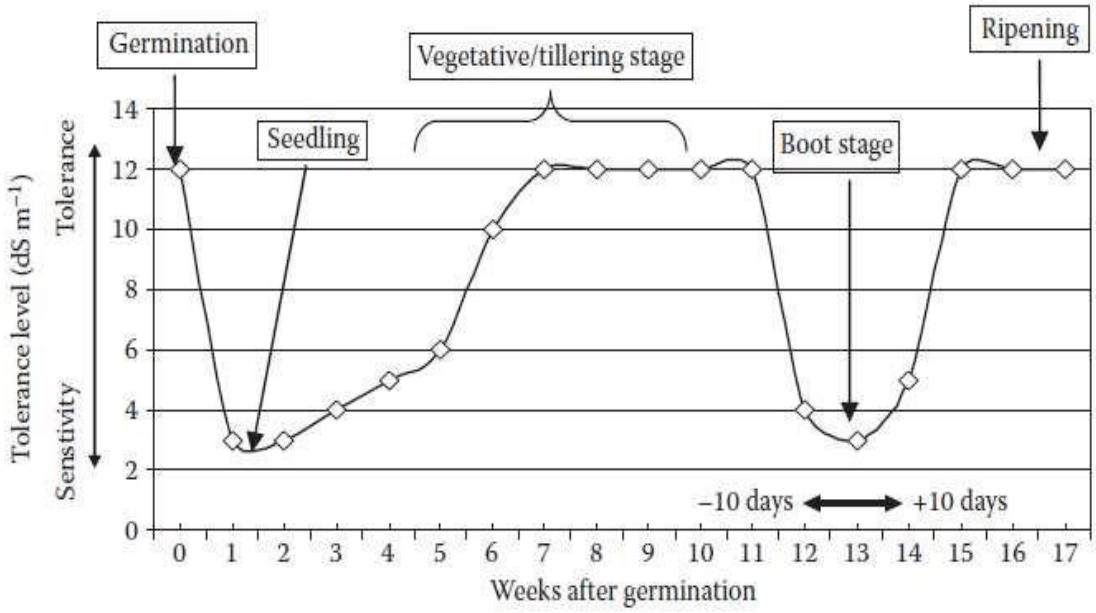


Figure 2.2. Variation in the sensitivity of rice to salt stress during its ontogeny. (Adapted from Singh *et al*., 2008)

2.2.2 Tolerance at early seedling stage

Rice is highly sensitive to salt stress during the first few weeks (1-3) after transplanting (Khatun and Flowers, 1995, Lutts *et al*., 1995, Makihara *et al*., 1999, Singh *et al*., 2004, Shereen *et al*., 2005, Singh *et al*., 2008; Singh and Flowers, 2010). The initial response of rice to salt stress is in the form of an osmotic stress where plants regulate water loss due to plasmolysis resulting from the hypertonic conditions created in the region of plants‟ roots (Munns and Tester, 2008). In rice and other cereals, salt exclusion from leaves is the most important salt tolerance mechanism during the seedling stage (Genc *et al*., 2007, James *et al*., 2011, Platten *et al*., 2013, Adem *et al*., 2014, Ismail and Horie, 2017). Selective ion uptake by compartmentation of harmful ions into older tissues (leaves and leaf sheaths) reduce Na^+ accumulation and prevent its building up to toxic concentrations in photosynthetically active tissues (Gerona *et al*., 2019). At the seedling stage, salt stress causes decreased cell division and growth which results in stunted leaf growth, decline in the rate at which new leaves and lateral buds emerge (Munns and Tester, 2008) and decreased concentration of chlorophyll content (Ravikiran *et al*., 2018) in rice. Under salt stress, ability of plants to maintain higher concentrations of antioxidants and osmoprotectants such as superoxide dismutase (Kao, 2017), ascorbate peroxidase (Vighi *et al*., 2017), reduced glutathione (Wu *et al*., 2015), catalase (Gill and Tuteja, 2010) glycine betaine (Hasanuzzaman *et al*., 2014), and trehalose (Mostofa *et al*., 2015) are vital in scavenging generated reactive oxygen species (ROS) thereby improving the protection of plants against salt stress. The ROS scavenging systems play a key role in mitigating salt stress effects in rice at the early seedling stage (Moradi and Ismail, 2007). Höller *et al*. (2015) reported an increase in ascorbate levels in leaves of rice plants under salt stress at the vegetative stage, which might be due to the physiological role of ascorbate in reproductive development. Accumulation of excessive ROS disrupts cellular

metabolism through oxidative damage of lipids. When ROS generation exceed the capacity of the plants to scavenge them, lipid peroxidation in biological membranes increases, causing serious damage to organelles like mitochondria, chloroplasts and plasma membranes. Reduced ascorbate is an important metabolite which can directly scavenge ROS through enzymes of the ascorbate-glutathione cycle involving ascorbate peroxidase, or non- enzymatically by reducing H_2O_2 directly to water (Evans *et al*., 2016). In addition to the upregulation of ROS-scavenging systems, synthesis and accumulation of compatible solutes such as proline, which stabilizes the structure of proteins, is another strategy for tolerance of salinity (Szabados and Savouré, 2010). Kibria *et al*. (2017) reported significant increase of proline accumulation in the salt-tolerant genotype „BINA dhan 10" during the vegetative stage, which might be associated
with its salt tolerance.

The sensitivity of rice to salt stress at the early seedling stage has resulted in numerous studies imposing salt stress within the first few weeks after germination in an attempt to discover tolerant genotypes (Islam and Karim, 2011; Shakeela *et al*., 2015; El-Mokhtar *et al*., 2015; Chunthaburee *et al*., 2016; Bizimana *et al*., 2017; Rhaman *et al*., 2017). The international Rice Research Institute (IRRI) has developed a simple hydropic protocol for this purpose (Gregorio *et al*., 1997). Following this protocol, several studies have discriminated tolerant genotypes from susceptible ones using different rice populations including F_2 (Sabouri *et al*., 2009), F_3 (Kaushik *et al.*, 2003); F_6 (Bizimana *et al*., 2017; De Leon *et al*., 2017), recombinant inbred lines RIL (Wang *et al*., 2012; Rhaman *et al*., 2017), induced mutant lines (Zeng and Shannon, 2000) and landraces (Ali *et al*., 2014; Shakeela *et al*., 2015). Bimpong *et al*. (2014) evaluated 300 recombinant inbred lines (RILs) for tolerance to salt stress at

ECe of 12dS/m and reported varying response of the progenies to salt stress. Based on salt injury score, plant vigour and biomass, the authors observed transgressive segregation among the RIL population where 10% of the RILs performed better than the donor parent, Hasawi. Transgressive segregation which indicates genetic variability and scope for improving tolerance to salt stress beyond parental fitness has been reported in different rice populations (Kaushik *et al.*, 2003; Sabouri *et al*., 2009; Wang *et al*., 2012; Ali *et al*., 2014; Bizimana *et al*., 2017). Contrasting findings has been reported by De Leon et al. (2016) where none of the F_6 progenies studied outperformed the donor parent „Pokkali".

2.2.3 Tolerance at the reproductive stage

The deleterious effect of salt stress on rice at the reproductive stage has been demonstrated by several studies (Rana *et al*., 2009; Thomson *et al*., 2010; Nakhoda *et al*. 2012; Bimpong *et al*., 2014; Tiwari *et al*., 2016). At the reproductive stage, reduction in chlorophyll concentration caused by salt stress reduces photosynthetic rate of rice plants (Dionisio-Sese and Tobita, 2000, Moradi and Ismail, 2007, Morales *et al*., 2012). Accumulation of sodium to levels that are toxic to rice genotypes disrupts ionic balance and membrane stability, leading to the detachment of plasma membrane from the cell wall (Mitsuya *et al*., 2002), altered orientation of the grana, swelling of thylakoids and distortion of grana lamellae (Zahra *et al*., 2014). These impact the chlorophyll content in leaves which in turn has direct implications on photosynthetic capacity of plants under salt stress conditions. It has been proposed that a GA-dependent pathway plays an important role in flowering time under salt stress by regulating the biosynthesis levels of GA_4 (Achard *et al*., 2006). Li et al. (2007) reported a rapid restoration of flowering in *Arabidopsis* when GA_4 was applied to plants under salt-stressed conditions. The researchers also argued

that, delayed flowering under salt stressed conditions could be an adaptive mechanism where plants conserve energy to maintain ion homeostasis rather than transition from vegetative to reproductive stage. Salt stress also disrupts the normal development of spikelets (Asch *et al*., 1999, Saeedipour, 2014). Two separate pathways have been proposed for the uptake of Na^+ into rice panicles; one independent of transpiration and the other driven by panicle transpiration (Asch *et al*., 1999). The Na^+/K^+ ratios in the reproductive stage of rice genotypes have been found to be lower than those in vegetative stage under salt stress (Gerona *et al*., 2019; Abdullah *et al*., 2001). These reports show that sodium accumulation in rice genotypes decreases progressively while moving up the plant towards the reproductive apex, with K^+ concentration gradient opposite to Na^+ concentration. Lower Na^+/K^+ ratios in reproductive tissues is critical during pollen development, pollination and seed formation under salt stress environments (Gerona *et al*., 2019). The increase in the biosynthesis of proline in plant tissues has been suggested to have a protective role against salt-induced oxidative damage in several plant species (Gerona *et al*., 2019; Bhusan *et al*., 2016; Biancucci *et al*., 2015, Ghosh *et al*., 2011). Proline plays an important role in protecting subcellular structures and mediating osmotic adjustment under stress conditions (Rao *et al*., 2013). Furthermore, proline has multifunctional roles including protection against ROS damage (Hoque *et al*., 2008), acting as a signaling molecule for plant recovery from stress (Szabados and Savouré, 2010), and in stabilizing proteins (Bozorgmehr and Monhemi, 2015). Studies has suggested proline to play a vital role in pollen development, since it is the most abundant amino acid in the tapetum layer supplying nutrients during microsporogenesis (Mariani *et al*., 1990, Paupière *et al*., 2014, Biancucci *et al*., 2015). This was confirmed by Mattioli *et al*., (2012) who reported male gametophytes of proline-deficient *Arabidopsis* mutants were severely

compromised. However, the precise role of proline during salt stress remains to be clearly outlined.

Susceptibility of rice to salt stress has been observed to vary among genotypes. For example, Bimpong *et al*. (2016) identified 16 introgression lines (ILs) with reduced yield penalties between 3%-26% under stress while remaining ILs suffered yield loss in the excess of 50%. Higher yield losses between 65%-73% among some F_2-derived rice populations have been reported (Bimpong *et al*., 2014). Nakhoda *et al*. (2012) observed as high as 77% yield reduction among some mutant rice lines but identified a progeny that yielded almost 140% more than the recipient parent IR64 under salt stress. A rice cultivar with 14% yield penalty was reported by Hakim *et al*. (2014) when some Malaysian rice cultivars were evaluated for tolerance to salt stress at an ECe of 12 dS/m. Surprisingly, the authors reported a yield reduction as high as 75% for „Pokkali", a popular salinity donor. Several other studies have established the varying effect of salt stress on rice at the reproductive stage, albeit, with severe yield reduction (Mahmood et al. 1999; Zeng and Shannon, 2000; Zeng *et al*., 2002; Moradi and Ismail, 2007; Rad *et al*., 2012; Mishra *et al*., 2012)

2.3 Morphological and Physiological changes caused by Salt Stress on the Rice plant

The severity of morphological expression of the effect of salt stress, to a large extent, depends on intensity of stress. At first, tips of leaves begin to burn which advances towards the inner circles causing leaf rolling and eventually reduction in the leaf"s photosynthetic area. This leads to a reduction in general growth of the rice plant. In very extreme cases, the leaves „vomits" the salts and could be seen at the tips.

Flowering is delayed and when it finally flowers, most abort so much that the florets on the panicles appear as though papery, whitish in colour (Fig 2.2). Number of grains per panicle and seed weight reduces drastically leading to a massive reduction in grain yield. Salt stress, when it occurs at the seedling stage leads to a patchy field establishment.

Figure 2.3. Morphological effect of salt stress on rice plant; (a) Poor plant establishment; (b) Leaf rolling/burning tips and (c) Aborted papery-like spikelet. *(Pictures taken at research fields of AfricaRice Sahel Regional Center, NdiayeSenegal).*

Salt stress causes a reduction in cell size and eventually a general retardation in plant growth as a result of reduced uptake of water. Hours after incidence of stress, plant cells are able to regain their volume and turgor through osmotic adjustment. However, there is a continuous retardation in cell division and elongation which then leads to slower emergence of leaves (Fricke and Peters, 2002). The resulting leaves become smaller but thicker due to decrease in area relative to depth (Munns and Tester 2008). The osmotic imbalance caused by salt stress affects the rate at which growing leaves expand whiles new leaves and lateral buds emerge more slowly or in the extreme, remain quiescent (Munns and Tester 2008). In older leaves, where no expansion occurs, dilution of accumulated salts ceases. This leads to a considerable build-up of salts in leaves and eventually causes their death. When this is not compensated for by development of new leaves, the photosynthetic capacity of plants is critically reduced,

causing further reduction in growth and eventual death of plant. Ability of certain rice cultivars to maintain vigorous growth under saline conditions has been suggested as an important tolerance mechanism. As suggested by Yeo *et al.* (1990), vigorous growth has the tendency to dilute salt concentrations in plant tissues, hence reduces salt accumulation in plant tissues. This mechanism has long been linked with adaptations of landraces such as „Pokkali" and „Nona Bokra" in saline environments (Yeo *et al*., 1990; Richards, 1992; Bohra and Doerffling, 1993).

2.4 Improving tolerance to salt stress in rice

Several approaches have been used to improve salt prone soils including proper irrigation and drainage schemes through leaching (Willardson *et al*., 1981; Kara and Willardson, 2006), phytoremediation (Salt *et al*., 1998; Qadir *et al*., 2007; Hasanuzzaman *et al*., 2014), organic amendments through the application of farm manure (Tejada *et al*., 2006; Cha-um and Kirdmanee, 2011) and chemical remediation through the supply of gypsum, calcite or calcium chloride (Mitchell *et al*., 2000; Hanay *et al*., 2004). However, the most sustainable approach has been suggested to be the development of salt tolerant rice cultivars through breeding
(Ashraf and Wu, 1994; Silvey, 1981; Poehlman, 1978; Ashraf *et al*., 1986; Watt, 1983; Ray and Islam 2008; Osei *et al*., 2014). To effectively achieve progress in breeding, there needs to be in place, genetic variability in tolerance among germplasm, well defined traits and reliable protocols that are easy to replicate.

2.4.1. Variability in germplasm with regards to salt stress tolerance

Maas and Hoffman (1977) characterized crop plants into four categories (viz tolerant, moderately tolerant, moderately sensitive and sensitive) based on yield response under varying salt stress. Though the authors classified rice as a sensitive crop, which may

be true for a broader group of cultivated rice, its generalization seems inconsistent with observations made by other researchers because the crop has demonstrated to possess natural variability in adaptability to salt stress (Zeng *et al*., 2004; Munns *et al*., 2006). More compelling in rice is the existence of intra-species variability for salt stress tolerance; a clear case of „Pokkali" (a highly salt tolerant indica cultivar) and IR29 (a highly susceptible indica cultivar), which presents a good opportunity for varietal improvement. Evidently, the development of FL478 (IR66946-3R-178-1-1), also a highly tolerant line especially at the seedling stage, from a cross between these two contrasting parents (Thomson *et al*., 2010) goes to show the importance of the inherent variability in rice. With the majority of cultivated rice falling under irrigated ecology, constant availability of water also enhances the crop"s adaptability to salt stress with the possibility of salt dilution, making rice a suitable crop for coastal saline environments (Lafitte *et al*., 2004; Singh *et al*., 2009). Genebanks of rice around the world has in storage several thousands of different rice accessions which makes the prospect of the crop"s improvement for tolerance to salt stress promising. Approximately 100,000 rice genotypes were evaluated for salt tolerance between 1969–1984 and identified about

20% of the tested entries to be tolerant with a salt injury score of 1.0 (Senadhira,

1994). In Africa, some rice landraces (Madina Koyo, Condeh Mano, Many Fingo, Jarmissa, Gold Coast Fingo, Nafisatu and Camaro) were identified to have better adaptability to salt stress following an exploration trial (Bimpong *et al*., 2011). Some cultivars from India („Nona Bokra", Getu, Cheriviruppu, Damodor, Kalarata 1-24 and Pat), Bangladesh (Ashfal, Patnai Balam, Jamainaru, Horkuch, Lakshmikajal and Morichshail) Saudi Arabia (Hasawi), Thialand (Khao Seetha), Indonesia (Ketumbar) and Vietnam (Soc Nau) and their derivatives such as IR4630-22-2-5-1-3, IR45427-

2B-2-2B-1-1, IR51500-AC11-1 (PSBRc50), IR51500-AC17 (CSR21), IR51485AC6534-4 (CSR28), IR52724-2B-6-2B-1-1, IR60167-129-3-4, IR63731-1-1-4-3-2, IR65192-4B-10-3, IR66946-3R-78-1-1 (FL378), CSR10, CSR13, CSR27 and CSR30

have shown to be tolerant to salt stress.

2.4.2 Physiological traits related to salt stress

Tolerance to salt stress is a complex trait that is controlled by multiple factors. This makes the ability to confer tolerance by a single mechanism or trait in rice difficult. Thus, it is possible for a genotype to perform well for a particular trait yet fail under another (Yeo *et al*., 1990). Taking this into account, De Leon *et al*. (2015) did a combined multivariate analysis of 6 traits measured on some 49 genotypes and reported a high correlation between salt injury score (SIS) and the other 5 traits (ion leakage, chlorophyll reduction, shoot length reduction, shoot K^+ concentration, and shoot Na^+/K^+ ratio), making these traits unbiased estimate of a variety"s performance in response to salt stress. Earlier, SIS based on the Standard Evaluation Score (IRRI, 2014) was proposed by Gregorio *et al*. (1997) to be a good measure of tolerance to salt stress in rice as it reflects the overall performance of plants under stress. This assessment of plants, however, may suffer evaluator subjectivity (De Leon *et al*., 2015) making the direct comparison of results from other studies complicated. Root related traits under salt stress has been suggested to be less important compared to shoot parameters by De Leon *et al*. (2015) as they reported that genotypes varied significantly for shoot traits but not for root parameters. Similar to earlier findings by Yeo *et al*. (1990), De Leon *et al*. (2015) observed that, accumulation of shoot Na^+ was not any different among both sensitive and tolerant entries and that, correlation between SIS and shoot Na^+ concentration was not significant. This finding, thus, moderates the use of shoot Na^+ concentration as a measure of tolerance even though

accumulation of Na^+ has been reported to decrease K^+ concentration and K^+/Na^+. Interestingly, they proposed the use of shoot K^+ and K^+/Na^+ as important traits for evaluating rice for salt stress tolerance. Contrary, the use of K^+ concentration as a useful measure of tolerance was opposed by Pires *et al*. (2015) whiles investigating salt stress tolerance among some 56 rice genotypes. However, the use of K^+ and

K^+/Na^+ as critical traits has been suggested by several studies (Gregorio and Senadhira,1993; Koyama *et al*., 2001; Bonilla *et al*., 2002; Ren *et al*., 2005; Pushparajan *et al*.,2011). Nonetheless, it might be interesting to look at overall growth and productive traits such as plant establishment, plant height, days to flowering, leaf burning/senescence, number of productive tillers and grain yield because salt stress has been established to retard growth and plant productivity.

2.4.3 Screening Techniques/Protocol

Under field conditions, salt stress occurs erratically and creates heterogeneity in discrete patches on field plots. Complicating the problem is the possible occurrence of nutrient toxicities and deficiencies associated with salt stress. These make standardization of screening techniques difficult and has contributed to the retardation in the establishment of reliable field screening protocols. Chowdhury *et al*. (1995) developed a method to monitor Na^+ exclusion by growing rice in culture solution supplied with varying NaCl. They measured plant growth as well as internal Na^+ concentration at the roots after 14 days. By measuring the electrical potential difference between the external solution and vacuoles of outer root cells, the authors were able to estimate the driving force of Na^+ in the cell membranes, hence were able to distinguish between tolerant and susceptible genotypes. Other researchers have designed screening techniques based on salinized nutrient solution to evaluate rice at

the early seedling stage (Aslam *et al*., 1993; Gregorio *et al*., 1997), where holes were drilled into styrofaom floats supported by nylon insect proof net at the base to create wells where individual test entries were sown and evaluated. This protocol allows the evaluation at one of the critical growth stages of the crop (early seedling stage) permitting the advance of only tolerant entries for further evaluation at the second critical phase; reproductive stage. Another advantage of this protocol is its rapidness (whole experiment finishes in about a month) and the ability to handle large number of samples at a go. However, this protocol was observed not mimicking exact field conditions with regards to transpiration rate (as experiment is set up in screen/greenhouses) and that, stress induction was not gradual (Flowers *et al*., 2000).

A greenhouse pot plant experiment where soil was used as the growth medium was designed to evaluate rice genotypes for tolerance to salt stress at the reproductive stage screening (Gregorio *et al*., 1997). The researchers drilled holes 3cm from the rim of pots downwards all around the pots and lined them with fitting cloth bags made of cotton materials containing soil. Several of these pots were then arranged in larger trays initially filled with normal water and later changed to salinized water to the desired EC, at the desired growth stage. Another field technique where salt (NaCl) was dissolved in irrigation water and used to irrigate demarcated stress plots at the reproductive stage has been reported (Bimpong *et al*., 2014; Bimpong *et al*., 2016). An extension of this technique would be to create artificial fields in the form of controlled rectangular tanks where the sides and base (about 1m) from the surface are cemented and filled with typical soils pertaining to the specific salt affected area. In this way, salt stress could be imposed uniformly and evaluated under normal field conditions. However, this may be expensive to build and maintain.

Another approach based on non-destructive imaging method was proposed by Rajendran *et al*. (2009) in *Triticum*, and adapted by other researchers for different purposes (Furbank and Tester 2011; Berger *et al*. 2012; Jansen *et al*. 2014). This technique has been applied to evaluation of rice for tolerance to salt stress (Hairmansis *et al*., 2014). The authors successfully quantified the total shoot and senescent shoot areas based on visible red-green-blue and florescent imaging and distinguish between osmotic and ionic stress among the tested cultivars which themselves showed varying degrees of tolerance. The authors concluded that, imagebased technology could be an efficient method in breeding salt tolerant rice varieties.

2.5 Breeding for Tolerance in Rice

2.5.1 Parental Selection (donor/recipient parents)

The success of breeding for tolerance to salt stress depends on the accurate identification of parents that are contrasting in their adaptability to the stress. This may involve rigorous testing of available fixed germplasm already available onstation or introductions from other research facilities or germplasm collected from farmers‟ fields delineated to be salt stress hotspots. It is equally important to elucidate the mechanism underlying the tolerance so that, controlled trait or multiple trait introgression could be pursued however deemed fit by the breeder. Once a genotype has been identified as a donor source, steps should be taken to select a recipient parent that is genetically distant from the donor parent. The recipient parent is usually one that is widely accepted and cultivated by farmers due to its large number of desirable attributes but is deficient in only a few characteristics (Allard, 1960). However, most donor parents are unimproved landraces and possess several undesirable agronomic traits which may result in yield penalty while slowing the breeding cycle through linkage drag (Hasan *et al*., 2015). Recently, it has been suggested that, using elite by

elite salt tolerant varieties as parents could shorten the breeding cycle and improve genetic gain per cycle (Atlin *et al*., 2017). Nonetheless, genetic variability among initial population has been shown severally to be the basis for any successful breeding program (Akbar *et al*., 1972; Flowers and Yeo, 1981; Dudley, 1994; Brondani *et al*., 2005; Singh *et al*., 2016).

2.5.2 Genetics of Salt stress tolerance in rice

The genetics of salt stress tolerance in rice was investigated using nine-parent (3 each susceptible, moderately tolerant and tolerant cultivars) in a complete diallel where low Na^+/K^+ was found to be controlled by both additive and dominance gene effects as well as overdominance with low heritability estimates for the trait (Gregorio and Senadhira, 1993). Akbar *et al.* (1985) reported that both additive and dominance effects were present with high heritability for some traits under salt stress in rice and that, shoot length, shoot dry weight, shoot Na^+ and Ca^+ content, root dry weight, plant height and yield per plant exhibited significant additive effect with a high degree of heritability. The authors suggested these traits as promising parameters in breeding for salt stress tolerant cultivars. In a related study, estimates of genetic parameters were reported to show significant additive and non-additive gene actions for SIS, K^+, and Na/K ratio with 65%, 55% and 41% as narrow sense heritability respectively (Mishra *et al*., 2003). The authors reported that average dominance was within the range of incomplete dominance and that recessive alleles were more concentrated than dominant genes in tolerant varieties for SIS and K^+ uptake at the reproductive stage. Sterility of rice under salt stress is controlled by dominant genes (Akbar and Yabuno, 1972; 1975; 1977) and has been reported to follow a normal distribution in different populations, indicating its polygenic inheritance. Example of such is an inheritance study conducted under artificial saline conditions using F_1 and bulked F_3 generations

generated from three crosses between two tolerant and two susceptible parents (Mishra *et al.,* 1998). It is likely that, several gene actins come into play when rice plants are stressed by salinity. This goes to confirm the quantitative nature and inheritance of salt tolerance.

2.5.3 Conventional Breeding for Tolerance to Salt Stress

Breeding for salt tolerance in rice has not achieved much progress mainly because of: (1) its heterogenous occurrence in the field (Richards, 1983; Flowers and Yeo, 1995); (2) high genotype by environment (G × E) interaction, which affects heritability and renders the assessment of a genotype‟s actual breeding value unreliable (Ceccarelli 1994, Ceccarelli *et al*., 1994); (3) unavailability of precise and repeatable phenotyping protocols and facilities especially for reproductive stage field testing; (4) complications from mineral deficiencies or toxicities (Talei *et al*., 2012) and (5) limited knowledge about the mechanisms (Lin *et al*., 2004; Ghomi *et al*., 2013) for salt tolerance and associated difficulty in pyramiding several physiological mechanisms into one whiles ensuring optimum stability of other quality traits such as yield and good plant architecture. Towards breeding for salt stress tolerance in rice, nearly all conventional breeding methods such as mass, pureline, pedigree, single seed descent (SSD), backcross and recurrent selection methods have been exploited. Majority of the initial salt tolerant rice varieties including „Pokkali‟ were developed through systematic selection (Singh *et al*., 2010) such as mass and pureline selection without hybridization. The cultivars Damodar (CSR1), Dasal (CSR2), Getu (CSR3) followed similar pattern from the local traditional cultivars (IRRI, 2007; Singh *et al*., 2010). Later, other salt tolerant varieties like CSR10, CSR13 CSR23, CSR27, CSR30, CSR36, PSBRc48, PSBRc84, PSBRc86, PSBRc88 and NSIC Rc106 were developed

through hybridization breeding following methods such as pedigree breeding, shuttle breeding and other selection methods (IRRI, 2007).

Modified versions of the pedigree method have been devised to shorten the breeding cycle and save cost of breeding (Singh *et al*., 2010). Another selection method that has seen progress in salt tolerance breeding is the shuttle breeding. This method operates on the principle that, specific plant-types are needed for specific environments, so advanced breeding lines are tested in several sites to select best adaptable lines. Crosses are then made among selected promising lines and field tested again in different locations. Shuttle breeding has been used to develop salt tolerant cultivars CSR23 and CSR27 in India (Mishra, 1994). Through mutation breeding, the Central Soil Salt Stress Research Institute (CSSRI), Karnal, India, developed a salt tolerant cultivar CSR10 by crossing M40-431-24-114 and Jaya whiles advancing subsequent progeny through the pedigree method.

Some techniques such as the Single tiller approach has been used to simultaneously evaluate segregation populations under salt stress and stress-free conditions (Bimpong *et al*., 2014) where the researchers separated tillers from already established plots to re-constitute alternate plots for evaluation and mapped 75 QTLs using three different genetic backgrounds. Similarly, Khan *et al*. (2016) and Kanbar *et al*. (2010) used the Single Tiller Approach to successfully evaluate different segregating populations for tolerance to salt stress at the reproductive stage.

2.5.4 Anther Culture

Anther culture (AC) has the combined advantages of increasing speed through early attainment of homozygosity and improving breeding efficiency. Following crosses, resulting F_1 pollen are regenerated into a homozygous plant through AC techniques,

considerably reducing the breeding cycle from five years to just a year (Sripichitt *et al*., 2000). Plants generated through this procedure are called double haploids. The technique offers the opportunity to screen haploid materials at an early stage using tissue culture and allows the identification of recessive mutants. Again, AC permits the execution of several selection pressures. Singh *et al*. (2010) in their review recounted the success story of AC techniques in raising salt tolerant rice varieties involving IRRI and collaborations with other institutes. Several salt tolerant varieties have been developed through AC (Singh *et al*., 1992; Singh and Mishra 1995). Perhaps, the major contribution of AC to the development of varieties tolerant to salt stress is the development and release of the variety „Bicol" (PSB Rc50) and its extensive use as donor source for salt stress tolerance in several breeding programs in Bangladesh, Dominican Republic, Egypt, Mexico, Myanmar, Philippines and Thailand (Senadhira *et al*., 2002; Gregorio *et al*., 2002). Bicol is reported to be the first salt tolerant cultivar developed from F_1 AC following an indica-indica cross (IR5657-33-2 X IR4630-22-2-5-1-3, Senadhira *et al*., 2002).

2.5.5 Marker Assisted Selection (MAS)

Marker assisted selection as the name implies complements phenotypic selection through the use of molecular markers to (1) guide the introgression of traits and (2) select recombinants with less linkage drag (Francia *et al*., 2005). Marker assisted selection has received much consideration in the breeding of salt tolerant rice varieties largely due to the fact that they are unaffected by environment, are growth stage independent, dominance effect is absent, can screen larger numbers for particular traits in a relatively shorter period and efficient to use in early generations. Among the selection methods, it is backcrossing that has received most application of MAS. The rationale behind backcross breeding is to replace a specific undesirable gene with a

desirable alternative, while preserving all other qualities (adaptation, productivity, etc.) of an adapted cultivar or breeding line (called the recurrent parent) (Allard, 1960; Reyes-Valde´s, 2000). Instead of inbreeding the F_1 as normally done, it is repeatedly crossed with the desirable parent to retrieve desirable genotype(s). Rice, being a self-pollinating crop, fits well into backcross method because inbreeding depression which is otherwise associated with outbreeding crops (such as maize) is not an issue (Mackill *et al*., 2005).

There are three principal applications of molecular markers in MAS; (a) foreground selection (Hospital and Charcosset, 1997), (b) recombinant selection (Collard and Mackill 2007) and (c) background selection (Frisch *et al*., 1999) (Fig 2.5). Foreground selection refers to using markers that are tightly linked (5cM genetic distance preferably) to the gene of interest in order to select for the target allele or gene. The objective is to maintain the target locus in a heterozygous state (one donor allele and one RP allele) until the final backcross is completed. Then, the selected plants are self-pollinated and progeny plants identified that are homozygous for the donor allele. Markers which are tightly linked to the target gene or QTL is used to select the target locus of donor parent in early (BC) progenies on foreground selection. Recombinant selection involves selecting backcross progeny that has received the target gene as well as tightly-linked flanking markers in order to minimize linkage drag (Hasan *et al*., 2015). This could be seen as the first step in regaining the genome of the recurrent parent hence background selection. However, markers used are those that closely flank the trait. Background selection on the other hand, refers to using markers that are not tightly linked to the gene of interest with the objective of selecting against other DNA from the donor (Collard and Mackill,

2007) in an attempt to accelerate the recovery of the recurrent parent genome (i.e., to select for the recurrent parent alleles at all loci other than the target locus/trait among progenies that have already been selected for using foreground and recombinant markers).

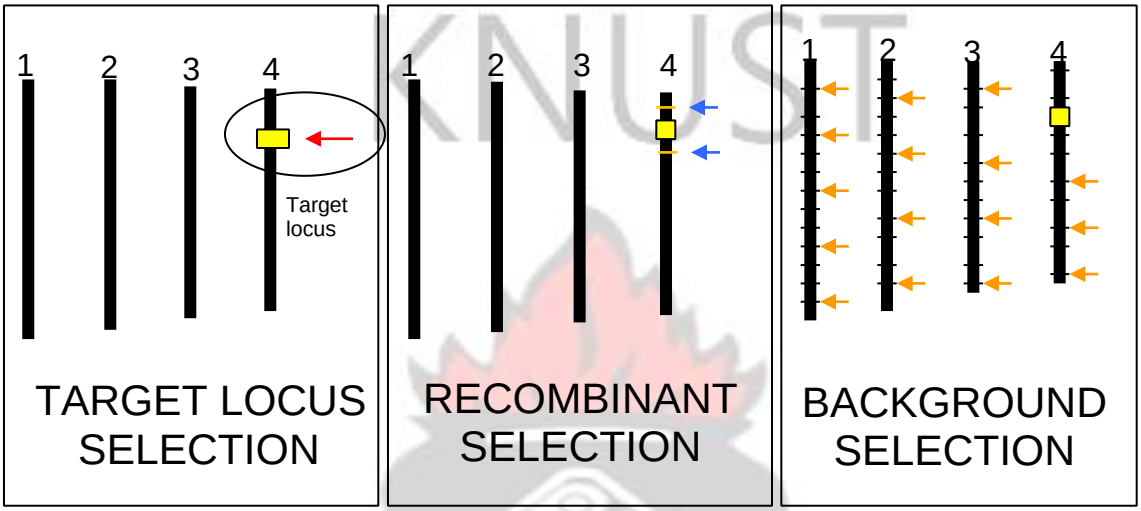


Figure 2.4. Schematic illustration of the three stages of selection applied in MAS (Collard and Mackill, 2008).

2.5.5.1 QTL Mapping for Tolerance to Salt stress in Rice

Perhaps, the most beneficial use of molecular markers in breeding is this possibility to guide chromosomal section(s) of donor parental genome dictating the expression of desirable traits. In traits that are inherited dominantly as monogenic/qualitative traits, the use of MAS is quite straightforward (Pan *et al*., 2003; Singh *et al*., 2012; Fu *et al*., 2012). However, in traits that are inherited in a continuous manner (quantitatively) or are governed by several genes acting together, MAS holds tremendous potential in guiding one to several pre-identified genes into resulting mapping population (Xiao *et al*., 1996). The regions or loci on chromosomes that dictate the expression of quantitative traits are called quantitative trait loci (QTL). The concept of QTL in breeding has brought speed and accuracy to designing climate smart rice varieties.

As a major breakthrough in the characterization of quantitative traits, QTL detection is realized through genetic linkage analysis based on genetic recombination during meiosis (Tanksley, 1993). This is accomplished through statistical methods such as single marker analysis, composite interval mapping and multiple interval mapping. These analyses are based on two main statistical approaches: regression (Korol *et al*., 1995; Calinski *et al*., 2000; Hackett *et al*., 2001; Knott and Haley 2000; Korol *et al*., 1998) and maximum likelihood, ML (Jiang and Zeng, 1995). Regression QTLmapping methods, though easier to implement and faster to compute, give biased parameter estimates with sparse markers (Xu, 1995) or when QTLs interact and are closely linked (Kao, 2000). Maximum likelihood methods are devoid of these shortcomings (Kao, 2000).

In rice, several QTLs controlling salt stress tolerance have been reported and majority have been mapped onto chromosome 1. Prominent among these are *Saltol* (Gregorio *et al*, 2002; Bonilla *et al*, 2002), QNa for Na uptake (Flowers, *et al*., 2000), QTL for Na+ uptake, K+ concentration and Na^+/K^+ ratio (Koyama *et al*, 2001); SKC1 or OsHKT8, RNTQ1, SDS1 (Lin *et al*, 2004; Ren *et al*, 2005); and *qST1* (Lee *et al*, 2006). Others with equally big-effect on other chromosomes such as chromosomes 3, 4, 10 and 12 (Gregorio, 1997); 10 (chromosomes 4, 6 and 9 (Flowers *et al*, 2000; Koyama *et al*, 2001); Chromosomes 4, 6, 7 and 9 (Lin *et al*, 2004); and Chromosome 3 (Lee *et al*, 2006) have been documented.

A total of 75 QTLs for 9 different traits have been mapped under 3 different genetic backgrounds using „Hasawi" as a donor source (Bimpong *et al*., 2014). Of these, 24 were common among all the 3 backgrounds while 31 were identified in 2 backgrounds, and 17 in a single background. The researchers identified 2 QTLs related to grain yield which were common in all 3 backgrounds and observed that, „Hasawi" contributed on

average 49 % alleles to these QTLs. Using a set of introgression lines (ILs) from salt tolerant donor line „„Pokkali““ and a susceptible high yielding rice cultivar „Bengal“, De Leon *et al*. (2017) mapped a total of 18 and 32 QTLs were detected using SSR and SNP markers, respectively. They went further to test these same markers on previously developed RIL population from these same two parents and identified 14 common QTLs among the two populations. From their analysis, they suggested that the mechanisms of tolerance could be due to Na^+ dilution in leaves, vacuolar Na^+ compartmentation, and possibly synthesis of compatible solutes. The researchers emphasized the use of salt injury score (SIS)

QTLs in marker-assisted breeding to improve salt stress tolerance. Rahman *et al*. (2017) evaluated some RIL population derived from IR29 by Hasawi population for seedling stage tolerance to salt stress in 3 locations (The Philippines, Senegal and Tanzania) and conducted QTL mapping using SNP markers and identified 34 QTLs on 10 chromosomes for SIS, shoot & root lengths and fresh & dry shoot weights. Of these, 14 QTLs on chromosomes 1, 4, 6, 8 and 12 colocalized across the three locations suggesting their stability. Using F_6 RIL of same parentage, Pascual *et al*.

(2017) mapped 17 QTLs for SIS, shoot and root lengths as well as their weights. Located on chromosome 1 were two large effect QTLs explaining 41.1% and 37.6% variation in shoot length and SIS respectively. With a similar objective, Wang *et al*. (2017) developed ILs between wild rice (*Oryza rufipogon* Griff.) and a widely grown *O. sativa indica* cultivar 93-11 as the recipient. They mapped a total of 10 quantitative trait loci (QTLs) related to salt tolerance on chromosomes 1, 5, 7, 9, 10,

11 and 12, whuch explained 2–8% of phenotypic variance. In a related study, 300 $F_{5:6}$ recombinant-inbred lines derived from a cross between IR29 (highly sensitive *indica*), Hasawi (salt tolerant *aus*) were evaluated to identify quantitative trait loci (QTLs) linked to salt stress tolerance using 194 polymorphic SNP markers that covered 1441.96

cM genome with an average distance of 7.88 cM between loci (Bizimana *et al*., 2017). The researchers identified 20 new QTLs (LOD > 3) on chromosomes 1, 2, 4, 6, 8, 9 and 12 using composite interval mapping with phenotypic variance as high as >20%. None of the identified QTLs was mapped in the *Saltol* region which made the researchers conclude that, Hasawi conferred salt stress tolerance in RILs due to novel QTLs.

2.5.6.2 The Large Effect Salinity Tolerance QTL *Saltol*

Between AFLP markers P3/M9-8 and P1/M9-3, Gregorio (1997) mapped a QTL with a large effect on chromosome 1 spanning from 14.7 cM to 18.6 cM using a tolerant F_8 recombinant inbred line (RIL) „IR66946" which was developed from IR29 x „Pokkali" cross. This QTL controlled three quantitative traits for salt stress tolerance (high K absorption, low Na absorption, and low Na^+/K^+ ratio) and explained 64.3-80.2% of the phenotypic variation with LOD of 14.5. Subsequently, Bonilla *et al*. (2002) saturated this region with restriction fragment length polymorphism (RFLP) and simple sequence length polymorphism (SSLP) and constructed linkage maps using same RIL population. They mapped *Saltol* between two RFLP markers C52903S and C1733S, (with 10.1 and 22.6 cM distance, respectively) and again between microsatellite markers, RM140 (16.4 cM) and RM23 (10.1 cM). Later, Lin *et al*. (2004) did similar studies but this time, using tolerant indica variety („Nona Bokra") and a susceptible japonica variety (Koshihikari). In addition to three QTLs identified for seedling survival days on each of chromosomes 1, 6 and 7, they identified 2 major effect QTLs; qSNC-7 for shoot Na^+ concentration on chromosome 7 and qSKC-1 for shoot K^+ concentration in the *Saltol* region on chromosome 1, explaining 48.5% and 40.1% of the total phenotypic variance, respectively. Ren *et al*. (2005) went further to isolate the SKC1 gene by map-based cloning and found that it encodes a member of HKT-type transporters. They reported that SKC1 is preferentially expressed in the

parenchyma cells surrounding xylem vessels. Voltage-clamp analysis showed that SKC1 protein functions as a Na^+-selective transporter (Ren *et al*., 2005). From their physiological analysis, it was suggested that SKC1 was involved in regulating K^+/Na^+ homeostasis under salt stress. The researchers went on to develop two very close flanking CAPS

(cleaved amplified polymorphic sequence) markers, K159 and K061. Thomson *et al*. (2010) fine mapped the *Saltol* QTL using NILs derived from the cross IR29 × „Pokkali". They saturated the peak region (10.7-12.5 Mb) with SSR and customdesigned insertion/deletion (indel) markers. They targeted four major genes for the purpose of designing gene-based markers that could be useful in fine mapping of *Saltol* and other exploits aimed at developing varieties with better adaptability to salt stress. Thomson *et al*. (2010) characterized *Saltol* for salt stress tolerance using 140 IR29/"Pokkali" RILs. In addition to the confirmation of the gene"s location on chromosome 1, they identified other QTLs associated with salt tolerance. To better characterize the effect of *Saltol* locus, they developed and analyzed a series of RILs, backcross lines and NILs and revealed that *Saltol* mainly acted to control shoot Na^+/K^+ homeostasis. On a whole, the researchers recounted that, introgression of multiple QTLs was required to achieve a high level of tolerance. Since then, several studies have mapped *Saltol* using different mapping populations (Aliyu, *et. al.* 2011; Alam *et al*., 2011; Islam *et al*., 2011; Mondal *et al*., 2013).

2.5.7 Genome-Wide Association Mapping

Complementary to bi-parental (related individuals) mapping (or linkage analysis), association mapping, involves the search for genotype-phenotype correlations among unrelated individuals (Zhao *et al*., 2007; Brachi *et al*., 2010). Linkage mapping identifies regions controlling a trait among related individuals (parents and offspring)

whiles association mapping tries to answer the question; which markers are related to the trait in question in unrelated population. Association mapping is usually faster and cost effective compared to linkage mapping. One critical advantage GWAS has over linkage mapping is the ability of the former to identify QTL regions more precisely through the identification of associated markers (Korte and Farlow, 2013). This higher precision might probably be as a result of the characteristically lower linkage disequilibrium that exist in natural populations vis a vis that of mapping populations (Balasubramanian *et al*., 2009). With the rapid increase and availability of cheap DNA sequencing and other genotyping technologies which has resulted in a boost in the development of SNP (single nucleotide polymorphism) and InDel (Insertion/Deletion) markers, GWAS is conducted with much more precision and accuracy. GWAS, thus, has great potential for identification of valuable natural variations in trait-associated loci, as well as allelic variations in candidate genes underlying quantitative and complex traits, including those related to growth, development, stress tolerance and nutritional quality (Kumar *et al*., 2015). Rice being homozygous in nature, GWAS makes it possible to genotype or sequence once and build the so called „training population" whose genotypic information will subsequently be used to validate phenotypic calls from other populations (Zhao *et al*., 2011). GWAS thus has tremendous significance for the identification of genes, particularly, those of complex quantitative traits in rice such as tolerance to salt stress. For example, Kumar *et al*. (2015) used 220 rice accessions and over 6000 SNPs for GWAS and identified 20 SNPs (loci) significantly associated with Na^+/K^+ ratio and another 44 associated with other traits observed under salt stress condition. In their study, the *Saltol* locus, which controls tolerance at the seedling stage was found to associate with reproductive stage Na^+/K^+ ratio. Additionally, GWAS peaks were observed on chromosomes 4, 6 and 7 preempting the presence of new QTLs on these chromosomes. Based on temporal

imaging data from 378 diverse rice genotypes maintained under 14 days of 90 mm NaCl stress, Campbell *et al*. (2015) developed a statistical model to assess the genetic architecture of dynamic salt stressinduced growth responses in rice germplasm and reported a strong association on chromosome 3 for early growth response. The researchers also observed 4 genomic regions linked to salt stress-induced fluorescence responses. Based on their findings, a region on chromosome 1 which regulates both the ionic stress and the early growth rate decline during salt stress was also identified. Al-Tamimi *et al*., (2016) conducted an association study using a diversity panel of 553 individuals. Using a new association model that takes into account the interaction between treatment (control and salt) based on high throughput non-invasive phenotyping together with a 700k SNP high-density genotypic data, the researchers mapped a new locus associated with transpiration use efficiency under salt stress on chromosome 11. Recently,

Meyer *et al*. (2016) studied the domestication history and geographic adaptation of *Oryza glaberrima* Steud., and conducted GWAS for tolerance to salt stress following the sequencing of 93 landraces. Among six salt tolerance traits studied, 11 significant loci were identified.

CHAPTER THREE

3.0 EVALUATION OF $F_{2:3}$ POPULATION FROM SAHEL 317/MADINA KOYO FOR TOLERANCE TO SALT STRESS AT THE REPRODUCTIVE STAGE.

3.1 Introduction

Rice (*Oryza sativa* L.), a leading world staple has limited adaptability to salt stress. During the seedling stage, salt stress levels of 6 dSm^{-1} and above can cause severe injury and even total crop death (Munns *et al*., 2006) resulting in a poor crop stand

while salt stress levels of 3.5 dSm^{-1} is enough to cause yield loss as high as 90% during the productive stage (Asch *et al*., 2000). Previous studies have established that the tolerance at these two most susceptible growth stages are uncorrelated, hence controlled by different genes (Singh *et al*., 2008; Singh and Flowers 2010). Therefore, to better study tolerance to salt stress in rice, it is important to impose stress separately at the early seedling (within the first three weeks after germination) and reproductive stages (after maximum tillering stage prior to the booting stage) (Singh *et al*., 2008).

Salt stress is most prevalent along the coast as well as semi-arid and arid regions where crop production relies heavily on irrigation. In areas where it occurs, salt stress may not be homogenous throughout the entire field but rather patches of heterogenous spots with varying degree of intensity, making it a difficult problem to tackle (Ceccarelli *et al*., 1994; Richards, 1983). Among all options, development of salt stress tolerant varieties that are high yielding through reconstitution of genes, appears to be the most economically viable and sustainable option (Ashraf and Wu,
1994; Silvey, 1981; Ashraf *et al*., 1986; Watt, 1983; Ray and Islam 2008; Osei *et al*., 2014) in reducing the yield gap deficit, which in turn, would improve the income of resource poor farmers and everyone else in the rice value chain. However, salt stress tolerance is a complex trait and phenotypic responses of plants to salt stress are highly affected by the environment (Gregorio and Senadhira, 1993; Koyama *et al*., 2001; Flowers, 2004). Fortunately, there exists considerable genetic variability in cultivated rice from very susceptible to highly tolerant (mostly traditional cultivars) genotypes that provide opportunities to improve salt stress tolerance of rice through breeding. Of the tolerant genotypes, two („Pokkali“ and „Nona Bokra“ and their derivatives such as FL478) have been used in several breeding programs to improve the adaptability of farmer preferred varieties (Gregorio 1997; Singh *et al*., 2007;

Thomson *et al*., 2010; Linh *et al*., 2012; Bimpong *et al*., 2016; De Leon *et al*., 2017). However, these donor parents are low yielding, tillers much less, lodge easily and possess many other undesirable agro-morphological features (Gregorio *et al*., 2002). These undesirable traits lead to unwanted linkage drag which tends to make their use in breeding unattractive. Therefore, there is a need to find other donor sources that are equally tolerant, but with better agro-morphological features. Such a donor will accelerate the breeding cycle hence save time and money.

Bimpong *et al*. (2011) embarked on a germplasm collection exercise in farmers‟ fields in The Gambia and Cassamance Region of Senegal where they collected over 300 accessions and pre-screened for agro-morphological characteristics and tolerance to salt stress. Among 5 other accessions, a landrace called "Madina Koyo" combined good adaptability to salt stress with good agro-morphological characters such as earliness, medium plant height and good grain characteristics. Initial comparison with „Pokkali‟ (a *Saltol* donor) using SSR and SNP markers revealed that the two were genetically different (Bimpong *et al*., 2011), suggesting that, tolerance in

Madina Koyo may be controlled by alleles different from that of „Pokkali‟. This presents an opportunity to widen the gene pool of salt stress tolerance in breeding programs, while lessening the otherwise undesirable background noise associated with „Pokkali‟. The objectives for this study were to:

i. Develop a mapping population using Madina Koyo as a donor source ii. Evaluate resulting breeding lines for tolerance to salt stress at the reproductive stage iii. Identify and select best breeding lines for generation advancement

3.2 Materials and Methods

3.2.1 Development of a mapping population using Madina Koyo as a donor parent

Madina Koyo, a landrace with no documented pedigree but with better adaptability to salt stress was used as a pollen donor while Sahel 317 (WAS 62-B-B-17-1-1), a lowland irrigated indica type developed by the Sahel Regional station of the then West Africa Rice Development Association, WARDA (now Africa Rice Center) was used as the pollen recipient. Sahel 317 was developed from a cross between parents 4456 and 32 Xuan 5C and is widely cultivated in Senegal due to its exceptional grain qualities and high yield.

3.2.2 Generation of crosses and development of mapping population

Sahel 317 was crossed with Madina Koyo to generate 8 F_1 seeds at Africa Rice Center, Sahel Regional Station in Saint Louis-Senegal in 2015. Of these, 3 were successful (based on quality control SNP marker analysis; results not shown) and were selfed to generate F_2 seeds. In total 300 F_2 plants were reconstituted from the 3

F_1 plants based on phenotypic selection. These were grown to raise seeds for next season‟s field testing during the 2016 dry season. However, due to a severe bird and rat damage, seeds were harvested from only 48 F_2 plants. About 2180 $F_{2:3}$ seeds were retrieved and bulked of which half were pregerminated for field testing.

3.2.3 Experimental site and conditions

Evaluation of $F_{2:3}$ progenies was conducted during the dry season (February-June) of 2017 at the research fields at Africa Rice Sahel research station in Ndaiye (16^o 14‟N, 16^o 14‟W) located in the Senegal River Delta. The region is purely Sahelian with a uni-modal rainfall pattern spanning barely three months in a year (August-October). Temperature ranges from 10^oC to 35^oC. Ndiaye and Ndiol are located in a depression

along one branch of the Senegal River and have saline ground water (EC ≥ 20 dSm^{-1}) due to deposits of marine origin (Haefele, 2001).

3.2.4 Screening for salt stress tolerance at the reproductive stage

A total of 1150 $F_{2:3}$ seeds were soaked in water overnight and then removed for incubation at room temperature for 48 hours. After this period, when germination had begun, the seeds were transferred unto nursery beds, where they were maintained for 21 days during which time, field plots were cleared, ploughed and leveled. Approximately 868 seedlings (21-day old), were transplanted following a spacing of 0.2 meters within and between rows. Due to the large number of lines and fewer seeds per line, the trial was randomized following an augmented design (Federer, 1961) with nested randomized complete block for the parents and checks. In total, the stress experiment was made up of 14 blocks, each with 70 plots. Out of these, eight were sown to the two parents Sahel 317 and Madina Koyo. The remaining 6 were sown to tolerant checks (FL478 and NERICA-L9) and susceptible checks (Sahel 108, Sahel 134, SMK3 and SMK87). Each block was sandwiched between the two parents for accuracy and ease of comparison. A month later, when seedlings were well established, a tiller from each entry was harvested from the stress experiment and used to re-constitute the control experiment through the Single Tiller Approach. Thus, progeny number one under control set-up was a tiller from progeny number one under stress hence were the same genotype. This technique reduced bias associated with evaluating segregating populations. Both experimental set-ups were treated alike with regards to irrigation, nutrient, pest and weed management until after maximum tillering, around the booting stage. Salt (NaCl in the ratio of 3g:1L water) was dissolved in water and used to irrigate the stress experiment for 4 weeks while the control experiment was continuously irrigated with fresh water. Salt stress levels were monitored every two days using a handheld conductivity meter (Orion star A222) and

maintained approximately at 6.0 dsm^{-1}. When the average salt stress levels had significantly exceeded this threshold, the stress plot was irrigated with fresh water to dilute the concentration of salt. More salts were added to bring EC up to 6 dsm^{-1} when salt stress levels went too low.

3.2.5 Data Collection

The following data was collected:

- **Days to 50% flowering**: the number of days from seeding till 50% of the panicles flowered, in days.
- **Number of effective tillers:** the number of grain-bearing tillers per plant (n=1)
- **Plant height at maturity**: height of plant measured from the soil surface to the tip of the tallest panicle/leaf, in cm.
- **Grain yield**: yield expressed as the total weight of seeds per plant in grams.

3.2.6 Statistical Analysis

Data analysis was done using a generalized mixed-model in „CAR" package of R (Fox and Weisberg 2011) where blocks were considered as random effects and genotypes and environment as fixed effects. First, a combined analysis of variance (ANOVA) was done using the repeated checks in each block to test if differences among their phenotypic performance were significant, and if so, for which trait(s). Secondly, the residual (experimental) error was estimated from ANOVA and used in the adjustment of phenotypic values of unreplicated entries to derive adjusted means. Thirdly, the computed residual error was used in separating the adjusted means through the computation of $LSD_{(0.05)}$. Subsequently, adjusted means were used in computing percent reductions and trait stability through the formula;

$$Reduction\ (\%)\ in\ Yield\ (Z) = 100\frac{(Yc - Ys)}{Yc}$$

$$Stability\ in\ Yield = 100 - Z$$

Where: Y*c* and Y*s* are yield performance under control and stress conditions respectively and Z is Percentage Reduction in yield.

Lastly, simple Pearson"s correlation coefficients were calculated to study the association among the parameters studied.

3.3 RESULTS

Average EC readings are illustrated in Fig 1a and 1b below. The average salinity levels were 0.8 dsm^{-1} and 6.1 dsm^{-1} under the control and stress experiments respectively.

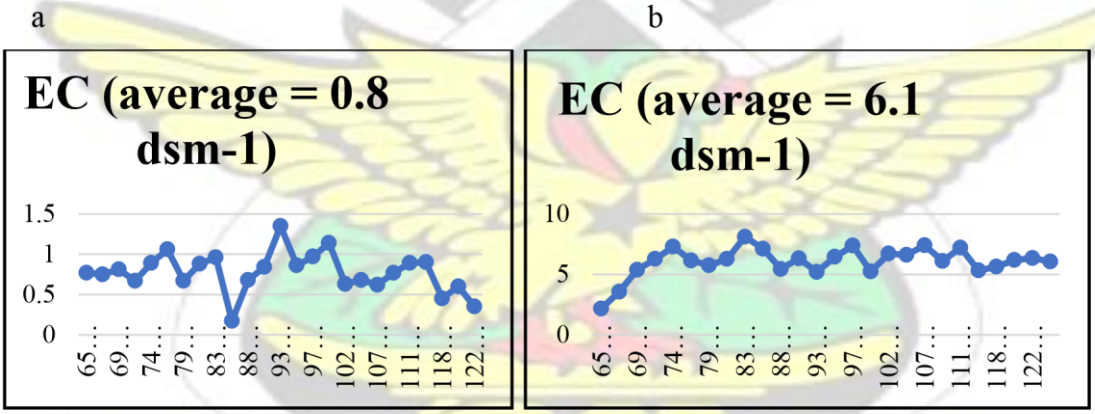


Figure 3.1. EC readings from the 65th day to the 122nd day after transplanting under control (a) and stress (b).

3.3.1. Analysis of variance for trait scores among genotypes

Combined analysis of variance for the repeated checks showed a significant site by genotype (GxE) interaction for all traits measured (Table 3.1). Site specific analysis of variance also revealed significant differences for all traits among genotypes.

Table 3.1. Analysis of variance for combined and single site for parents and checks.

Source of Variation	df	DTF	Height	eTiller	Yield
Site (E)	1	<0.00001	<0.00001	<0.00001	<0.00001
Varieties (G)	864	<0.00001	<0.00001	<0.00001	<0.00001
Interaction GxE	864	<0.00001	<0.00001	<0.00001	<0.00001
Control					
Genotype Significance	7	<0.00001	<0.00001	<0.00001	<0.00001
Stress					
Genotype Significance	7	<0.00001	<0.00001	<0.00001	<0.00001

Df- Degree of Freedom DTF=Days to 50% flowering eTiller= Effective Tillers

3.3.2 Days to Flowering

Significant difference was observed in the number of days to flowering under stress compared to control (Table 3.2). Under control, the progenies flowered in 77 days after transplanting, same as the recipient parent (Sahel 317) whiles the donor parent (Madina Koyo) flowered five days earlier. However, a total of 218 and 353 progenies flowered earlier than the two parents. Under stress conditions, both parents flowered earlier than mean of the progenies. However, some progenies flowered earlier than both parents. All the checks except NERICA-L9 flowered before the mean progeny days to flowering under both control and stress conditions.

Table 3.2. Performance of genotypes for number of days to flowering under control and stress conditions

No. of Days to Flowering	Contr					Stress				
	No lines	of . Mean (Days)	SD	Min	Max	No of lines	Mean (Days)	SD	Min	Max
Progeny Performance	868	77	7.27	53	100	868	78	9.04	52	105
Earlier than Sahel317 (RP)	353	68	4.64	53	76	234	68	5.37	52	75
Earlier than Madina Koyo (DP)	218	63	6.38	53	72	196	63	6.23	52	70
Parents										
Sahel317	14	77	0.76	75	78	14	75	0.78	73	77
Madina Koyo	14	72	0.61	71	73	14	70	0.39	69	71
Checks										
Sahel 108	14	72	0.94	69	73	14	70	0.83	68	72
Sahel 134	14	68	1.29	66	70	14	67	1.38	65	68
SMK3	14	65	0.77	64	66	14	63	0.47	63	64
SMK87	14	65	0.8	64	66	14	63	0.47	63	64

FL478	14	75	1.4	73	77	14	74	1.38	72	75
NERICA-L9	14	87	1.29	86	91	14	85	1.09	85	89
LSD for 2 Checks		0.64					0.59			
Heritability		0.98					0.98			

RP= Recipient Parent DP= Donor Parent

3.3.3 Number of Effective Tillers

Under both control and stress conditions, the progenies had a mean number of effective tillers higher than the donor but lower than the recipient parent (Sahel 317) though 24 progenies produced higher number of tillers compared to Sahel 317 (Table 3.3). Among the checks, only SMK3 had comparable tiller numbers to the progeny mean.

Table 3.3. Performance of genotypes for number of effective tillers under control and stress conditions

No. of Tillers	Control					Stress				
	No of lines	Mean	SD	Min	Max	No of lines	Mean	SD	Min	Max
Progeny Performance	868	24	7.00	1	44	868	23	4.9	1	34
Better than Sahel317 (RP)	24	32	4.39	28	44	140	30	3.4	17	34
Better than Madina Koyo (DP)	118	25	4.34	21	44	220	25	3.3	15	34
Parents										
Sahel317	14	28	8.87	15	44	14	27	1.56	15	45
Madina Koyo	14	21	4.92	14	36	14	22	1.71	11	37
Checks										
Sahel 108	14	20	5.75	14	35	14	18	2.56	15	25
Sahel 134	14	17	2.94	11	22	14	14	1.02	12	15
SMK3	14	25	8.21	11	39	14	23	1.39	10	35
SMK87	14	23	5.09	14	36	14	18	2.99	15	25
FL478	14	15	1.98	12	19	14	16	1.22	10	18
NERICA-L9	14	18	4.29	12	25	14	17	1.12	15	19
LSD for 2 Checks		2.07					1.16			
Heritability		0.99					0.98			

RP= Recipient Parent DP= Donor Parent

3.3.4 Grain Yield

Under control conditions, progenies recorded mean grain yield lower than their parents (Sahel 317 and Madina Koyo) and all checks except FL478 (Table 3.4). However, 106 and 193 progenies had grain yields better than the two parents and all checks. Under stress conditions, mean progeny performance for grain yield was less than the donor parent (Madina Koyo) but better than the recipient parent (Sahel 317). However, some 41 progenies recorded a mean grain yield of 25.2g and were better than Madina Koyo. Among the checks, only FL478 and NERICA-L9 were better than the mean of all progenies.

Table 3.4. Performance of genotypes for grain yield under control and stress conditions

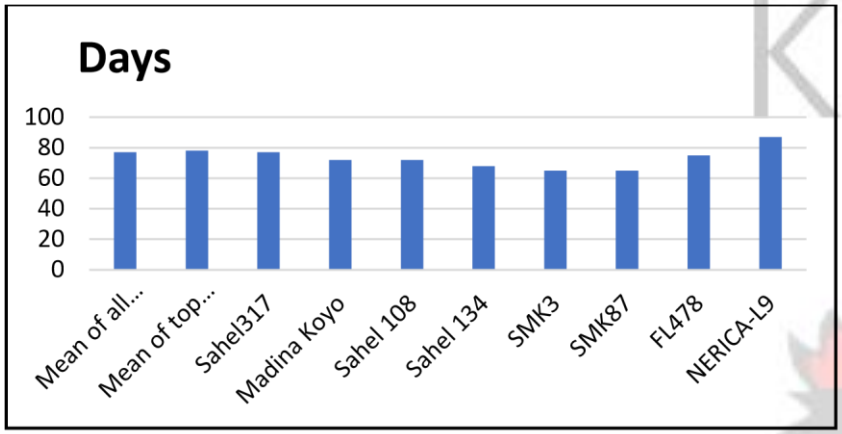
Grain Weight/Hill (g)	Control					Stress				
	No of lines	Mean (g)	SD	Min	Max	No of lines	Mean (g)	SD	Min	Max
Progeny Performance	868	29.9	21.2	1.09	161	868	8.33	7.47	0.18	30.2
Better than RP Sahel317	106	71.7	15.9	54.2	161	438	15.4	4.41	5.2	30.2
Better than DP Madina Koyo	193	62.8	17.1	41.1	161	41	25.2	2.3	21.7	30.2
Parents										
Sahel317 (RP)	14	54.2	15.4	25.7	81.0	14	5.00	3.40	-	9.0
Madina Koyo (DP)	14	41.1	7.99	26.9	57.5	14	21.6	6.16	12	32.0
Checks										
Sahel 108	14	61.0	16.8	26.3	82.0	14	4.50	2.28	-	8.0
Sahel 134	14	40.5	9.79	26.9	56.6	14	5.07	2.59	-	9.0
SMK3	14	59.5	26.5	20.3	103	14	1.57	1.45	-	5.0
SMK87	14	39.2	7.4	26.9	57.5	14	2.07	2.13	-	6.0
FL478	14	25.1	3.14	21.3	30.1	14	13.5	6.81	-	23.0

NERICA-L9	14	34.7	10.3	17.8	45.4	14	22.1	4.69	13	29.0
LSD for 2 Checks		8.98					2.56			
Heritability		0.94					0.97			

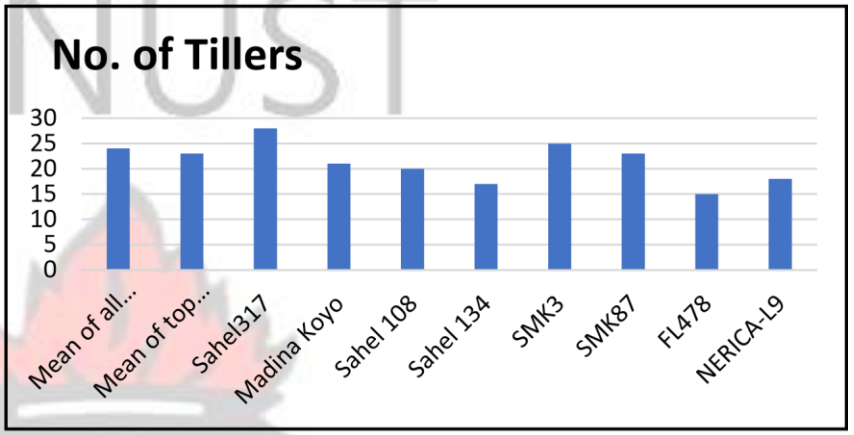
RP= Recipient Parent DP= Donor Parent

3.3.5 Summary of Top 5% Progenies for Grain Yield under control conditions

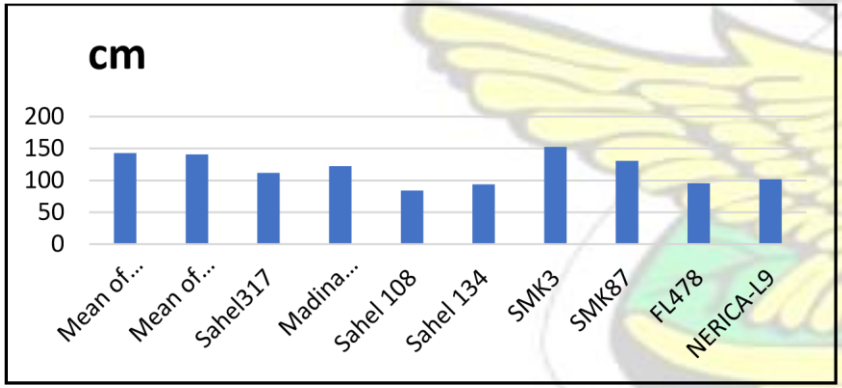
The top 5% performers (representing 45 progenies) selected based on their superior grain yield under control conditions had similar number of days to flowering as the recipient parent (Sahel 317) but flowered late compared to the donor parent (Madina Koyo) and all checks except NERICA-L9 (Figure 3.2). These 45 progenies recorded mean number of effective tillers less than Sahel 317 but greater than the number recorded by Madina Koyo and were also taller than all the parents and checks except SMK3.



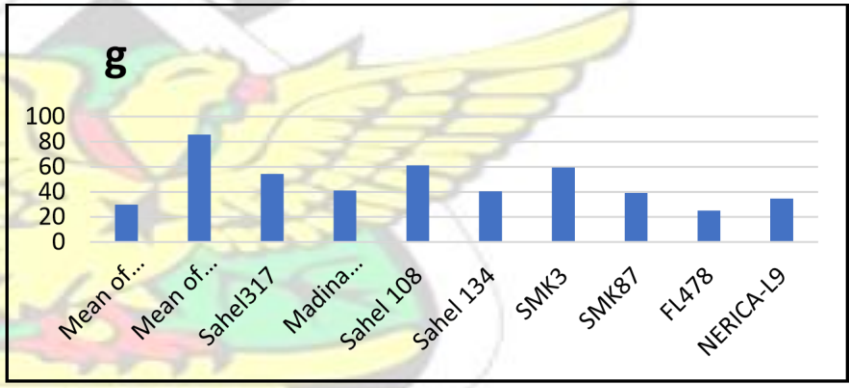
(a) Number of days to flowering



(b) Number of Effective Tillers



(c) Plant Height



(d) Grain Yield

Figure 3.3. Trait by trait comparison between the mean of progenies, parents, checks and the top 5% progenies based on yield under control conditions

3.3.6 Summary of Top 5% Progenies for Grain Yield under stress conditions All parents and checks (with the exception of NERICA-L9) flowered earlier than the top 5% progenies under stress conditions. These top performing progenies recorded lower plant height compared to all parents and checks (Figure 3.3, Appendix 2). The donor parent (Madina Koyo) had the same number of effective tillers as the top 5% progenies while the recipient parent (Sahel 317) recorded a slightly higher number of tillers.

3.3.7 Yield stability among the progenies.

The 5% most stable progenies had less reduction in grain yield under stress conditions and with better stability compared to the two parents and all the checks (Figure 3.4, Appendix 3). Five progenies (ARS1181-1-7-263, ARS1181-1-4-7, ARS1181-1-3-8, ARS1181-1-6-105 and ARS1181-1-6-45) which were among the top high yielders under stress were also found to be among the top most stable progenies (Appendix 2 and 3).

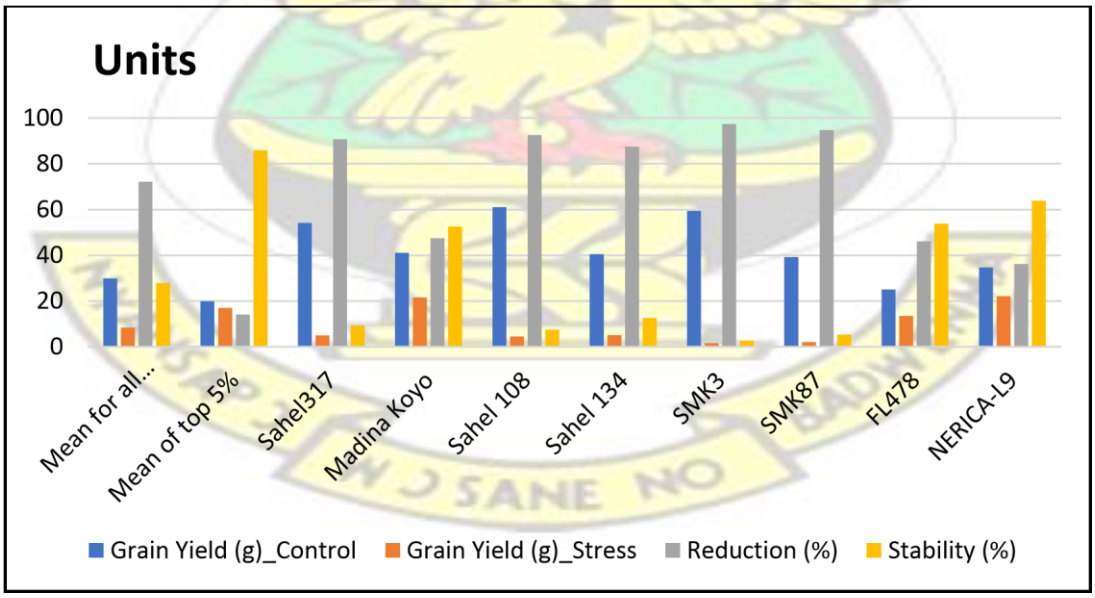
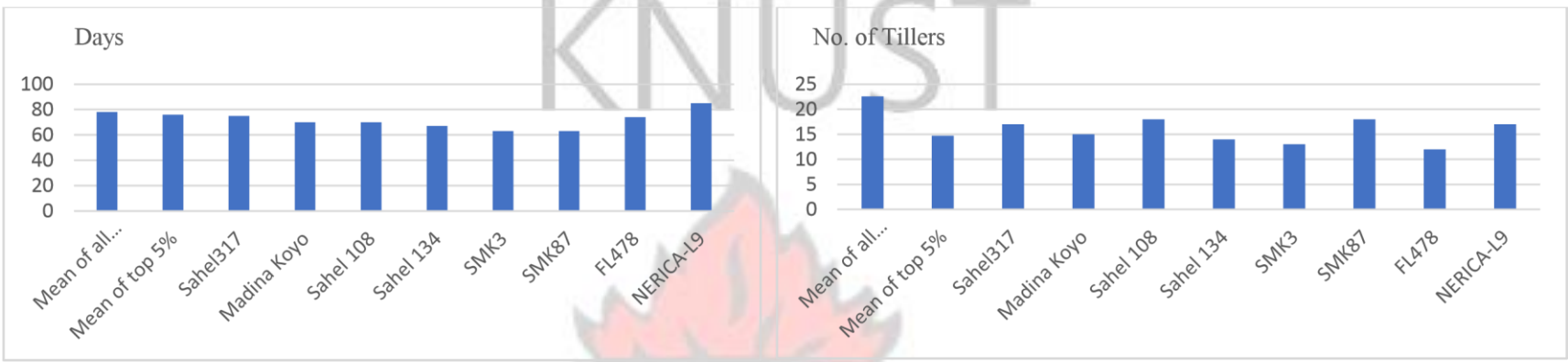
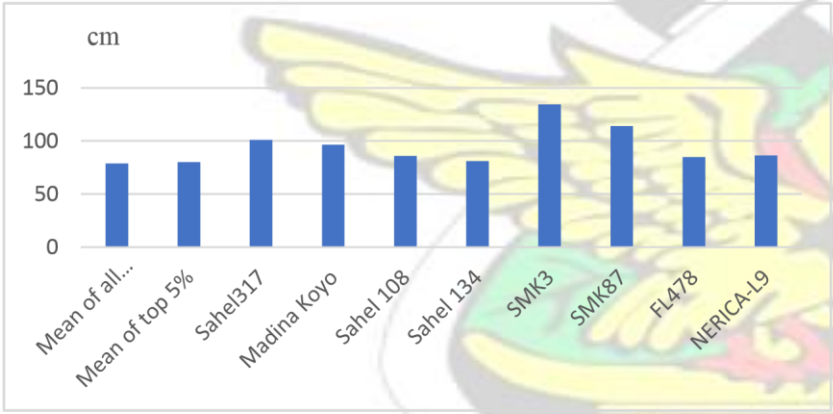


Figure 3.4. Yield stability for progenies, parents and checks

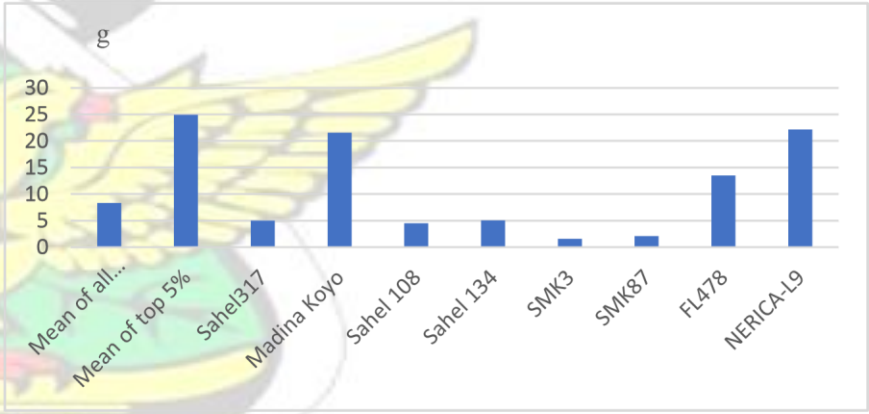


(a) Number of days to flowering

(b) Number of Effective Tillers



(c) Plant Height



(d) Grain Yield

Figure 3.3. Trait by trait comparison between the mean of progenies, parents, checks and the top 5% progenies based on yield under stress conditions

3.3.8 Correlation among Traits.

Linear correlation revealed weak association among measured traits under both control and stress conditions (Table 3.5). Under control conditions, plant height and number of effective tillers recorded the highest correlation but in opposite directions. Correlation between grain yield and number of effective tillers was at 36% whiles days to flowering correlated with number of effective tillers and plant height at -25% and 30% respectively under control. Under stress, correlation between grain yield and number of effective tillers was 8.6% while plant height correlated with grain yield at -17.9%.

Table 3.5. Correlation among measured traits under control and stress

Variables	Grain Yield	Days to Flowering	Number of eTillers	Plant Height
Grain Yield	*	0.013	0.086	-0.179
Days to Flowering	-0.044	*	0.017	0.039
Number of Tillers	0.360	-0.251	*	0.071
Plant Height	-0.089	0.291	-0.711	*

Values below diagonal are for correlation estimated under control while above diagonal are for stress.

3.4 Discussion

In rice, the number of seeds generated through selfing of F_1 generation and subsequent generations under normal growth conditions is exceptionally high. However, simultaneous evaluation of segregating breeding lines for tolerance to stresses, and generation of enough seeds for testing in subsequent generations is challenging, because, some stresses such as salt stress can cause total crop failure (Munns *et al*., 2006; Asch *et al*., 2000). Combining the single tiller approach with augmented design, this study evaluated more than 800 different $F_{2:3}$ progenies under salt stressed

conditions with meaningful comparison with the control experiment while seed increase was achieved under control conditions. This approach has been used by Bimpong *et al*. (2014) and Khan *et al*. (2016) to successfully screen for tolerance to salt stress at the reproductive stage in different F_2 populations. Kanbar *et al*. (2010) also adopted this approach to identify deep rooted F_2 rice progenies.

Salt stress at EC levels of 3 dSm^{-1} is enough to cause reduction in rice yields (Maas and Hoffman 1977). In this study, average EC levels were approximately 6.0 dSm^{-1} which caused a drastic reduction of 72% in grain yield among the test entries. This observation is comparable to the findings of Bimpong *et al*. (2016) who reported over 50% reduction in grain yield under stress among some *Saltol* introgression lines. Much earlier, the authors had reported a salt stress-induced yield deficit of 73% among some F_2 derived progenies (Bimpong *et al*., 2014). Similar results were reported by Nakhoda *et al*. (2012) and Zeng and Shannon (2000). Of the total progenies evaluated, 45 yielded better than the donor parent Madina Koyo suggesting that segregation was transgressive and in both directions. This observed transgressive segregation suggests the availability of sufficient genetic variability and ample opportunity to develop varieties that are more adaptable than their parents under salt prone environments. Similar transgressing segregation for grain yield under salt stress conditions has been reported among various rice bi-parental populations including F_2 (Rana *et al*., 2009; Mohammadi *et al*., 2014), BC_3F_2
(Bimpong *et al*., 2016) and RILs (Thomson *et al*., 2010; Tiwari *et al*., 2016).

Further, salt stress caused a general delay in flowering up to 7 days on the average in the current study. It has been proposed that a GA-dependent pathway plays an

important role in flowering time control under salt stress by regulating the biosynthesis levels of GA_4 (Achard *et al*., 2006). Flowering was greatly restored when GA_4 was applied to salt-stressed Arabidopsis plants (Li *et al*., 2007). The researchers also argued that, delayed flowering under salt stressed conditions could be an adaptive mechanism where plants conserve energy to maintain ion homeostasis rather than transition from vegetative to reproductive stage. Castillo *et al*. (2003) reported similar salt stress-induced delay in flowering until a maximum of 12 days in rice cv. IR64. Later, the researchers reported an average delay of 11 days in the same cultivar (Castillo *et al*., 2007). Khan *et al*. (2016) and Bimpong *et al*. (2014) reported similar delayed flowering among some rice mapping populations they evaluated for tolerance to salt stress. On the contrary, salt stress was reported to speed flowering among rice genotypes (Bimpong *et al*., 2016). Salt stress has been reported to prolong flowering in sensitive cultivars whereas in tolerant genotypes, salt stress induces early flowering (Safitri *et al*., 2016). Castillo *et al*. (2007) attributed the prolonged flowering under stress conditions to the osmotic stress imposed by salt

stress.

Direct selection for yield has been proposed to be more effective under stress conditions in rice (Manneh, 2004). In this study, more than half (440) of the progenies yielded better than the recipient parent Sahel 317 under stress conditions. Among these, 41 were better than the donor parent Madina Koyo. Some of these promising progenies had yield stability ranging between as high as 99.6% to 73.9%. Some progenies even combined high yielding under stress with good yield stability. Taking these into consideration, direct selection for grain yield was applied to compose individual F_3 derived F_4 families for further testing at the early seedling stage. This

included progenies from the top yielding 5% (representing 45 progenies) each under both control and stress as well as the top 5% that were most stable for grain yield. Since tolerance to salt stress at the reproductive stage does not necessarily translate into tolerance at the early seedling stage (Singh *et al*., 2008; Singh and Flowers 2010), another 365 progenies were selected at random to

constitute a total of 500 $F_{3:4}$ families to be evaluated for tolerance to salt stress at the early seedling stage.

High magnitude and negative correlation between plant height and tiller number is an indication of an inverse association between the two traits, suggesting that, tall genotypes produced fewer tillers and vice versa. Mishra *et al*. (2014) reported similar negative association between plant height and number of tillers under saline conditions. A positive correlation between grain yield and number of effective tillers in this study, confirms number of effective tillers as a component of grain yield and that, an increase in number of tillers will cause an increase in grain yield of that genotype. Hence, an indirect selection of yield could be made by selecting directly for effective tiller numbers in salt stress breeding programs. Grain yield and tiller number were also found to be positively correlated under salt stress by Tiwari *et al*. (2016) and Zeng *et al*. (2002). On the contrary, Mishra *et al*. (2014) observed a negative association between grain yield and tiller number.

CHAPTER FOUR

4.0 EVALUATION OF $F_{3:4}$ POPULATION DERIVED FROM A TOLERANT DONOR (MADINA KOYO) FOR TOLERANCE TO SALT STRESS AT THE EARLY SEEDLING STAGE.

4.1 Introduction

Salt stress causes leaf damage leading to considerable reduction in photosynthesis which results in decline in growth and eventual death of the crop (Yeo and Flowers, 1989). Resulting from natural causes and excessive use of irrigation water coupled with poor or improper drainage, the problem of salt stress is likely to worsen due to the numerous effects of climate change (Ismail *et al*., 2008; Ismail and Toung 2009; Wassmann *et al*., 2009). As a result, several studies have focused on improving tolerance to salt stress in different cereal crops including rice (Thomson *et al*., 2010; Platten *et al*., 2013; Bimpong *et al*., 2016), wheat (Nia *et al*., 2012), barley (Saade *et al*., 2016), millet (Satish *et al*., 2016) and sorghum (Roy et al., 2018).

Rice, a major world staple, has limited adaptability to saline conditions by reducing the amount of water that is absorbed through rice roots which eventually lead to an initial osmotic stress (Maas *et al*., 1986). If the crop is not relieved off the stress, ionic concentrations of salts, mainly sodium, accumulate in excessive amounts which causes ionic stress, resulting in drastic reduction in growth (Yeo and Flowers, 1986; Flowers *et al*., 1991). In an attempt to control the rate of loss of tissue moisture, plants may shed older leaves through leaf senescence, hence, reduce the photosynthetic leaf area of affected plants (Munns and Tester, 2008). If rate of regeneration of new leaves do not match up to the rate of loss of older leaves, photosynthetic activity reduces to levels that can no longer sustain growth and plants eventually die (Amirjani, 2011).

Tolerance to the stress during the early seedling growth phase does not necessarily lead to tolerance at the reproductive stage in rice (Singh and Flowers, 2010). This suggests the involvement of separate control mechanism each probably with different sets of genes (Ahmadizadeh *et al*., 2016). Though it is the reproductive stage salt stress

that causes direct yield loss, early seedling stage salt stress is equally important as it dictates the actual crop stand per plot which is also directly related to grain yield. Therefore, it is important to identify genotypes with better adaptability to salt stress at the seedling stage and combine with those that tolerate the stress better at the reproductive stage.

With the development of screening protocol based on hydroponic culture (Gregorio *et al*., 1997), it has become possible to efficiently evaluate larger number of genotypes for tolerance to salt stress at the early seedling stage in a relatively shorter period based on visual salt injury score. Current studies have combined physiological, morphological, biochemical and molecular traits to evaluate rice genotypes for tolerance to salt stress. De Leon *et al*., (2015) used a combination of morphological and physiological traits in multivariate analyses to elucidate the phenotypic and genetic variation in salt stress tolerance of some 30 Southern USA rice genotypes, along with 19 donor genotypes with varying degree of tolerance. They observed significant genotypic variation and correlations among the physiological and morphological while their combined analysis validated salt stress response of already known genotypes. Subsequently, the researchers used same traits to evaluate, map QTLs and select promising introgression lines from a mapping population developed between the popular salt tolerant donor „„Pokkali““ and a high yielding variety Bengal (De Leon *et al*., 2017). Other studies have used similar morphological and physiological traits to successfully distinguish between salt tolerant and susceptible rice genotypes (Razzaque *et al*., 2009; Gholizadeh and Navabpour, 2011; Safitri *et al*., 2016).

The objectives of this study were to:

i. Evaluate selected $F_{2:4}$ breeding lines derived from salt tolerant donor Madina Koyo for tolerance to salt stress at the early seedling stage.

ii. Identify and select best lines to be used as subsequent parents.

4.2. Materials and Methods

4.2.1 Screening for tolerance to salt stress at the early seedling stage

A total of 308 F_3 derived F_4 families were evaluated for tolerance to salt stress at the early seedling stage using a hydroponic system established during the dry season in a screenhouse at the M‟be Station at the headquarters of Africa Rice Centre in Bouake, Cote d‟Ivoire. Typically, conditions around this period of year ranges from 20˚C to 25˚C during early parts of the day and 28˚C to 38˚C during later hours of the day. The average relative humidity varies from 35% to 95%. Evaluation of test entries followed a method described by Gregorio *et al*., (1997). Four-day old pre-germinated seeds were sown in holes on plastic floats with a net bottom suspended on trays filled with distilled water for 3 days. This was to allow for the repair of any damage to radicles while transferring onto the Styrofoam before salinization. Each plastic float had 64 lanes/plots (each sown to one entry) made up of 7 slots/holes and was considered as a block. The experiment was laid out in an alpha lattice design with slight modification (where the two checks IR29 and FL478 were repeated in each block/plastic float to aid in accessing the performance of the test entries.). There were two replications each for saline and non-saline (control) experimental setups.

Thus, in summary, each replication contained 5 blocks, each block contained 62 families (plus the two checks) and each family was made up of 7 hills. After the three-day recuperation period, salinized Yoshida‟s solution (Yoshida *et al*., 1976; Table

4.1), with slight modifications as per the protocol by Singh and Flower (2010), replaced the distilled water in the stress set-up while the control set-up received Yoshida‟s solution with no salt. The initial salinization was maintained at an electrical conductivity of 6.0 dSm^{-1} by adding approximately 5.13 mM of NaCl to the solution. This concentration was increased to 12.0 dSm^{-1} after 3 days and 18 dSm^{-1} another 3 days after. The pH of the nutrient solution was maintained at approximately 5.0 with a weekly renewal of the solution throughout the experiment.

Table 4.1. Chemical composition of nutrient solution

No.	Element	Reagent	Stock Solution	Working Solution
			Qty to be used (g/L)	Qty of stock solution / 1L nutrient solution (ml)
		Macronutrient		
1	N	Ammonium nitrate (NH_4N0_3)	91.4	1.25
2	P	Potassium dihydrogen phosphate (KH_2PO_4)	29	1.25
		Potassium hydrogen phosphate (K_2HPO_4)	8	1.25
3	K	Potassium sulfate (K_2SO_4)	97.8	1.25
4	Ca	Calcium Chloride, diydrate ($CaC1_2.2H_2O$)	175	1.25
5	Mg	Mg Magnesium sulfate, 7-hydrate ($MgS0_4.7H_2O$)	324	1.25
		Micronutrient		
6	Mn	Manganous chloride, 4-hydrate ($MnC1_3.4H_20$)	1.5	
7	Mo Zn	Ammonium molybdate, 4-hydrate [$(NH4)_6Mo_7O_{24}$-$4H_2O$] Zinc	0.074	
8		sulfate, 7-hydrate ($ZnS0_4.7H_20$)	0.035	1.25
9	B	Boric acid (H_3BO_3)	0.93	
10	Cu	Cupric sulfate, 5-hydrate ($CuS0_4.5H_20$)	0.03	
11	Fe	Ferrous Sulfate, 7-Hydrate ($FeSO_4.7H_20$)	2.5	

4.2.2 Data Collection

Salt Evaluation Score

The test entries were assessed based on visual salt evaluation score (SES) using IRRI‟s standard evaluation system for rice (IRRI-SES 2014), with ratings from 1.0 (highly tolerant), 3.0 (Tolerant), 5.0 (Moderately tolerant), 7.0 (Susceptible) to 9.0 (highly susceptible) (Table 4.2). Ten days after the initial application of salt, the first assessment was done while the second and final assessments were done 6 days after the initial assessment.

Table 4.2. IRRI‟s 5th edition Standard Evaluation System for rice (IRRI 2014)

Score	Symptom/observation	Degree of tolerance
1	Normal growth, only the old leaves show white tips while no symptoms on young leaves	Highly tolerant
3	Near normal growth, but only leaf tips burn, few older leaves become whitish partially and rolled	Tolerant
5	Growth severely retarded; most leaves severely injured, few young leaves elongating	Moderately tolerant
7	Complete cessation of growth; most leaves dried; only a few young leaves still green	Sensitive
9	Almost all plants dead or dying	Highly sensitive

Shoot and Root Length

For each accession, data was recorded on all 7 plants for shoot length and root length. Shoot length was estimated as length from the base to the tip of the longest leaf while root length was estimated as length of roots from the base of shoots to the tip of the deepest root in centimeters. Shoot and root lengths were taken as a mean of all 7 measurements for each entry.

Shoot and Root Dry Weight

All 7 shoots and roots from each entry were bulked in an envelope and dried in an oven at 50^{o}C for 5 days. Shoot and root dry weights were recorded in grams after the 5th day.

Ion Leakage

Response of the test entries to salt stress was studied by measuring the concentration of the ions that leaked from the leaf tissue using a conductivity meter (Orion Star A222) following methods described by De Leon *et al*. (2015). After 2 days in saline solution, 100 mg of leaf tissue was collected from the second youngest leaf of each entry. The tissue was cut into 10 mm lengths, placed in 10 ml distilled deionized water, and incubated at room temperature for 2h before autoclaving. The electrical conductance of the solution was measured before and after autoclaving for initial and final EC values, respectively. Since ion leakage could vary between entries, the index of salt injury was estimated with respect to the ion leakage of the corresponding entries grown in control conditions, following the formula suggested by Flint *et al*. (1967):

$$Ion\ Leakage = 100 * [\frac{Rt - Ro}{1 - Ro}]$$

where ion leakage is the index of injury by ion leakage;

R_o = Initial EC/Final EC of the control plant, and

R_t = Initial EC/Final EC of the stressed plant.

4.2.3 Statistical Analysis

To evaluate the genotypic differences for each trait, and to test whether or not these differences under control and stress conditions were statistically significant, analysis of variance ANOVA was computed using the linear mixed model with a normal distribution for Shoot length, Root length, Shoot dry weight, Root dry weight an Ion Leakage and a generalized linear mixed model (McCullagh and Nelder, 1989) under the assumption of a multinomial distribution for salt stress. In summary, the model used is as below:

$$y_{ijkl} = \mu + G_i + E_j + R_{k(j)} + B_{l(jk)} + (GxE)_{ij} + \epsilon_{ijkl}$$

Where y_{ijklt} is the measurement on the plot t in the site *i*, bloc j of repetition k containing genotype l. μ is the general mean of all plots within all sites. G_l is the effect of genotype l. E_i is the effect of site *i*. $R(E)_{k(i)}$ is the effect of repetition k within the site *i*. $B_{j(ik)}$ is effect of block j of repetition k in the site *i*. $(GxE)_{il}$ is the interaction of genotype l with the site *i*. ϵ_{ijklt} is the plot residual. A significant genotype × environment interaction effect was tested, and where this was the case, a model for each environment (control, and stress) was fitted separately. Genotype, environment, and genotype × environment were considered as fixed factors with replicate nested into environment and block nested into replication and environment as random factor. Comparison of means was done by LSD for data with normal distribution. For the SES, where a multinomial distribution was assumed, we modelled the probability levels of scores having lower ordered values. Odds ratios were calculated to indicate relative differences between entries and the two parents (Sahel 317 and Madina Koyo) as well as the two international checks, IR29 or FL478 respectively. Variation due to genotype and environment (residual) as well as heritability were computed to estimate the level of genotypic contribution to the phenotypic variation of measured traits.

Relationship among traits was computed using genetic correlation, based on the pooled least square (LS) mean of two

replications per trait.

4.2.3.1 Stability of genotypes under stress and control experiments

Comparisons of genotypes for all measured traits (except SES) were computed as the percentage reduction of genotypic performance under stress conditions relative to their performance under control using their generated LS means. These were computed as below:

Shoot and root length stability;

$$Stability(shoot\ length) = [1 - \frac{a-c}{a}] * 100\%$$

Similarly, $$Stability\ (root\ length) = [1 - \frac{b-d}{b}] * 100\%$$

Percentage reduction in growth was then computed as summation of shoot and root lengths under control less that under stress and expressed as a proportion to the summation of shoot and root lengths under control;

Where; a = shoot length measured under control setup
b = root length measured under control setup c
= shoot length measured under salinized setup
d = root length measured under salinized setup

Stability for dry weights:

$$Stability\ (shoot\ dry\ weight) = [1 - \frac{x-q}{x}] * 100\%$$
$$Stability\ (root\ dry\ weight) = [1 - \frac{y-r}{y}] * 100\%$$

Where; x = shoot dry weight measured under control setup

y = root dry weight measured under control setup q

= shoot dry weight measured under salinized setup r

= root dry weight measured under salinized setup

Except for computations to which formula are shown, all analysis done using R statistical package (R Core Team, 2018).

4.3. Results

There were significant ($P < 0.05$) genotype x environment interaction effects and also among genotypes for all traits except for ion leakage (Table 4.3). Since genotype x site interaction was significant, data from control and stress were analyzed separately. Under both control and stress conditions, genotypic differences were significant except for Ion Leakage. Differences for stability among traits were however significant for all traits.

Table 4.3. Analysis of Variance for SES, Shoot and Root lengths, Shoot and Root Dry weights and Ion Leakage.

Source of Variation	SES	Shoot Length	Root Length	Shoot Dry Weight	Root Dry Weight	Ion Leakage
Combined						
Genotype significance		0.0001	<0.0001	0.5828	0.3806	0.6741
Site significance		0.0122	0.0181	0.0034	0.0102	0.001
Genotype x Site significance		<0.0001	<0.0001	<0.0001	<0.0001	0.1873
Genotype significance (Control)		<0.0001	<0.0001	<0.0001	<0.0001	0.2986
Genotype significance (Stress)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.1594
Trait Stability						
Genotype Significance		<0.0001	0.02745	<0.0001	<0.0001	0.02549

4.3.1 Salt Evaluation Score (SES)

Symptoms of salt injury were visible 5-10 days after imposing salt stress, though, with varied response among the test entries (Figures 4.2 – 4.4). Mean SES for all progenies was 5 with a range of 1-9. A total of 46 progenies had a score ranging from 1-3 and were better than the donor parent Madina Koyo but were comparable to the tolerant check FL478 (Appendix 4). Among these, six progenies (ARS1181-1-69-B-9, ARS1181-1-7-2-B-2, ARS1181-1-9-6-B-6, ARS1181-1-9-30-B-30, ARS1181-1-2-13-B-13 and ARS1181-1-2-11-B-11) recovered fully from salt injury and had a final score of 1. Some 88 progenies scored between 7-9 and were comparable to the recipient parent Sahel 317 and the susceptible check IR29 (Figure 4.2). The most tolerant 5% progenies (representing 16 progenies) had an average score of 1.6 which was significantly different from the Madina Koyo and the FL478 with odd ratios ranging 817-312 and 442-59.88 respectively (Table 4.4)

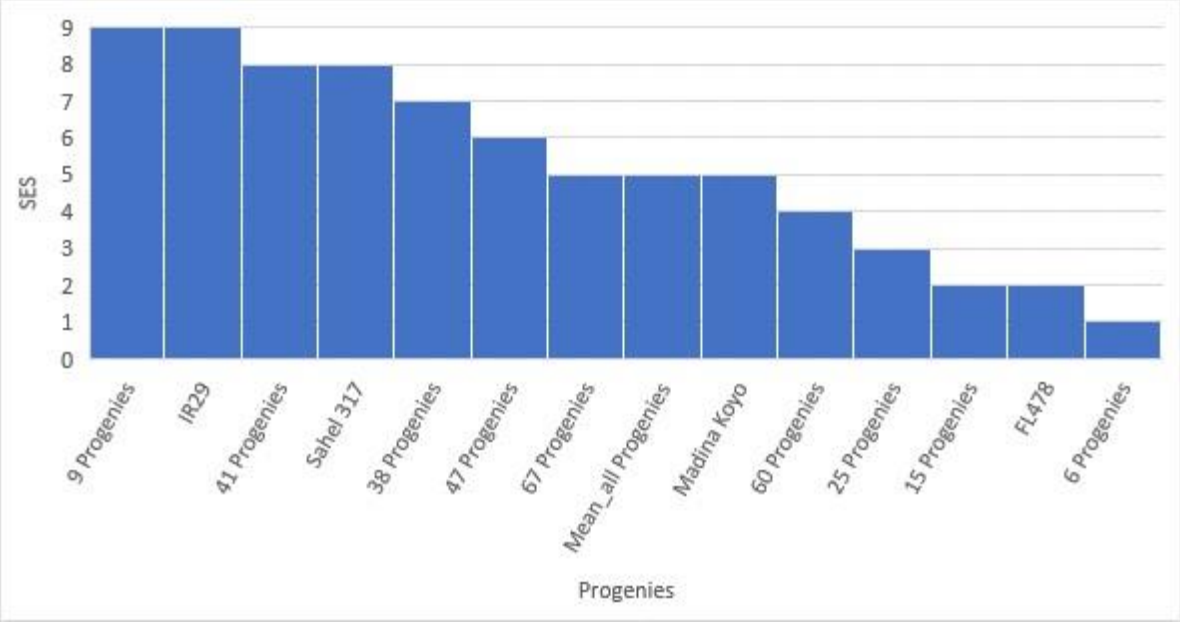


Fig. 4.2 Distribution of salt evaluation score among the test entries

Table 4.4. SES, P-values and odd ratios of entries of the top 5% progenies

Genotype	Mean SES	Genotypes vs Madina Koyo		Genotypes vs FL478	
		P-value	Odds ratio	P-value	Odds ratio
ARS1181-1-9-6-B-6	1	<0.0001	817.61	<0.0001	442.42
ARS1181-1-2-11-B-11	1	<0.0001	639.84	<0.0001	156.81
ARS1181-1-2-13-B-13	1	<0.0001	639.84	<0.0001	122.71
ARS1181-1-7-2-B-2	1	<0.0001	553.94	<0.0001	122.71
ARS1181-1-9-30-B-30	1	<0.0001	515.21	<0.0001	106.24
ARS1181-1-6-9-B-9	1	<0.0001	493.8	<0.0001	98.81
ARS1181-1-12-3-B-3	2	<0.0001	489.24	0.01	94.71
ARS1181-1-12-14-B-14	2	<0.0001	469.16	0.01	93.83
ARS1181-1-13-1-B-1	2	<0.0001	371.8	0.01	71.31
ARS1181-1-13-18-B-18	2	<0.0001	371.8	0.01	89.98
ARS1181-1-1-12-B-12	2	<0.0001	353.48	0.01	71.31
ARS1181-1-3-17-B-17	2	<0.0001	332.31	0.01	67.79
ARS1181-1-4-30-B-30	2	<0.0001	332.31	0.02	63.73
ARS1181-1-4-38-B-38	2	<0.0001	318.66	0.02	63.73
ARS1181-1-7-6-B-6	2	<0.0001	312.2	0.02	61.12
ARS1181-1-8-26-B-26	2	<0.0001	312.2	0.02	59.88
Mean of top 5% Progenies	1.6				
Mean of all Progenies	5.3				
Madina Koyo	4				
FL478	2.4				
Sahel 317	8				
IR 29	8.4				

Figure 4.3 Comparison between a tolerant progeny, the two parents and the two checks.

4.3.2 Shoot Length

Generally, salt stress caused a significant reduction in shoot length among all the test entries. (Table 4.5). Under control conditions, the donor parent Madina Koyo recorded shoot length higher than mean performance of the progenies while the progenies out-performed the recipient parent Sahel 317. Mean progeny performance was higher than both tolerant (FL478) and susceptible (IR29) checks. Under stress conditions, progenies recorded higher shoot lengths than the two parents and the two checks. The top 5% progenies had the highest mean shoot length under stress conditions. Some progenies out performed both parents whiles other progenies measured lower shoot lengths than the parents under both control and stress conditions. Mean progeny stability (including the top 5%) was higher than the two parents and the susceptible check but lower than the tolerant FL478.

Table 4.5. Mean Performance for Shoot Length under control and stress conditions

Genotype	Control			Stress			Stability
	Mean	Min	Max	Mean	Min	Max	Mean
All Progenies	43.3	28.6	59.0	24.5	20.3	30.5	56.6
Top 5% Progenies	40.9	29.0	54.4	25.5	22.3	30.5	63.3
Madina Koyo (Donor Parent)	44.5	40.2	49.6	22.6	17.4	22.3	50.8
Sahel 317 (Recipient Parent)	38.1	35.7	37.1	21.1	12.4	21.2	55.4
FL478 (Tolerant Check)	35.6	21.2	42.8	23.3	18.5	26.1	65.4
IR29 (Susceptible Check)	37.2	26.6	51.5	20.0	13.7	23.8	53.8
Mean	39.9			22.8			57.6
SD	6.67			1.42			12.6
LSD	7.37			1.92			21.3
Heritability	0.76			0.50			0.25

4.3.3 Root Length

Mean root length for all progenies including the top 5% progenies was higher than their two parents (Madina Koyo and Sahel 317) and the two checks (FL478 and IR29) under both control and stress conditions (Table 4.6). However, the two parents recorded higher root length stability, than the progenies.

Table 4.6. Mean Performance for Root Length under control and stress conditions

Genotype	Control			Stress			Stability
	Mean	Min	Max	Mean	Min	Max	Mean
All Progenies	12.8	9.6	18.9	10.0	8.3	11.6	78.1
Top 5% Progenies	13.6	11.8	16.8	10.5	9.4	11.4	78.0
Madina Koyo (Donor Parent)	11.1	8.5	11.9	10.3	10.6	10.7	92.8
Sahel 317 (Recipient Parent)	11.2	9.6	11.3	8.9	5.1	8.4	79.5
FL478 (Tolerant Check)	10.9	8.9	12.8	8.3	3.9	10.8	76.1
IR29 (Susceptible Check)	11.6	8.9	17.4	7.6	4.3	10	65.5
	11.9			9.3			78.3
SD	0.27			0.07			11.4
LSD	0.24			0.07			24.2
Heritability	0.68			0.36			0.48

4.3.4 Shoot Dry Weight

Under control conditions, the progenies were outperformed by the donor parent Madina Koyo. However, mean performance of progenies was higher than the recipient parent (Sahel 317) and the two checks FL478 and IR29 (Table 4.7). Under stress conditions, the progenies performed better than the two parents and check. The top 5% progenies had higher shoot dry weights compared to the entire progenies.

The progenies had better stability than the parents and the checks.

Table 4.7. Mean Performance for Shoot Dry Weight under control and stress conditions

Genotype	Control			Stress			Stability
	Mean	Min	Max	Mean	Min	Max	Mean
All Progenies	0.79	0.34	1.38	0.28	0.20	0.38	49.0
Top 5% Progenies	0.76	0.38	1.37	0.30	0.26	0.38	45.0
Madina Koyo (Donor Parent)	0.91	0.81	1.05	0.27	0.15	0.35	30.0
Sahel 317 (Recipient Parent)	0.62	0.55	0.62	0.23	0.09	0.19	36.4
FL478 (Tolerant Check)	0.74	0.28	1.24	0.27	0.04	0.35	35.8
IR29 (Susceptible Check)	0.64	0.19	1.31	0.23	0.09	0.39	35.4
	0.74			0.26			38.6
SD	0.26			0.01			17.4
LSD	3.80			0.02			24.9
Heritability	0.82			0.42			0.39

4.3.5 Root Dry Weight

Differences in root dry weight among the entries under control and stress conditions were significant. The donor parent (Madina Koyo) measured heavier root dry weight than the progenies while the recipient parent (Sahel 317) and the two checks (FL478 and IR29) were outperformed by the progenies under control conditions (Table 4.8). A similar trend was observed under stress conditions. The top 5% progenies had better root dry weight stability than the entire progenies as well as the parents and the checks.

Table 4.8. Mean Performance for Root Dry Weight under control and stress conditions

Genotype	Control			Stress			Stability
	Mean	Min	Max	Mean	Min	Max	Mean
All Progenies	0.20	0.09	0.435	0.07	0.05	0.10	34.5
Top 5% Progenies	0.21	0.11	0.435	0.08	0.06	0.10	41.0
Madina Koyo (Donor Parent)	0.22	0.10	0.29	0.09	0.08	0.09	38.7
Sahel 317 (Recipient Parent)	0.16	0.14	0.15	0.06	0.03	0.05	36.3
FL478 (Tolerant Check)	0.18	0.17	0.36	0.06	0.04	0.10	32.2
IR29 (Susceptible Check)	0.18	0.12	0.32	0.05	0.03	0.07	30.3
	0.19			0.07			35.5
SD	0.07			0.01			16.3
LSD	0.06			0.02			24.6
Heritability	0.76			0.42			0.61

4.3.6 Ion Leakage

Though ion leakage under both control and stress were not significant, stability in the trait showed significant differences among genotypes. Mean stability for the entire progenies was 53.1% while that of the donor (Madina Koyo) and the recipient (Sahel 317) parents were 73.3% and 67.6% respectively (Table 4.9).

Table 4.9. Mean Ion Leakage under control and stress conditions

Genotype	Control			Stress			Stability		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
All Progenies	0.168	0.160	0.17	0.640	0.615	0.665	53.1	23.4	88.9
Top 5% Progenies	0.168	0.165	0.17	0.640	0.625	0.655	52.0	34.2	79.2
Madina Koyo (Donor Parent)	0.166	0.150	0.18	0.651	0.600	0.780	73.3	64.5	70.6
Sahel 317 (Recipient Parent)	0.170	0.170	0.19	0.656	0.710	0.760	67.6	72.8	73.8
FL478 (Tolerant Check)	0.169	0.170	0.18	0.615	0.520	0.610	47.9	42.3	53.5
IR 29 (Susceptible Check)	0.172	0.170	0.18	0.654	0.660	0.670	59.3	58.7	59.9
SD	0.002			0.009			22.75		
LSD	NS			NS			47.05		
Heritability	0.100			0.110			0.62		

4.3.7. Broad Sense Heritability

Heritability ranged from 68% to 82% under control conditions while under stress, the range was 36% to 50% (Tables 4.5 – 4.8). Generally, heritability estimates under control conditions were higher than estimates under stress conditions. Heritability estimates for trait stability ranged from 25% to 61%.

4.3.8 Correlation among traits

Correlation among traits measured under control conditions were significant (Table 4.9). Significant and positive association were observed between shoot and root dry weights, shoot dry weight and shoot length and between root dry weight and shoot length. Significant but negative association were observed between shoot and root lengths, shoot dry weight and root length and between root dry weight and root length. Except for a positive correlation with shoot length, ion leakage demonstrated a weak and negative correlation with all other traits. Under stress, all correlation among traits were significant except between SES and shoot length as well as correlations between ion leakage and every other trait but SES. Significant and positive correlations were observed between SES and Ion leakage and between shoot and root dry weights under stress. Shoot length associated positively with root length, shoot dry weight and root dry weight while SES associated negatively with these three traits. While root length associated negatively with shoot length, shoot dry weight, root dry weight and ion leakage under control, association among these traits were positive under stress.

Table 4.10. Genetic correlation with P-values for measured traits under control and stress conditions

	Genetic Correlations					**P-value**				
	Control Conditions									
Traits	SES Score	Shoot Length	Root Length	Shoot Dry Weight	Root Dry Weight	SES Score	Shoot Length	Root Length	Shoot Dry Weight	Root Dry Weight
Shoot Length										
Root Length	-	-0.54					0.00^{***}			
Shoot Dry Weight	-	0.71	-0.45				0.00^{***}	0.00^{***}		
Root Dry Weight	-	0.52	-0.36	0.93			0.00^{***}	0.00^{***}	0.00^{***}	
Ion Leakage	-	0.14	-0.15	-0.13	-0.26		0.01^{**}	0.01^{**}	0.03^{*}	0.00^{***}
	Stress Conditions									
Shoot Length	0.07					0.24^{ns}				
Root Length	-0.22	0.47				0.00^{***}	0.00^{***}			
Shoot Dry Weight	-0.53	0.59	0.50			0.00^{***}	0.00^{***}	0.00^{***}		
Root Dry Weight	-0.56	0.44	0.54	0.92		0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}	
Ion Leakage	0.92	-0.07	0.04	0.02	-0.10	0.00^{***}	0.19^{ns}	0.52^{ns}	0.70^{ns}	0.07^{ns}

****Highly significant at <0.0001 **Significant at 1% *Significant at 5% ^{ns} Not Significant

4.4 Discussion

The problem posed by salt stress only seems to worsen with time due to human intrusion and climate change. Considering that rice feeds more than half of the world populace (Seck *et al*., 2012; FAO 1995), the need to develop salt stress tolerant varieties that are high yielding cannot be overemphasized. The best approach in doing this would be to test parents and breeding lines under field conditions at the two most sensitive stages (early vegetative and reproductive). However, field testing for tolerance could be difficult considering the heterogenous nature of the occurrence of salt stress in the field as well as its ability to influence deficiencies or toxicities of other essential ions in the soil (Richards, 1983; Flowers and Yeo, 1995; Talei *et al*., 2012). Hydroponic screening offers a better option that allows repeatability of experiment devoid of spatial variation. Lines with better adaptability to salt stress at the early seedling stage ensures good plant stand and may result in better grain yields.

In this study, significant ($P<0.0001$) GxE interaction was observed for shoot length, root length, shoot dry weight and root dry weight. Genotypic performance for these traits were also significantly different under control and stress conditions. These results indicate that the entries responded differently to salt stress than they did under control conditions suggesting a wide genetic variability for shoot length, root length, shoot dry weight and root dry weight among the entries. This gives an indication of how effective these traits were in correctly discriminating among progenies for adaptation to salt stress at the early seedling stage. Shoot length and dry weight as well as root length and dry weight have been suggested as good indicators for selecting cultivars adapted to saline systems in rice and is well documented (Kashenge-Killenga *et al*., 2013; Chunthaburee *et al*., 2016; De Leon *et al*., 2015;

Shakeela *et al*., 2015).

Salt stress caused a general reduction in growth among the genotypes evaluated in this study. This is a common phenomenon in most crops such as rice (Bimpong *et al*. 2016), barley (Yousofinia *et al*. 2012), wheat (Sharma 2015) and sorghum (Almodares *et al*. 2014). However, some progenies performed better than the two parents for all traits suggesting that segregation was transgressive. Transgressive segregation has been suggested to be common among morphological traits in plants and its occurrence has been attributed to the complementary gene action of additive alleles that are dispersed between the parental lines (Rieseberg *et al*., 1999). In rice, transgressive segregation under saline conditions has been reported in different populations such as F_2 (Sabouri *et al*., 2009), F_3 (Kaushik *et al.*, 2003), F_6 (Bizimana *et al*., 2017) and RIL (Wang *et al*., 2012; Rhaman *et al*., 2017) populations. However, some 16 progenies recorded mean SES between 1-2 and were classified to have significantly better tolerance (based on odd ratios) than the donor parent (Madina Koyo) which had an SES score of 4. Odd ratios estimate the degree of chance that an individual will perform differently from another individual. For example, ARS1181-1-9-6-B-6 had an odd ratio of 817.61 against Madina Koyo, which implies that the chances of ARS1181-1-9-6-B-6 having a lower score is 817.61 times more than the chance of Madina Koyo having a lower score than this progeny. Contrary to some progenies having better score than FL478 in this study, De Leon *et al*. (2016) found that, none of the F_6 Bengal/"Pokkali" progeny registered a better SES than „Pokkali", the donor source (parent) of FL478. These contrasting results could be due to the different populations used by the different studies.

Heritability estimates were lower for all traits measured under stress conditions compared to estimates under control as well as their corresponding stability values suggesting that, salt stress influenced the expression of traits (Bhadru *et al.,* 2012). On the contrary, Salam *et al*. (2011) observed higher heritability estimates under stress conditions compared to control. Under stress conditions, heritability for shoot length was high (50%) suggesting the possibility of genetic improvement for tolerance to salt stress through selection for shoot length (Bhadru *et al.,* 2012; Prajapati *et al*., 2011). Heritability for traits associated with fitness in natural populations varies between 10–20% (Visscher *et al*., 2008). In this study, heritability estimates for fitness traits (shoot and root lengths and their dry weights) ranged from
36%-50% confirming that heritability for the measured traits were high in this study. High heritability observed under stress in this study suggests that a greater proportion of the phenotypic variation is due to genotypic effects which provides opportunities for genetic improvement through selection based on these traits.

Correlation among all traits measured under control conditions and some associations under stress conditions were significant, confirming the existence of true relationships among these traits. Association between ion leakage and SES was high and positive suggesting a strong association between these two traits and that progenies with high ion leakage will have high SES. Similar association between ion leakage and SES has been previously reported (De Leon *et al*., 2015). SES correlated negatively with root length, root dry weight and shoot dry weight in this study. This implies that progenies with long and dense root system coupled with heavy dry shoot had the least SES and were the most tolerant. The implication is that, indirect selection for long and dense root systems capable of exploring deep and wide into the soil for

water (during initial osmotic stress caused by salt stress) and nutrients (in the face of deficiency or toxicity on the near surface) can be achieved through selection based on visual SES. Salt stress is known to limit the amount of water absorption through the roots, leading to accumulation of salt concentrations which eventually inhibit cell growth (Munn and Tester 2008; Bimpong *et al* 2016). Therefore, only tolerant genotypes (with lower SES) will be able to maintain healthy cell growth to produce enough biomass as observed in this study. Similar significant and negative correlation between SES and root length, root dry weight and shoot dry weight had earlier been reported by Barua *et al*. (2015) and De Leon *et al*. (2016). While root length associated negatively with shoot length, shoot dry weight and root dry weight under control, association among these traits were positive under stress. This suggests that increase in root length had an increasing effect on root dry weight, shoot dry weight and shoot length under stress conditions, with the reverse being true under control conditions. In the absence of salt stress, it is probable that roots did not need to grow deeper to sustain healthy supply of nutrients for proper growth, hence the negative association. However, under stress, roots had to grow deeper, with the anticipation that, there might be some sort of relief to the much saline conditions near the surface of the growth medium. A large increase in root length under stress compared to the control in hydroponics has been reported in tomato (Lovelli *et al*., 2012). In rice, increased root length has been observed to increase under stress especially for drought (Li *et al*., 2017; Uga *et al*., 2013; Lou *et al*., 2010).

CHAPTER FIVE

5.0 IDENTIFICATION OF QUANTITATIVE TRAIT LOCI FOR SALT STRESS TOLERANCE USING A NEWLY IDENTIFIED DONOR MADINA

KOYO MAPPING

5.1 INTRODUCTION

The complex nature of quantitative traits has been a bottleneck in conventional plant breeding. Consequently, progress through phenotypic selection has been marginal due to the polygenic nature of quantitative traits as well as heterogenous environmental conditions within which plants are tested. Molecular marker technology overcomes these challenges, allowing for the selection of target genomes, devoid of any interference. Since its inception, several studies have employed the technology in diverse ways to complement conventional breeding including genetic diversity studies, MAS and QTL studies. By regressing phenotypic data on its molecular data, it is possible to detect regions in the genome that dictate tolerance of the rather complex quantitative traits and subsequent dissection of the genetic status of tolerance mechanisms (Ismail *et al*., 2007; Thomson *et al*., 2010).

In breeding for salt stress tolerance in rice, several QTL mapping studies used linkage maps constructed based on Amplified Fragment Length Polymorphism (Gregorio, 1997), Restriction Fragment Length Polymorphism (Koyama *et al*., 2001; Bonilla *et al*., 2002; Lin *et al*., 2004), and SSR markers (Thomson *et al*., 2010; Wang *et al*., 2012; Bimpong *et al*., 2014). However, marker density in these studies were low due to low polymorphism between parents and the respective population sizes used were small. Recent advances in genome research have provided a range of molecular-marker techniques for constructing high-density genetic maps including microarrays and single feature polymorphism (SFPs) (Borevitz *et al*., 2003; Rostoks *et al*., 2005; Gupta *et al*., 2008; Xie *et al*., 2009). However, the recovery of polymorphisms depends on the probes fixed on the microarrays which restricts the markers used in the study and the technique for SFP analysis is costly and time consuming if a large segregating

population is genotyped (Yu *et al*., 2011). The development of new sequencing technologies has made it practical to use DNA sequencing technology for directly obtaining single nucleotide polymorphism (SNP) markers for population genotyping (Mardis, 2008; Schuster, 2008; Varshney *et al*., 2009). This marker technology has rapidly become the marker of choice due to its robustness, dense marker size, high precision, rapid turnaround time and high informative status. Bimpong *et al*. (2014) used 384 SNP chip to map 7 significant QTLs related to salt stress tolerance in rice. Recently, 20 fitness related QTLs under salt stress at the early seedling stage have been mapped in rice from a novel donor source Hasawi using a 384 SNP chip (Bizimana *et al*., 2017). However, identified QTLs in these studies are delimited by rather large flanking markers making their use quite difficult. There is the need to use high density marker size to ensure better saturation and accurate prediction of QTLs with less false positives.

The most promising QTL so far reported for seedling stage salt stress tolerance in rice is *Saltol* QTL that was mapped to the position of 10.7–12.2 Mb region on the short arm of the chromosome 1 explaining 43 % of phenotypic variation in shoot Na^+/K^+ ratio (Bonilla *et al*. 2002; Gregorio 1997). Lin *et al*. (2004) also identified a major QTL for shoot K^+ concentration on chromosome 1 (*qSKC-1*) using a F_2 derived F_3 population derived from a cross of „Nona Bokra" and Koshihikari within the *Saltol* region. Further, Ren *et al*. (2005) identified the *SKC1* gene underlying the *qSKC-1* QTL by map-based cloning and found that it encodes HKT-type Na^+ transporter. Subsequently, several other studies have identified and mapped QTLs for salt stress tolerance (Lee *et al*, 2006; Singh *et al*. 2007; Kim *et al*. 2009; Sabouri *et al*. 2009; Haq *et al*. 2010; Bimpong *et al*., 2014; Bizimana *et al*. 2017). However, little progress has

been made in using these QTLs to improve tolerance among elite rice varieties because several of such studies have sought to achieve improvement using *Saltol* as the only donor even though tolerance to salt stress is a complex trait controlled by several traits. The identification and pyramiding of several QTLs for different traits may be a better option in improving tolerance among rice varieties

(Rahman *et al.,* 2016). Moreso, majority of these *Saltol* donor parents such as „Pokkali" and „Nona Bokra" possess many undesirable traits (tall, lodge, photosensitive, low yielding, and red pericarp) which may prolong the breeding process due to linkage drag. There is therefore the need to identify new donor sources for tolerance to salt stress which will make it possible to identify and pyramid several QTLs for different traits to increase chances of developing rice varieties that are tolerant to salt stress.

In this study, a new donor source Madina Koyo has been identified and used in the development of a mapping population. The objectives for this study, therefore, were to:

i. genotype by sequencing using high density SNP markers for the newly developed mapping population.
ii. identify genomic region(s) controlling different salt stress traits iii. map QTLs controlling tolerance to salt stress

5.2 Material and Methods

Plant material used for QTL mapping included 308 $F_{3:4}$ progenies evaluated for tolerance at the early seedling stage and their 2 parents Sahel 317 and Madina Koyo.

5.2.1 DNA Extraction and Molecular Analysis

Eight leaf discs of 6 mm diameter were punched from 15-day old leaves and collected into 96 deep well PCR plates. Prior to sampling, leaves were dried at 50^{o}C for 2 days to prevent possible mold development during shipment to the outsourced laboratory. DNA extraction and genotyping were done by sequencing using the Integrated Genotyping Service and Support (IGSS) based in the Biosciences eastern and central Africa (BecA) hosted by the international livestock research institute (ILRI) in Kenya. IGSS uses DArTseq™ technology from DArT (Diversity Arrays Technology Pty Ltd) in Australia. DArTseq™ represents a combination of a DArT complexity reduction methods and next generation sequencing platforms (Kilian *et al*., 2012; Altshuler *et al*., 2000). Compared to other similar approaches, DArTseq™ yields a lower density of markers (10‟s of thousands and up to 35,000 loci versus >800,000 loci with a GBS approach) but has substantially higher coverage and results in less missing data (Chen *et al*., 2016). An added advantage is the fact that DArTseq™ can directly score samples as either heterozygous/homozygous at each locus with the lower density approach (Chen *et al*., 2016) and has the capacity to produce several short, high quality polymorphic loci using a custom analytical pipeline (Sansaloni *et al*., 2011; Raman *et al*., 2014; Al-Beyroutiova *et al*., 2016) making it easier to optimize the platform species-specific projects.

5.2.2 Data filtering process and DArTseq SNP calling

DArTseq SNP derived markers were filtered to remove uncallable SNPs and genotypes using FlapJack software (Milne *et al*., 2010), where genotypes with more than 30% missing data, SNP loci with more than 20% missing data and rare SNPs with less than 5% minor allele frequencies (MAF) were pruned. Only 3698 DArTseq

informative SNPs (out of 10336) and 310 genotypes were considered after filtering and data quality control process. Missing data were imputed using KDCompute, an online analysis plugin that runs on R scripts.

5.2.3 Construction of Linkage Maps and Detection of QTLs

The SNP data was used to construct a genetic linkage map for the F_3 population derived from new salinity donor Madina Koyo and Sahel 317. Only traits for which the progenies showed significant differences were maintained for QTL analysis (thus Ion Leakage was dropped). QTL analysis was performed by Windows QTL Cartographer Version 2.5_011 (Wang *et al*., 2012) using default settings for Composite Interval Mapping, CIM (map function= Haldane, Model= 6, Regression method= backward) with a permutation time of 1000. MapChart Version 2.3 (Voorrips, 2002) was used for the construction of detailed linkage maps showing positions of QTLs. Due to the highly dense linkage maps generated, construction of maps showing QTL positions focused on markers within QTL regions only. QTLs were named following nomenclature proposed by McCouch *et al*. (1997). Basically, QTL names started with a lowercase "*q*", which was followed by a 2-3 initials of the corresponding measured trait (in upper cases) and then followed by the chromosome number. If there were more than one QTL on any chromosome, a "." was used to uniquely identify them.

5.3 Results

5.3.1 Summary of SNP Marker Information.

A total of 3698 SNP markers were used to genotype 308 progenies and their two parents (Sahel 317 and Madina Koyo). Table 5.1 shows summary of genotypic proportions of the two parents among the progenies. The amount of recipient parent

genome retained among the progenies genotyped was 28% while that of the donor parent was 50%. The remaining 22% of the collective progeny genome was heterozygous between the two parents. This distribution represents a 1:1:2 homozygote dominant: heterozygote: homozygote recessive distribution for the F_3 population.

Table 5.1 Percentage of Parental Genome among the progenies

Marker-genotype	Marker-genotype-translation	Number	Percentage
AA	Recipient Parent Genotype	315131	28
Aa	Heterozygous	249961	22
aa	Donor Parent Genotype	573892	50
Total			100

5.3.2 Genetic Linkage map for the F_3 derived between Sahel 317 and Madina Koyo

The genetic linkage map is presented in Figure 5.1. Summary of the genetic linkage groups is presented in Table 5.2. Linkage analysis was performed on 308 F_3 families genotyped by sequencing with 3698 SNP markers. Twelve (12) linkage groups (LG) were constructed which corresponded to the 12 gametic rice chromosomes, spanning a total length of 1617.1 cM (369131487bp) at an average marker interval of 0.46 cM (Table 5.2). Linkage group 1 was the longest (203.9 cM) and had the most markers (532 markers) with an average marker interval of 0.38 cM, while 4 LGs (9, 10, 11 and 12) had lengths less than 100 cM. Linkage group 10, the second shortest group had the fewest number of markers (188) with a marker at every 0.49 cM. Linkage group 4 with a length of 130.7 cM and 473 markers had the best coverage with a marker at every 0.28 cM. Linkage group 5 had the widest marker interval of 0.64 cM. the average marker density was 0.46 cM.

Table 5.2 Genetic linkage groups for the 3698 SNP markers

Linkage Group/ Chromosome No.	Physical Length (bp)	Genetic Length (cM)	Number of SNPs	Average Physical Distance (bp)	Average Linkage Distance (cM)
1	43229577	203.9	532	81259	0.38
2	35890520	175.1	292	122913	0.60
3	36362027	194.4	354	102718	0.55
4	34980319	130.7	473	73954	0.28
5	29270709	132.4	207	141404	0.64
6	30371849	134.5	270	112488	0.50
7	28924473	151.0	328	88184	0.46
8	28361417	117.3	250	113446	0.47
9	22614057	92.0	204	110853	0.45
10	23107718	93.0	188	122913	0.49
11	28912694	94.3	326	88689	0.29
12	27106127	98.7	274	98927	0.36

Total/Average	369131487	1617.1	3698	1257748	0.46

5.3.3 Identification and mapping of QTLs

A total of 46 QTLs were identified for 4 traits (SES, shoot length, root length, and shoot dry weight) studied across 10 chromosomes (Figure 5.1). Eight QTLs were identified for SES score, four for root dry weight, 14 for shoot dry weight and 19 for shoot length. While chromosome 1 harbored the most QTLs (11) with at least one QTL for each of the 4 traits, chromosomes 4 had the least number of QTLs (1) with chromosomes 5 and 6 not showing any QTL. Some chromosomes (1, 2, 3 and 7) were long and could not be fitted in one continuous LG by the program MapChart (Figure 5.1) hence had to be separated into two. For example, QTLs for chromosome 1 were displayed as Chromosome 1 (1) and Chromosome (2).

5.3.4 QTLs for SES Score.

A total of 8 QTLs were mapped for SES score; one on chromosome 1 (*qSES1* between 176.2-176.9 cM and peaked at 176.8 cM), three on chromosome 2 (*qSES2.1, qSES2.2* and *qSES2.3* at 54.5, 111 and 118.2 cM respectively), two on chromosome 7 (*qSES7.1 and qSES7.2* at 55 and 122.9 cM respectively), and one each on chromosomes 10 (*qSES10* at 44.3 cM) and 11 (*qSES11* at 60.0 cM) (Table 5.3). All 8 QTLs had minor effects and explained 0.7-4.4% of the phenotypic variance. The *qSES2.3* contributed most to the phenotypic variance (4.4%) and had the highest LOD score (3.37) with an additive effect of -0.38. This was followed by *qSES2.2* with R^2 of 3.8%, LOD of 2.67 and an additive effect of -0.37. The least contribution to the phenotypic variation came from *qSES2.1* (R^2 = 0.7%) with 2.86 LOD and additive effect of -0.16.

Table 5.3 QTLs identified for SES under salt stress conditions

QTL Name	Chr.	Position (cM)	LOD	Additive Effect	Source of Increasing Allele	R^2 (%)	Interval (cM)	Flanking Markers
qSES1	1	176.81	2.74	-0.78	Madina Koyo	3.0	175.6-176.9	3059508|F|0-16:T>A-16:T>A - 12386616|F|0-23:T>C-23:T>C
qSES2.1	2	54.51	2.86	-0.16	Madina Koyo	0.7	54.4-54.6	3448390|F|0-9:A>C-9:A>C - 10272514|F|0-16:T>A-16:T>A
qSES2.2	2	111.01	2.67	-0.37	Madina Koyo	3.8	110.9-111.1	9752116|F|0-43:A>T-43:A>T - 5140307|F|0-17:C>T-17:C>T
qSES2.3	2	118.21	3.37	-0.38	Madina Koyo	4.4	117.2-119.5	3056318|F|0-54:A>G-54:A>G - 5142709|F|0-32:A>G-32:A>G
qSES7.1	7	55.01	2.84	0.31	Madina Koyo	0.4	52.5-55.5	100147611|F|0-22:G>A-22:G>A - 100112465|F|0-18:T>C-18:T>C
qSES7.2	7	122.91	2.73	-0.69	Madina Koyo	1.7	120.9-124.2	3063473|F|0-29:A>G-29:A>G - 3048456|F|0-20:A>G-20:A>G
qSES10	10	44.31	2.66	-0.04	Madina Koyo	1.2	42.7-45.5	3453430|F|0-47:A>G-47:A>G - 100111501|F|0-36:T>G-36:T>G
qSES11	11	60.01	3.16	0.36	Sahel 317	1.4	59.5-62.0	100112475|F|0-34:C>T-34:C>T - 5381322|F|0-8:C>T-8:C>T

5.3.5 QTLs for Root Dry Weight.

Five QTLs were identified for root dry weight (Table 5.4). Among these five, one each was identified on chromosomes 1 (*qRDW1*) and 3 (*qRDW3*) while three were mapped to chromosome 7 (*qRDW7.1*, *qRDW7.2* and *qRDW7.3*). All five QTLs were of minor effects with no additive effect. The *qRDW1* with R^2= 6%, made the highest contribution to the phenotypic variance followed by *qRDW7.3* which explained 3% of the variance in root dry weight and recorded the highest LOD of 149.5. The remaining three QTLs; *qRDW3*, *qRDW7.1* and *qRDW7.2* explained 2.2%, 2.6% and 2.0% of the phenotypic variance respectively.

Table 5.4 QTLs identified for Root Dry Weight under salt stress conditions

QTL Name	Chr.	Position (cM)	LOD	Additive Effect	Source of Increasing Allele	R^2 (%)	Interval (cM)	Flanking Markers
qRDW1	1	84.31	3.4	0	-	6.1	82.6-84.4	3448991|F|0-64:C>T-64:C>T - 100109180|F|0-16:G>C-16:G>C
qRDW3	3	8.11	4.7	0	-	2.2	7.4-10.1	3055415|F|0-66:T>C-66:T>C - 3063054|F|0-25:C>T-25:C>T
qRDW7.1	7	133.91	3.2	0	-	2.6	133.4-133.9	100111619|F|0-55:G>A-55:G>A - 100050673|F|0-5:C>G-5:C>G
qRDW7.2	7	136.31	3.6	0	-	2.0	136.2-136.5	3056128|F|0-9:A>G-9:A>G - 9761229|F|0-17:T>C-17:T>C
qRDW7.3	7	149.51	3.6	0	-	3.0	148-149.8	3054699|F|0-35:T>C-35:T>C - 3999459|F|0-9:G>C-9:G>C

5.3.6 QTLs for Shoot Dry Weight.

Of the 14 QTLs identified for shoot dry weight, 3 were mapped on chromosome one (*qSDW1.1, qSDW1.2* & *qSDW1.3*), 1 each on chromosomes two (*qSDW2*), seven (*qSDW7*) and eleven (*qSDW11*), 2 each on chromosomes three (*qSDW3.1* & *qSDW3.2*) and twelve (*qSDW12.1* & *qSDW12.2*) and 4 on chromosome 9 (*qSDW9.1, qSDW9.2, qSDW9.3* & *qSDW9.4*) (Table 5.6). Only two out of the 14 (*qSDW1.1* at a peak of 25.7 cM and *qSDW2* at a peak of 55.6 cM) had major effect on the shoot dry weight. Respectively, *qSDW1.1* and *qSDW2* contributed 29.6% and 11.2% to the variance in shoot dry weight observed among the population. The remaining 12 shoot dry weight QTLs had minor effects with phenotypic variations ranging from 2.4% for *qSDW7* on chromosome 7 to 6.5% for *qSDW11* on chromosome 11. The *qSDW12.1* recorded the highest LOD of 5.6 followed by *qSDW9.2* at LOD of 5.1. The least LOD at which a QTL for shoot dry weight was detected was at 3.0 for *qSDW1.1*.

Table 5.5 QTLs identified for Shoot Dry Weight under salt stress conditions

QTL Name	Chr.	Position (cM)	LOD	Additive Effect	Source of Increasing Allele	R^2 (%)	Interval (cM)	Flanking Markers
qSDW1.1	1	25.71	3	0.03	Sahel 317	29.6	24.7-27.5	3058811|F|0-26:A>G-26:A>G - 5399201|F|0-66:A>C-66:A>C
qSDW1.2	1	55.41	3.22	-0.02	Madina Koyo	5.2	53-61.3	100164910|F|0-5:T>C-5:T>C - 5388554|F|0-57:A>G-57:A>G
qSDW1.3	1	93.21	3.64	-0.01	Madina Koyo	4.5	92.9-96.1	3049525|F|0-62:C>T-62:C>T - 3450375|F|0-23:A>G-23:A>G
qSDW2	2	55.61	4.07	-0.04	Madina Koyo	11.2	54.6-61.2	3762312|F|0-27:T>A-27:T>A - 100161114|F|0-7:A>T-7:A>T
qSDW3.1	3	160.91	4.19	0.01	Sahel 317	5.2	158.3-161.1	3450598|F|0-16:T>A-16:T>A - 5403448|F|0-10:C>T-10:C>T
qSDW3.2	3	161.81	4.65	0.01	Sahel 317	6.3	161.7-161.9	3058773|F|0-61:A>G-61:A>G - 3050772|F|0-22:T>C-22:T>C
qSDW7	7	149.51	3.88	0	-	2.4	148.2-149.8	3059821|F|0-67:A>G-67:A>G - 100186607|F|0-9:A>G-9:A>G
qSDW9.1	9	19.81	3.82	-0.02	Madina Koyo	4.1	17.1-20.2	3453872|F|0-53:C>T-53:C>T - 9752844|F|0-7:C>T-7:C>T
qSDW9.2	9	25.11	5.14	-0.02	Madina Koyo	3.3	24.8-25.2	9750028|F|0-52:A>C-52:A>C - 100134838|F|0-8:A>G-8:A>G
qSDW9.3	9	29.01	4.15	-0.02	Madina Koyo	2.8	27-29.2	3054699|F|0-35:T>C-35:T>C - 3999459|F|0-9:G>C-9:G>C
qSDW9.4	9	43.81	4.02	-0.02	Madina Koyo	5.9	43.6-43.9	100147949|F|0-35:A>G-35:A>G - 3993879|F|0-18:G>A-18:G>A
qSDW11	11	32.91	4.14	-0.02	Madina Koyo	6.5	32.8-33.0	5145308|F|0-17:A>T-17:A>T - 100109582|F|0-10:T>G-10:T>G
qSDW12.1	12	55.51	5.59	-0.02	Madina Koyo	5.1	55.4-55.7	9755603|F|0-40:C>T-40:C>T - 4387814|F|0-19:T>C-19:T>C
qSDW12.2	12	70.61	3.07	-0.02	Madina Koyo	2.6	70.2-70.8	3447933|F|0-26:G>A-26:G>A - 3443453|F|0-24:C>G-24:C>G

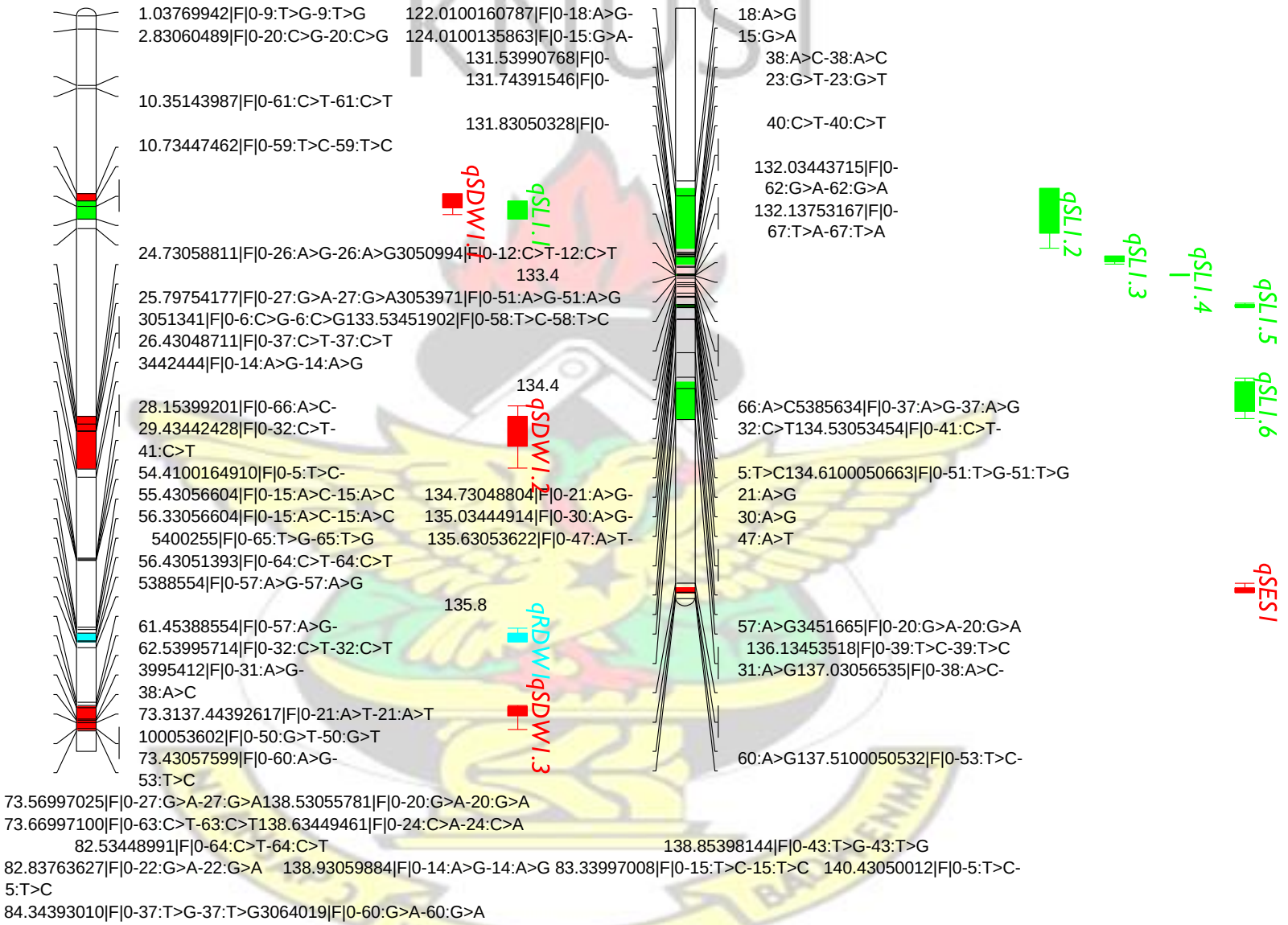
5.3.7 QTLs for Shoot Length.

A total of 19 QTLs were identified for Shoot length in this study. Six were mapped on chromosome one (*qSL1.1, qSL1.2, qSL1.3, qSL1.4, qSL1.5 and qSL1.6* at 26.4, 124, 132.1, 134.5, 138.6, and 149.7 cM respectively), 5 on chromosome two (*qSL2.1, qSL2.2, qSL2.3, qSL2.4 and qSL2.5* at peak positions of 45.7, 50.9, 54.6, 104.9 and 115.9 cM respectively), 1 each on chromosomes three (*qSL3* at 137.5 cM), four (*qSL4* at 64.5 cM), eight (*qSL8* at 109.1 cM), nine (*qSL9* at 43.9 cM), ten (*qSL10* at 29.7 cM) and twelve (*qSL12* at 91.9 cM) and another 2 on chromosome 11 (*qSL11.1* at 13.5 cM and *qSL11.1* at 48.3 cM) (Table 5.6). The LOD ranged between 3.77 to as high as 9.3 for these 19 shoot length QTLs. Of these, four (*qSL1.1, qSL2.2, qSL2.3* and *qSL2.4*) were major effect QTLs, with *qSL1.1* explaining almost 100% of the phenotypic variance. This QTL span a region of 25.7-28.1 cM with a peak at 26.41 cM on chromosome one and an additive effect of -3.41. The remaining three major effect QTLs were mapped on chromosome two between 47.8-53.4 cM (*qSL2.2*), 54.5-54.6 cM (*qSL2.3*) and 104.3-105 cM (*qSL2.4*). The *qSL2.2* explained 12% of the phenotypic variance while *qSL-2-3* and *qSL-2-4* contributed 12.4% and 11.2% respectively to the phenotypic variance. The remaining 15 QTLs with minor effects contributed between 0.4-8.4% to the variance in shoot length among the population.

Table 5.6 QTLs identified for Shoot Length under salt stress conditions

QTL Name	Chr.	Position (cM)	LOD	Additive Effect	Source of Increasing Allele	R^2 (%)	Interval (cM)	Flanking Markers
qSL1.1	1	26.41	3.88	-3.41	Madina Koyo	99.4	25.7-28.1	9754177|F|0-27:G>A-27:G>A - 5399201|F|0-66:A>C-66:A>C
qSL1.2	1	124.01	6.61	0.87	Sahel 317	4.6	123.0-131.0	100160787|F|0-18:A>G-18:A>G - 3990768|F|0-38:A>C-38:A>C
qSL1.3	1	132.11	5.48	0.88	Sahel 317	3.9	132-133.1	3443715|F|0-62:G>A-62:G>A - 3050994|F|0-12:C>T-12:C>T
qSL1.4	1	134.51	7.65	1.01	Sahel 317	8.3	134.4-134.5	3048711|F|0-37:C>T-37:C>T - 3444914|F|0-30:A>G-30:A>G
qSL1.5	1	138.61	9.3	1.01	Sahel 317	8.1	138.3-138.8	100050532|F|0-53:T>C-53:T>C - 3059884|F|0-14:A>G-14:A>G
qSL1.6	1	149.71	4.19	-0.58	Madina Koyo	4.9	148.3-153.7	3061076|F|0-15:A>T-15:A>T - 4391970|F|0-68:G>A-68:G>A
qSL2.1	2	45.71	4.39	-0.55	Madina Koyo	8.4	44.8-46.1	3056610|F|0-32:T>G-32:T>G - 5405148|F|0-63:T>C-63:T>C
qSL2.2	2	50.91	4.71	-0.89	Madina Koyo	12	47.8-53.4	100156096|F|0-61:G>A-61:G>A - 3049046|F|0-42:C>G-42:C>G
qSL2.3	2	54.61	5.34	-0.61	Madina Koyo	12.4	54.5-54.6	12388091|F|0-28:A>T-28:A>T - 4392723|F|0-31:A>C-31:A>C
qSL2.4	2	104.91	5.14	1.12	Sahel 317	11.2	104.3-105.0	4393469|F|0-24:T>C-24:T>C - 3062021|F|0-45:A>T-45:A>T
qSL2.5	2	115.91	4.63	0.08	Sahel 317	1.4	115.7-115.9	100110606|F|0-63:G>T-63:G>T - 3060184|F|0-46:T>C-46:T>C
qSL3	3	137.51	4.13	-0.86	Madina Koyo	5.9	137.4-137.5	3453354|F|0-18:A>T-18:A>T - 3447565|F|0-6:G>T-6:G>T
qSL4	4	64.51	4.11	0.51	Sahel 317	2.8	61.4-66.8	4388956|F|0-12:C>T-12:C>T - 3059821|F|0-67:A>G-67:A>G
qSL8	8	109.11	4.29	0.63	Sahel 317	3.8	109-110.4	3765663|F|0-11:C>T-11:C>T - 3449994|F|0-19:G>T-19:G>T
qSL9	9	43.91	4.25	0.48	Sahel 317	3.5	43.8-44.1	3054917|F|0-24:G>A-24:G>A - 6996219|F|0-19:T>C-19:T>C
qSL10	10	29.71	3.77	0.42	Sahel 317	0.4	29.6-30.7	5141542|F|0-53:A>T-53:A>T - 3445686|F|0-50:A>G-50:A>G
qSL11.1	11	13.51	4.54	0.45	Sahel 317	1.4	13.3-13.8	3057865|F|0-54:C>T-54:C>T - 3051953|F|0-33:C>T-33:C>T
qSL11.2	11	48.31	5.05	0.69	Sahel 317	6.3	47.6-49.3	3050151|F|0-19:T>A-19:T>A - 3059651|F|0-22:T>G-22:T>G
qSL12	12	91.91	4.14	0.46	Sahel 317	2.5	91.0-93.0	100047863|F|0-14:G>A-14:G>A - 4392609|F|0-26:G>A-26:G>A

Chromosome1 [1] **Chromosome1 [2]**


1.03769942|F|0-9:T>G-9:T>G
2.83060489|F|0-20:C>G-20:C>G
10.35143987|F|0-61:C>T-61:C>T
10.73447462|F|0-59:T>C-59:T>C
qSDW1.1
qSL1.1
24.73058811|F|0-26:A>G-26:A>G3050994|F|0-12:C>T-12:C>T
133.4
25.79754177|F|0-27:G>A-27:G>A3053971|F|0-51:A>G-51:A>G
3051341|F|0-6:C>G-6:C>G133.53451902|F|0-58:T>C-58:T>C
26.43048711|F|0-37:C>T-37:C>T
3442444|F|0-14:A>G-14:A>G
134.4
28.15399201|F|0-66:A>C-
29.43442428|F|0-32:C>T-
41:C>T
qSDW1.2
54.4100164910|F|0-5:T>C-
55.43056604|F|0-15:A>C-15:A>C
56.33056604|F|0-15:A>C-15:A>C
5400255|F|0-65:T>G-65:T>G
56.43051393|F|0-64:C>T-64:C>T
5388554|F|0-57:A>G-57:A>G
135.8
61.45388554|F|0-57:A>G-
62.53995714|F|0-32:C>T-32:C>T
3995412|F|0-31:A>G-
38:A>C
qRDW1
qSDW1.3
73.3137.44392617|F|0-21:A>T-21:A>T
100053602|F|0-50:G>T-50:G>T
73.43057599|F|0-60:A>G-
53:T>C
73.56997025|F|0-27:G>A-27:G>A138.53055781|F|0-20:G>A-20:G>A
73.66997100|F|0-63:C>T-63:C>T138.63449461|F|0-24:C>A-24:C>A
82.53448991|F|0-64:C>T-64:C>T
82.83763627|F|0-22:G>A-22:G>A
138.93059884|F|0-14:A>G-14:A>G
84.34393010|F|0-37:T>G-37:T>G3064019|F|0-60:G>A-60:G>A
122.0100160787|F|0-18:A>G-
124.0100135863|F|0-15:G>A-
131.53990768|F|0-
131.74391546|F|0-
131.83050328|F|0-
124.0100135863|F|0-15:G>A-
131.53990768|F|0-
131.74391546|F|0-
131.83050328|F|0-
18:A>G
15:G>A
38:A>C-38:A>C
23:G>T-23:G>T
40:C>T-40:C>T
132.03443715|F|0-
62:G>A-62:G>A
132.13753167|F|0-
67:T>A-67:T>A
qSL1.2
qSL1.3
qSL1.4
qSL1.5
qSL1.6
66:A>C5385634|F|0-37:A>G-37:A>G
32:C>T134.53053454|F|0-41:C>T-
5:T>C134.6100050663|F|0-51:T>G-51:T>G
21:A>G
30:A>G
47:A>T
qSES1
57:A>G3451665|F|0-20:G>A-20:G>A
136.13453518|F|0-39:T>C-39:T>C
31:A>G137.03056535|F|0-38:A>C-
60:A>G137.5100050532|F|0-53:T>C-
138.85398144|F|0-43:T>G-43:T>G
83.33997008|F|0-15:T>C-15:T>C
140.43050012|F|0-5:T>C-
5:T>C

140.5
84.4100109180|F|0-16:G>C-16:G>C3988359|F|0-24:T>A-24:T>A 85.23441464|F|0-62:C>G-62:C>G144.95142362|F|0-8:T>C-8:T>C
92.53757792|F|0-8:T>C-8:T>C148.23061076|F|0-15:A>T-15:A>T
92.93049525|F|0-62:C>T-62:C>T149.73442415|F|0-46:C>T-46:C>T
93.2100047407|F|0-31:C>T-31:C>T4391970|F|0-68:G>A-68:G>A
153.8
94.7100052709|F|0-20:T>G-20:T>G3764289|F|0-29:A>G-29:A>G
94.93439945|F|0-6:T>C-6:T>C175.63059508|F|0-16:T>A-16:T>A
95.23055667|F|0-47:G>A-47:G>A3048668|F|0-38:T>C-38:T>C
176.8
3453249|F|0-29:T>C-29:T>C3049442|F|0-7:G>C-7:G>C
96.0176.912386616|F|0-23:T>C-23:T>C
3051649|F|0-40:A>C-40:A>C
96.33450375|F|0-23:A>G-23:A>G 177.73756308|F|0-14:C>A-14:C>A

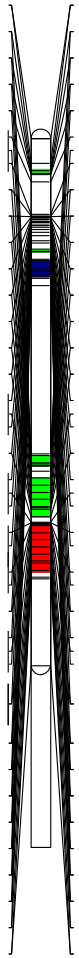
Figure 5.1. Linkage groups showing positions (in cM) of identified QTLs and chromosomes involved.

Chromosome2 [1] **Chromosome2 [2]**

4392608|F|0-10:A>T-10:A>T0.0
100110606|F|0-63:G>T-63:G>T44.5
.44.7
3051482|F|0-25:C>T-25:C>T45.7
3452415|F|0-7:A>G-
3060184|F|0-46:T>C-46:T>C3449994|F|0-
46.1
3056174|F|0-68:C>T-68:C>T3049768|F|0-
3453354|F|0-18:A>T-
22:G>A110.9 .46.9100112271|F|0-7:C>G-
.48.93449744|F|0-48:A>G-48:A>G111.3
.49.93055418|F|0-35:T>G-35:T>G111.4
5407353|F|0-26:T>C-26:T>C50.93448204|F|0-14:A>G-14:A>G111.6
.51.9
3052931|F|0-
16:T>A-16:T>A52.13048719|F|0-56:C>T-56:C>T111.8
52.3
3062378|F|0-59:T>C-59:T>C3053101|F|0-15:C>T-15:C>T112.2
.53.3
54.1
3051615|F|0-47:G>A-47:G>A4388527|F|0-60:T>C-60:T>C112.8 3990520|F|0-49:C>A-49:C>A54.2 3049606|F|0-62:C>T-62:C>T
113.2
3051912|F|0-48:A>T-48:A>T100147799|F|0-21:T>A-21:T>A
54.3
3050551|F|0-31:T>C-31:T>C9749803|F|0-64:C>T-64:C>T113.7
3056318|F|0-54:A>G-54:A>G54.4
4388956|F|0-12:C>T-12:C>T4393414|F|0-7:C>G-7:C>G114.7
54.5
3439917|F|0-18:A>G-18:A>G3054917|F|0-24:G>A-24:G>A115.6 3059821|F|0-67:A>G-67:A>G9760917|F|0-5:A>G-
5:A>G
100186607|F|0-9:A>G-9:A>G 62.1
54.6115.9
.3063684|F|0-37:C>T-37:C>T
5142709|F|0-32:A>G-32:A>G55.6 6996219|F|0-19:T>C-19:T>C116.0 .56.6

qSDW2
qSL2.3
qSES2.1
qSL2.2
qSL2.1
qSES2.3
qSL2.5
qSES2.2
qSL2.4

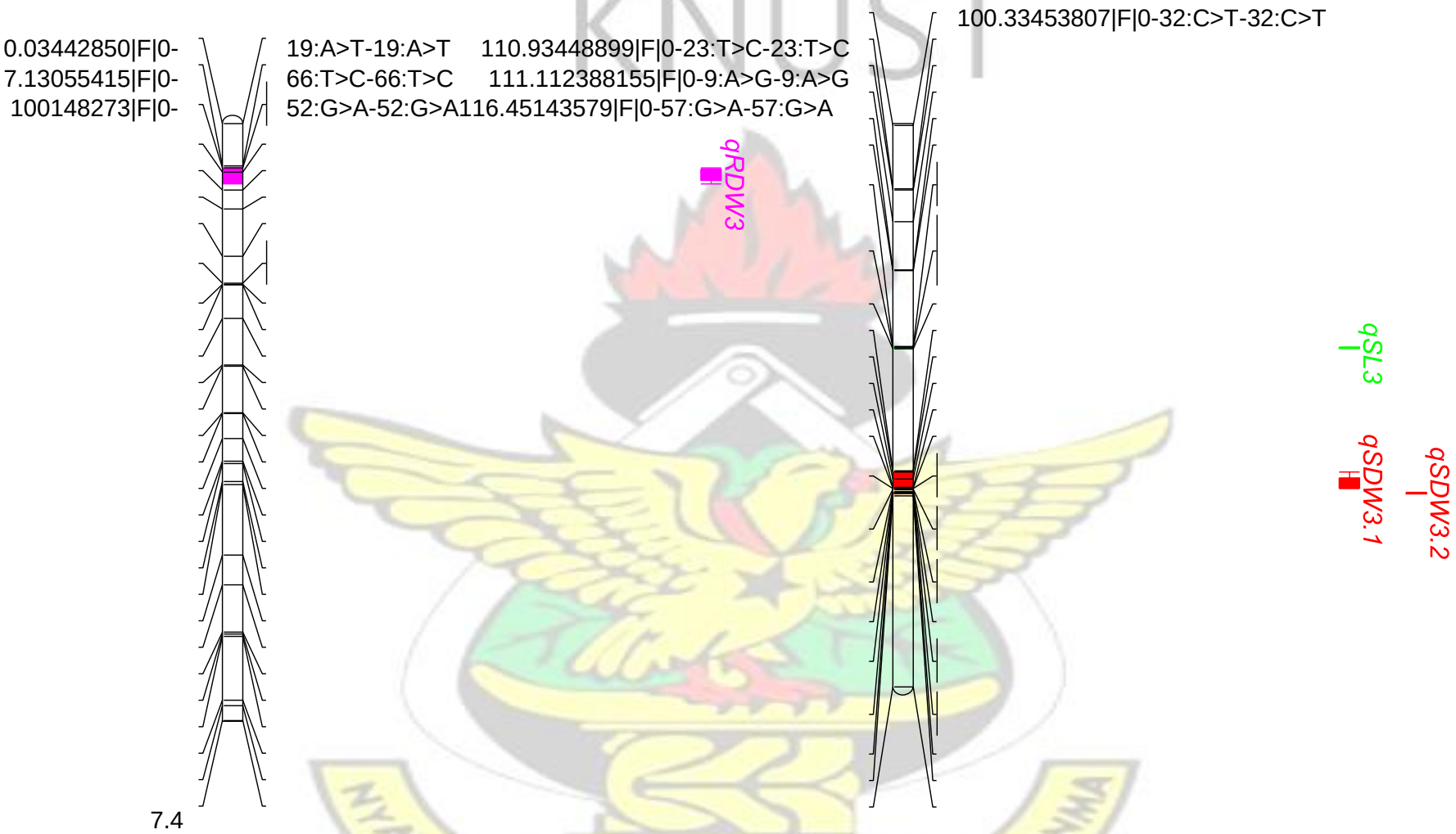
3765663|F|0-11:C>T-11:C>T103.5
.104.5
3050849|F|0-58:A>T-58:A>T104.9
3453969|F|0-49:A>G-49:A>G105.0
7:A>G45.93449994|F|0-19:G>T-19:G>T105.1
19:G>T-19:G>T106.1
38:T>G-38:T>G110.7
18:A>T46.2100147611|F|0-22:G>A-
7:C>G111.0
.47.9100112465|F|0-18:T>C-18:T>C111.1
3450921|F|0-43:T>G-43:T>G111.7
100047180|F|0-32:T>G-32:T>G3049319|F|0-48:G>A-48:G>A111.9
3054298|F|0-65:T>C-65:T>C112.3
3447565|F|0-6:G>T-6:G>T3061514|F|0-11:G>C-11:G>C112.5
3050175|F|0-29:C>T-29:C>T114.4
3050428|F|0-58:G>A-58:G>A 174.4
3063473|F|0-29:A>G-
29:A>G117.0



.57.6 .117.2
.58.6 3055769|F|0-50:A>C-50:A>C118.2
.59.6 3063102|F|0-30:C>T-30:C>T118.7
3453135|F|0-36:G>A-36:G>A59.9 3048456|F|0-20:A>G-20:A>G119.5 .60.9 3063177|F|0-46:G>C-46:G>C119.7
.61.9 .120.7

Figure 5.1 Cont"d.

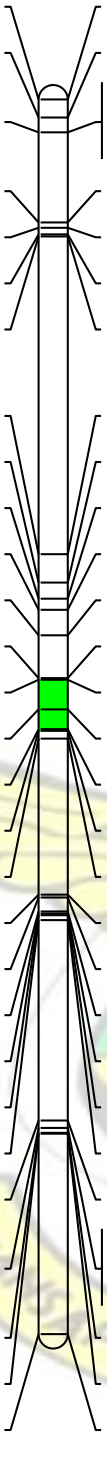
Chromosome3 [1] **Chromosome3 [2]**


0.03442850|F|0-
7.13055415|F|0-
100148273|F|0-
19:A>T-19:A>T
66:T>C-66:T>C
52:G>A-52:G>A
110.93448899|F|0-23:T>C-23:T>C
111.112388155|F|0-9:A>G-9:A>G
116.45143579|F|0-57:G>A-57:G>A
100.33453807|F|0-32:C>T-32:C>T
qRDW3
qSL3
qSDW3.1
qSDW3.2
7.4
3049544|F|0-10:C>A-10:C>A
8.13052451|F|0-7:C>T-7:C>T
11.13063054|F|0-25:C>T-25:C>T
3051531|F|0-57:T>C-57:T>C
124.53453166|F|0-54:A>T-54:A>T
124.63060365|F|0-16:A>T-16:A>T

14.35404956|F|0-47:A>T-47:A>T137.35141542|F|0-53:A>T-53:A>T
22.23763763|F|0-43:A>G-43:A>G3452296|F|0-38:G>A-38:G>A
4392750|F|0-30:C>A-30:C>A 137.53445686|F|0-50:A>G-50:A>G
26.7
3758798|F|0-20:C>A-20:C>A3445157|F|0-34:A>C-34:A>C
26.94391724|F|0-57:C>T-57:C>T 137.73452458|F|0-38:C>A-38:C>A
27.03755609|F|0-34:C>T-34:C>T 158.13453872|F|0-53:C>T-53:C>T
32.6100052377|F|0-43:C>G-43:C>G 158.33053665|F|0-30:C>T-30:C>T
40.4100049517|F|0-7:A>G-7:A>G 159.53051578|F|0-47:T>C-47:T>C
40.6100109503|F|0-11:C>T-11:C>T 160.93055188|F|0-47:C>T-47:C>T
48.43437425|F|0-19:C>T-19:C>T 161.03048477|F|0-19:T>G-19:T>G
48.56996147|F|0-21:G>A-21:G>A3453171|F|0-10:A>G-10:A>G
52.73449177|F|0-37:T>C-37:T>C 161.13453303|F|0-29:A>C-29:A>C
56.512386724|F|0-11:A>C-11:A>C9752844|F|0-7:C>T-7:C>T
56.93056373|F|0-68:A>G-68:A>G 161.29757595|F|0-32:A>T-32:A>T
59.93050512|F|0-63:T>C-63:T>C9754056|F|0-26:G>C-26:G>C
60.45141777|F|0-61:A>C-61:A>C 161.33050322|F|0-49:C>G-49:C>G
72.25398632|F|0-65:C>T-65:C>T 161.63059881|F|0-55:T>A-55:T>A
77.23447951|F|0-18:A>G-18:A>G3440241|F|0-28:A>G-28:A>G
85.13048799|F|0-10:T>A-10:T>A 161.79750028|F|0-52:A>C-52:A>C
85.43052982|F|0-9:A>C-9:A>C3054579|F|0-67:C>G-67:C>G
85.83763411|F|0-37:A>G-37:A>G 161.83061840|F|0-63:A>T-63:A>T
96.53059228|F|0-13:G>A-13:G>A 161.9100134838|F|0-8:A>G-8:A>G
97.43059727|F|0-31:G>T-31:G>T 162.33437477|F|0-59:C>T-59:C>T
99.912387877|F|0-28:C>A-28:C>A 194.33446472|F|0-30:T>A-30:T>A

Figure 5.1 Cont"d.

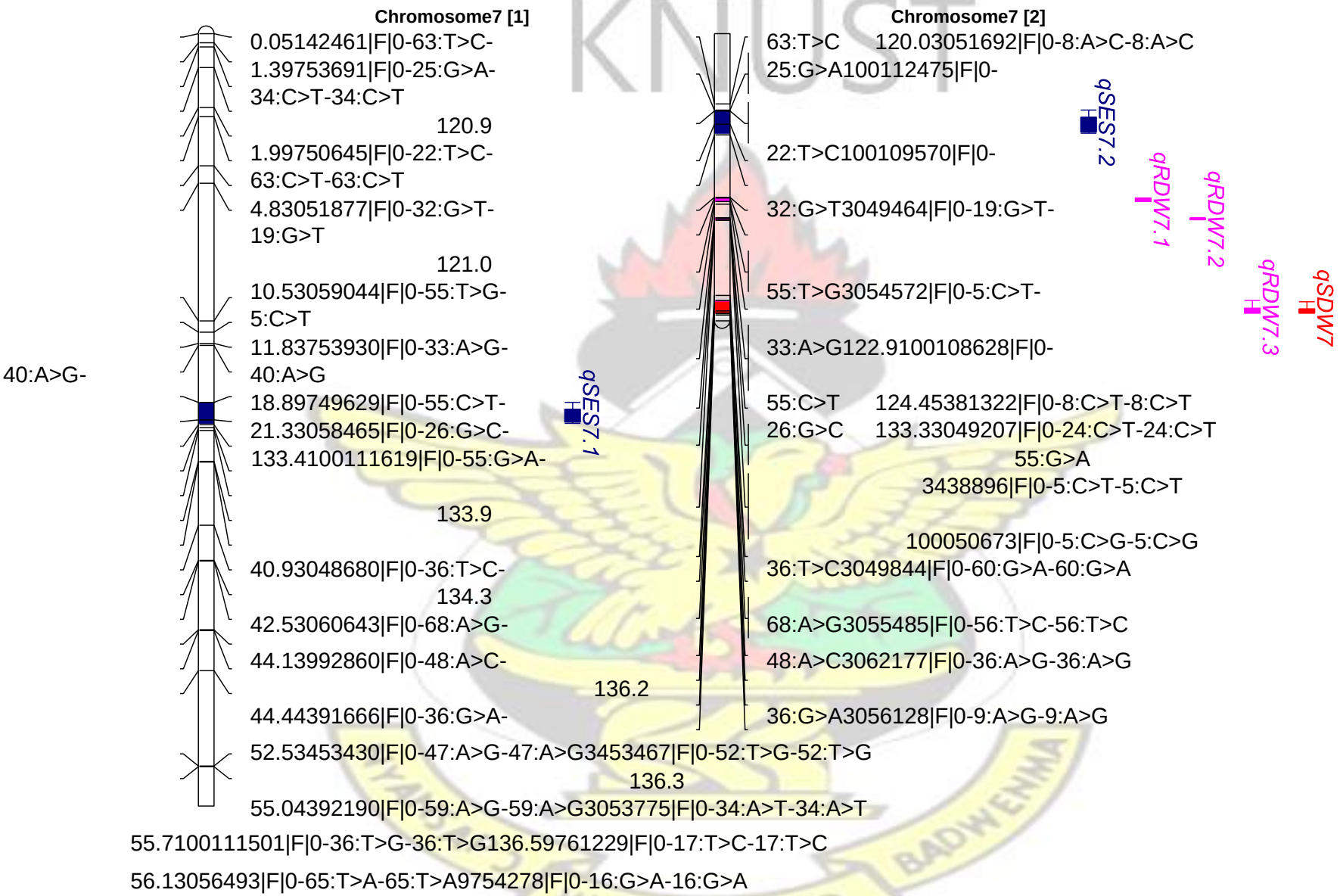
Chromosome4



0.03048376|F|0-56:T>G-56:T>G
1.94392873|F|0-26:G>T-26:G>T
3055181|F|0-27:A>G-27:A>G
3060670|F|0-10:G>C-10:G>C
13.0100183524|F|0-40:G>C-40:G>C
13.65141703|F|0-50:G>C-50:G>C
14.3100051719|F|0-20:G>A-20:G>A
14.5100108442|F|0-31:G>C-31:G>C

48.13057362|F|0-25:C>T-25:C>T
51.112388332|F|0-45:A>G-45:A>G
52.83440075|F|0-43:C>T-43:C>T
54.03751137|F|0-44:T>C-44:T>C
56.73762254|F|0-12:A>G-12:A>G
61.23453064|F|0-43:C>A-43:C>A
61.43057865|F|0-54:C>T-54:C>T
64.53052857|F|0-21:C>T-21:C>T
66.6100092824|F|0-26:C>T-26:C>T
66.83051953|F|0-33:C>T-33:C>T
67.63059212|F|0-39:G>A-39:G>A
84.13997024|F|0-60:A>C-60:A>C
84.45403297|F|0-67:G>T-67:G>T
85.93051666|F|0-51:A>T-51:A>T
86.13988776|F|0-32:G>T-32:G>T
86.39754388|F|0-12:G>T-12:G>T
86.83059815|F|0-54:G>A-54:G>A
108.03053527|F|0-31:T>C-31:T>C
100155954|F|0-49:A>G-49:A>G
108.8
3049962|F|0-64:C>A-64:C>A
109.3100174276|F|0-30:T>A-30:T>A
109.45145072|F|0-15:T>A-15:T>A
130.63049114|F|0-56:A>G-56:A>G

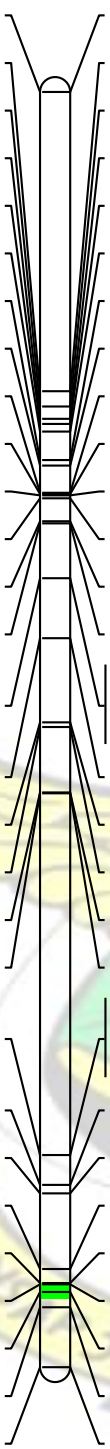
Figure 5.1 Cont"d.


Chromosome7 [1]
0.05142461|F|0-63:T>C-
1.39753691|F|0-25:G>A-
34:C>T-34:C>T
120.9
1.99750645|F|0-22:T>C-
63:C>T-63:C>T
4.83051877|F|0-32:G>T-
19:G>T
121.0
10.53059044|F|0-55:T>G-
5:C>T
11.83753930|F|0-33:A>G-
40:A>G
18.89749629|F|0-55:C>T-
21.33058465|F|0-26:G>C-
133.4100111619|F|0-55:G>A-
133.9
40.93048680|F|0-36:T>C-
134.3
42.53060643|F|0-68:A>G-
44.13992860|F|0-48:A>C-
136.2
44.44391666|F|0-36:G>A-
52.53453430|F|0-47:A>G-47:A>G3453467|F|0-52:T>G-52:T>G
136.3
55.04392190|F|0-59:A>G-59:A>G3053775|F|0-34:A>T-34:A>T
55.7100111501|F|0-36:T>G-36:T>G136.59761229|F|0-17:T>C-17:T>C
56.13056493|F|0-65:T>A-65:T>A9754278|F|0-16:G>A-16:G>A
40:A>G-
qSES7.1
Chromosome7 [2]
63:T>C 120.03051692|F|0-8:A>C-8:A>C
25:G>A100112475|F|0-
22:T>C100109570|F|0-
32:G>T3049464|F|0-19:G>T-
55:T>G3054572|F|0-5:C>T-
33:A>G122.9100108628|F|0-
55:C>T 124.45381322|F|0-8:C>T-8:C>T
26:G>C 133.33049207|F|0-24:C>T-24:C>T
55:G>A
3438896|F|0-5:C>T-5:C>T
100050673|F|0-5:C>G-5:C>G
36:T>C3049844|F|0-60:G>A-60:G>A
68:A>G3055485|F|0-56:T>C-56:T>C
48:A>C3062177|F|0-36:A>G-36:A>G
36:G>A3056128|F|0-9:A>G-9:A>G
qSES7.2
qRDW7.1
qRDW7.2
qRDW7.3
qSDW7

56.59750632|F|0-45:A>G-45:A>G3055948|F|0-28:A>G-28:A>G
136.6
60.94392811|F|0-26:C>T-26:C>T9747319|F|0-12:C>A-12:C>A
61.0100051704|F|0-13:A>G-13:A>G3442934|F|0-22:A>G-22:A>G
147.3
70.05408087|F|0-12:G>C-12:G>C3054699|F|0-35:T>C-35:T>C
148.0
75.04392508|F|0-20:C>A-20:C>A3447678|F|0-53:C>T-53:C>T
75.1100155684|F|0-19:A>G-19:A>G 149.53447678|F|0-53:C>T-53:C>T
84.93052274|F|0-62:G>T-62:G>T3999459|F|0-9:G>C-9:G>C
149.8
85.0100134743|F|0-5:T>C-5:T>C4392424|F|0-34:A>C-34:A>C
149.9
90.79743564|F|0-26:T>C-26:T>C3987754|F|0-57:T>C-57:T>C
150.1
150.93438968|F|0-41:G>A-41:G>A
104.36996340|F|0-56:G>A-56:G>A
104.43061015|F|0-48:T>C-48:T>C

Figure 5.1 Cont"d.

Chromosome8

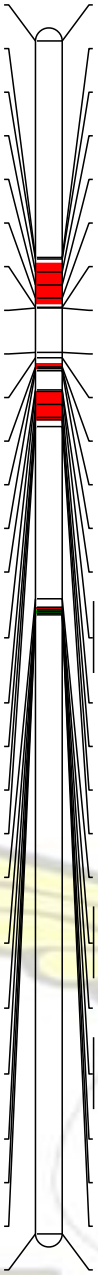


0.03054208|F|0-22:T>G-22:T>G
27.410272295|F|0-33:C>T-33:C>T
28.83048977|F|0-32:G>A-32:G>A
29.95401011|F|0-21:G>A-21:G>A
30.45148341|F|0-58:A>C-58:A>C
31.13054637|F|0-25:C>T-25:C>T
33.7100178002|F|0-16:C>T-16:C>T
34.23768847|F|0-26:T>C-26:T>C
36.73049299|F|0-23:T>A-23:T>A
36.8100110766|F|0-59:A>C-59:A>C
36.93058503|F|0-17:A>T-17:A>T
37.2100111124|F|0-42:C>T-42:C>T
39.33452930|F|0-30:G>A-30:G>A
39.53052802|F|0-13:A>T-13:A>T
3060023|F|0-39:T>C-39:T>C
44.59757810|F|0-6:T>A-6:T>A
50.05405965|F|0-35:A>C-35:A>C
57.73048932|F|0-58:G>T-58:G>T
58.1100155402|F|0-38:A>T-38:A>T

64.1100046390|F|0-57:G>A-57:G>A
64.210271338|F|0-54:G>A-54:G>A
3049745|F|0-37:A>C-37:A>C
97.3
3440840|F|0-23:A>T-23:A>T
100.03051170|F|0-13:T>C-13:T>C
100.83450883|F|0-41:T>C-41:T>C
107.79756282|F|0-67:G>A-67:G>A
109.03050151|F|0-19:T>A-19:T>A
109.15399638|F|0-17:C>A-17:C>A
109.83052871|F|0-63:T>C-63:T>C
111.23059651|F|0-22:T>G-22:T>G
116.714796097|F|0-19:A>C-19:A>C

Figure 5.1 Cont"d.

Chromosome9



0.03051687|F|0-17:G>A-17:G>A 16.7100147949|F|0-35:A>G-35:A>G

qSDW9.1 qSDW9.2 qSDW9.3 qSDW9.4 qSL9

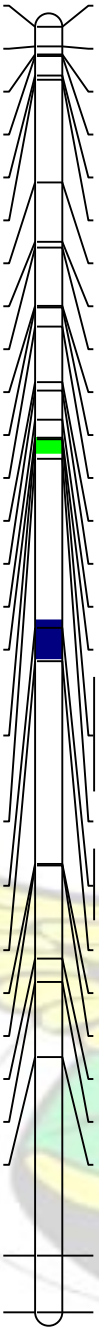
16.8. 17.8. 18.8.
19.83752887|F|0-10:T>C-10:T>C
20.53993879|F|0-18:G>A-18:G>A
20.65145005|F|0-59:T>A-59:T>A
24.0100135229|F|0-19:C>A-19:C>A
24.45145308|F|0-17:A>T-17:A>T
25.14392414|F|0-47:C>A-47:C>A
25.23453011|F|0-44:G>C-44:G>C
25.4100109582|F|0-10:T>G-10:T>G
26.93049446|F|0-19:A>G-19:A>G
9755603|F|0-40:C>T-40:C>T

27.0
.
28.0.
29.04386295|F|0-12:A>G-12:A>G
29.24387814|F|0-19:T>C-19:T>C
29.719320810|F|0-10:C>A-10:C>A
43.03994082|F|0-7:A>G-7:A>G
3447933|F|0-26:G>A-26:G>A
43.6
5392455|F|0-11:A>G-11:A>G
43.8100047863|F|0-14:G>A-14:G>A
3054747|F|0-26:T>C-26:T>C
43.9
3443453|F|0-24:C>G-24:C>G
44.06995776|F|0-28:G>T-28:G>T
44.13050341|F|0-8:A>G-8:A>G
44.24392609|F|0-26:G>A-26:G>A
91.93763275|F|0-65:T>A-65:T>A

Figure 5.1 Cont"d.

Chromosome 10

Chromosome10



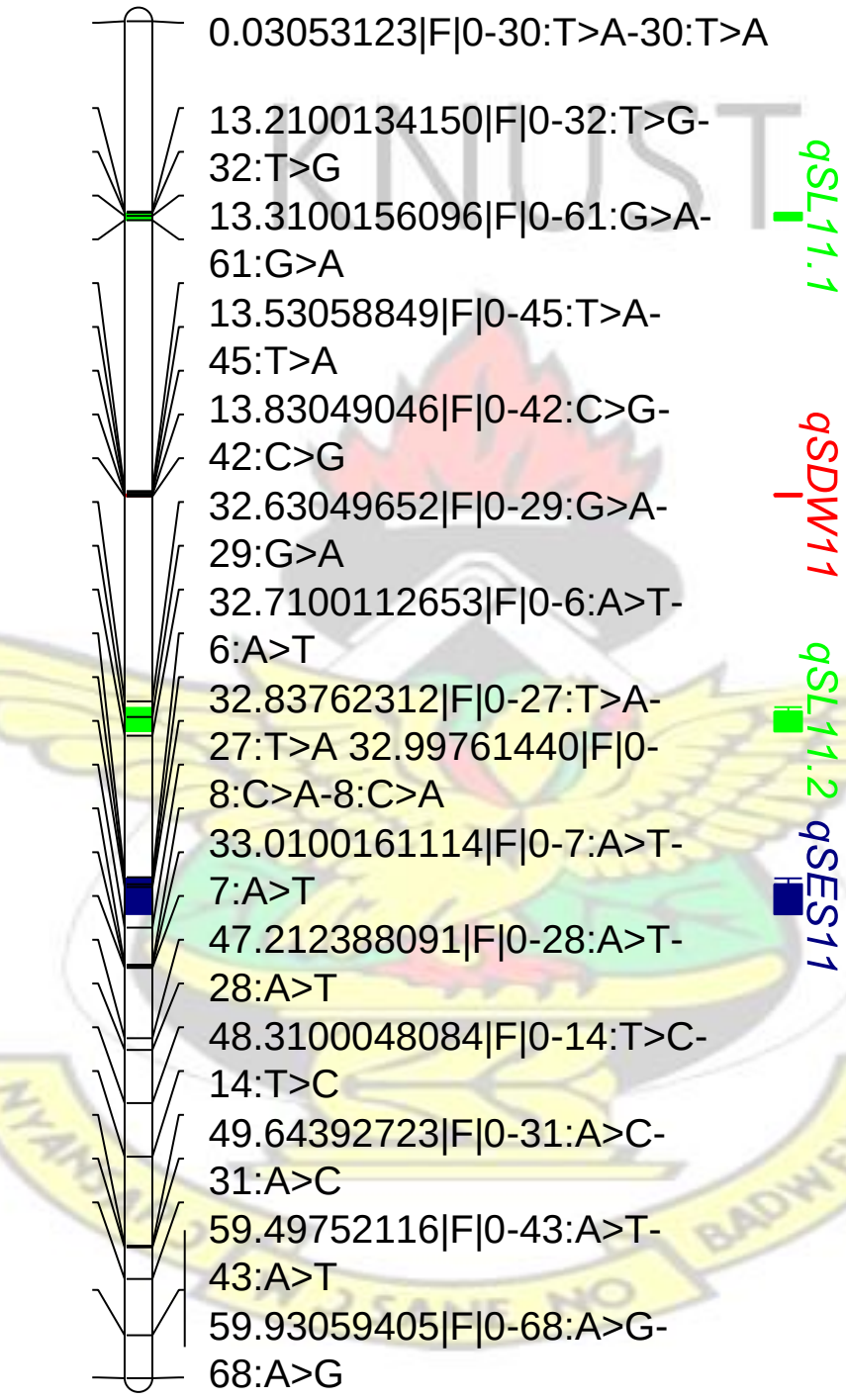
0.04392817|F|0-28:A>G-28:A>G
1.43055536|F|0-24:G>A-24:G>A
2.03050230|F|0-41:T>C-41:T>C
2.13059093|F|0-7:G>C-7:G>C
3.5100164784|F|0-43:T>G-43:T>G
3.83050853|F|0-60:C>T-60:C>T
11.26997084|F|0-45:G>A-45:G>A
15.5100052924|F|0-6:C>T-6:C>T
15.93057965|F|0-63:C>T-63:C>T
20.13771119|F|0-7:G>A-7:G>A
20.2100173227|F|0-45:C>T-45:C>T
21.63764233|F|0-45:T>G-45:T>G
25.63060966|F|0-32:T>C-32:T>C
26.25141850|F|0-11:T>C-11:T>C
28.35144748|F|0-59:A>C-59:A>C
29.63056610|F|0-32:T>G-32:T>G
3063126|F|0-10:C>T-10:C>T
29.73055206|F|0-17:A>C-17:A>C
5395323|F|0-54:T>C-54:T>C
31.15405148|F|0-63:T>C-63:T>C
3055041|F|0-27:C>A-27:C>A

43.3
100054868|F|0-18:C>T-18:C>T
45.710272514|F|0-16:T>A-16:T>A
60.33053177|F|0-26:T>A-26:T>A
60.4100093064|F|0-9:A>G-9:A>G
67.13055697|F|0-51:A>G-51:A>G
68.83053035|F|0-67:G>T-67:G>T
74.23453924|F|0-19:C>T-19:C>T

88.53053012|F|0-34:C>T-34:C>T
92.63054866|F|0-15:C>A-15:C>A

Figure 5.1 Cont"d.

Chromosome11

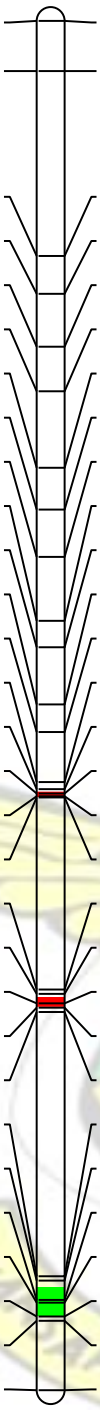


60.15142530|F|0-47:A>G-47:A>G
62.95140307|F|0-17:C>T-17:C>T
65.53445240|F|0-33:C>T-33:C>T
65.65388541|F|0-65:C>T-65:C>T

65.73056498|F|0-46:T>A-46:T>A
70.6100135684|F|0-9:T>C-9:T>C
71.45141849|F|0-17:T>G-17:T>G
75.13447259|F|0-34:G>A-34:G>A
78.83057114|F|0-44:A>G-44:A>G
85.03057228|F|0-7:G>A-7:G>A
85.13448685|F|0-26:T>G-26:T>G
87.35143724|F|0-10:T>A-10:T>A
3763421|F|0-62:C>T-62:C>T
91.24389262|F|0-50:G>T-50:G>T
100134371|F|0-5:A>C-5:A>C
94.23049608|F|0-16:G>A-16:G>A

Figure 5.1 Cont"d.

Chromosome12



0.04391847|F|0-46:G>C-46:G>C
3.63050919|F|0-22:C>T-22:C>T

16.93767523|F|0-68:G>A-68:G>A
19.6100164868|F|0-57:T>C-57:T>C
23.43452442|F|0-9:G>A-9:G>A
26.63054131|F|0-63:C>T-63:C>T
32.13050682|F|0-31:T>G-31:T>G
35.13060714|F|0-35:T>A-35:T>A
38.54393429|F|0-12:A>G-12:A>G
43.13053807|F|0-48:C>T-48:C>T
45.0100110011|F|0-45:C>T-45:C>T
49.14391520|F|0-64:T>A-64:T>A
51.15142146|F|0-24:G>T-24:G>T
54.73058734|F|0-28:T>C-28:T>C
55.23450598|F|0-16:T>A-16:T>A
55.5100048795|F|0-18:C>T-18:C>T
55.75403448|F|0-10:C>T-10:C>T
55.83452530|F|0-42:A>G-42:A>G
69.63444943|F|0-64:A>G-64:A>G
69.93058773|F|0-61:A>G-61:A>G

qSDW12.1
qSDW12.2
qSL12

70.6100135683|F|0-43:T>C-43:T>C
70.93050772|F|0-22:T>C-22:T>C
71.23053284|F|0-43:C>A-43:C>A
90.23056590|F|0-22:G>A-22:G>A
90.54393469|F|0-24:T>C-24:T>C
91.93452166|F|0-60:A>G-60:A>G
92.13052563|F|0-32:C>A-32:C>A

93.13062021|F|0-45:A>T-45:A>T
93.4100051999|F|0-39:G>T-39:G>T
98.43453132|F|0-23:C>T-23:C>T

Figure 5.1 Cont"d.

5.4 DISCUSSION

QTL mapping has been implemented in many breeding programs to discover genes underlying quantitative traits. However, many of these reported QTLs covered large chromosome intervals, thus, limiting the application of flanking markers in predicting the phenotype of the plant. A major constraint to previous QTL mapping studies is the number of available polymorphic markers. However, with reduction in DNA sequencing cost, high resolution QTL mapping is now possible using SNP markers. In this study, Genotype by Sequencing (GBS) approach was used to develop a high-density genetic linkage map of rice for identification of QTLs for traits related to salt stress tolerance, with a SNP at every 0.46 cM on the average.

A total of 46 QTLs were detected for all 4 studied traits. For each trait, more than one QTL was detected with varying contributions from both parents. Almost all SES QTLs (7 out of 8) were controlled by the donor parent Madina Koyo allele. The other 3 traits (shoot length, shoot dry weight and root dry weight) had contributions from the two parents, suggesting possible epistatic interactions across both parental genomes with all loci acting together to express the trait. This observation goes to buttress the point that salt tolerance in rice is a complex quantitative trait hence its mitigation requires the pyramiding of several different traits acting together. Similar

observation has been reported by several studies (Bizimana *et al*., 2017; Rahman *et al*., 2017; De Leon *et al*., 2017).

It was observed that, the same region on some chromosomes controlled more than one QTL in this study. For instance, 2 QTLs *qSL1.1* (shoot length) and *qSDW1.1* (shoot dry weight) were found in the 25.7-27,4 cM region on chromosome 1.

Similarly, on chromosome 2, five QTLs (*qSES2.1*, *qSDW2*, *qSL2.1*, *qSL2.2* and *qSL2.3*) for 3 traits (SES, shoot dry weight and shoot length) were mapped around the same region between 44.8 – 61.2 cM. On the same chromosome 2, three other QTLs (*qSES2.2*, *qSL2.4* and *qSL2.4*) colocalized between 104 – 115 cM. It is probable that, these regions where co-localization of QTLs were identified may either have a pleiotropic effect, or there might be the presence of several tightly linked genes acting together to express different effects. Similar pleiotropic or tightly linked gene effects has been reported by different studies on different chromosomes. In a study by Sabouri and Sabouri (2008), QTLs related to root length and Na^+ uptake colocalized around the same region on chromosome 1. Further, root dry weight and Na^+ uptake on chromosome 9, shoot dry weight and root length on chromosome 7, root length and Na^+ uptake on chromosome 1, root dry weight and root length on chromosome 5 all colocalized within the same regions on the respective chromosomes in their study. Bizimana *et al*. (2017) also reported similar instances where QTL affecting root length (*qRL12.1*) was closely linked to the SES QTL (*qSESF12.1*) and shoot dry weight QTL *qSDW12.1* in the same region on chromosome 12. Improving salt tolerance in rice cultivars through the introgression of this locus is likely to yield much progress because, this region seems to control three important traits (shoot length, shoot dry weight and ion leakage).

The SES reflects the overall plant‟s response to salt stress. It gives the clearest and first-hand information about the degree of tolerance for a particular genotype. Previously, a major QTL, *qSKC1*, which controls tolerance to salt stress at the seedling stage through shoot K^+ concentration homeostasis was mapped to chromosome one (Lin *et al*., 2004) and has direct control on the degree of expression of salt injury (SES). Fine mapping of *qSKC1* led to the cloning of *HKT1;5* gene located at 11.46 Mb region. In a separate RIL mapping population, *Saltol* QTL for low Na:K ratio was identified flanking the region of *qSKC1* (Gregorio, 1997; Bonilla *et al*., 2002). Further study on *Saltol* QTL assumed that the same *HKT1;5* gene was responsible for salt stress tolerance (Thomson *et al*., 2010). In this study however, none of the eight QTLs detected for SES colocalized in the *Saltol* region on chromosome one. Neither was any of the QTLs identified for the other traits mapped in this region. This suggests that tolerance genes from new donor Madina Koyo are controlled by different QTLs acting from a different locus (or loci). Similarly, De Leon *et al*. (2017) did not detect any significant QTL near or around *Saltol* or *qSKC1* region in spite of dense SSR and SNP marker availability at the locus. Using a different salt stress donor called Hasawi, both Bimpong *et al*. (2014) and Bizimana *et al*. (2017) reported similar results where no SES QTLs was detected on chromosome 1. Thomson *et al*. (2010), also identified 2 QTLs relates to SES but were mapped to chromosomes 4 and 9.

Of the eight QTLs detected for SES, only one *qSES1* was mapped to chromosome 1 and was about 23 Mb away from the *Saltol* region. This *qSES1* had a decreasing effect from the donor parent Madina Koyo and could lower SES score by 3 folds. The remaining seven were mapped to chromosomes 2, 7, 10 and 11; all with a decreasing

effect from the Madina Koyo except for *qSES-11* which showed an increasing effect to SES from the recipient parent Sahel 317. This suggests that, when *qSES11* is being considered in breeding for low SES, the corresponding Madina Koyo allele at *qSES11* should be targeted. De Leon *et al*. (2016) observed a similar increasing effect to salt injury score (SIS) from the recipient parent Bengal at *qSIS5.1b* on chromosome 5 which increased SIS by 13%. Later, the authors mapped a salt injury score QTL *qSIS1.39* at 39.5 Mb on chromosome 1 (De Leon *et al*., 2017). The *qSES1* mapped in the current study validates *qSIS1.39* even though different donor sources were used in developing the mapping populations in the two studies. Comparison of the other SES QTLs mapped on chromosomes 2, 7, 10 and 11 with several other QTL studies suggests the novelty of these QTLs. These could be targeted in QTL pyramiding to improve tolerance of farmer preferred cultivars.

The majority of QTLs identified in this study were mapped to shoot length on every chromosome except chromosome 7. A total of 19 QTLs were identified for shoot length. Several studies have identified QTLs associated with shoot length under salt stress on chromosomes 1, 2, 3, 4, 6, 7, 10 and 12 (Singh *et al*. 2007; Sabouri and Sabouri 2008; Thomson *et al*. 2010; De Leon *et al*. 2016; Bizimana *et al*., 2017). These findings suggested that there are several genes controlling shoot length under salt stress. Though there are disparity in the regions of these QTLs and those identified in the current study, the numbers and distribution among almost all chromosomes go to confirm the complexity and quantitative nature of salt tolerance mechanism (Singh *et al*. 2007, 2010; Baby *et al*. 2010; De Leon *et al*. 2016; Bizimana *et al*., 2017). It is evident that, several loci on different chromosomes contribute in an additive manner to maintain vigor in growth hence longer shoot under salt stress.

Vigorous growth has been suggested to have a dilution effect on accumulated salt concentrations in plant cells due to associated rapid cell division (Yeo *et al.*, 1990). This mechanism has long been linked with adaptation of landraces such as „Pokkali" and „Nona Bokra" in saline environments (Yeo *et al*., 1990; Richards, 1992; Bohra and Doerffling, 1993). In the current study, four QTLs with major effects were identified for shoot length on chromosomes 1 and 2. A major effect QTL (*qSL1.1*) that increased shoot length, hence seedling vigour under stress by almost 100% was identified on chromosome 1 at 26.4 cM. The increase in this QTL was influenced by alleles from the donor parent Madina Koyo. In a related study, *qSHL1.38* explaining more than 50% of the variation in shoot length was mapped at 168 cM on chromosome 1 by De Leon *et al*. (2016). Two of the other 3 major effect QTLs for shoot length identified in this study, *qSL2.2* and *qSL2.3*, all had the increasing effect from Madina koyo whiles *qSL2.4* had its increasing effect from Sahel 317. Given the high effect of the novel *qSL1.1* locus and the other two loci which had increasing growth effect from Madina Koyo, great progress could be made in breeding salt tolerant lines by pyramiding alleles at the Madina Koyo at these loci for these QTLs and the Sahel 317 allele at *qSL2.4* in an attempt to exploit the dilution effect associated with vigorous growth.

Koyama *et al*. (2001) and De Leon *et al*. (2016) detected QTLs for dry mass on chromosome 6 but at different loci. A combined total of 24 shoot and root dry weight QTLs were detected in this study but none on chromosome 6. All root dry weight QTLs were of minor effects and had no additive effect making them unsuitable in improving genetic gain in breeding. On the contrary, almost all shoot dry weight (except 1) had additive effects with two having major effects. The *qSDW1.1* on

chromosome 1, with allele contribution from the recipient parent Sahel 317 at 25.7 cM, could improve the weight of shoots by 30 folds. Another shoot related QTL *qSL1.1* for shoot length was identified around the same region (26.4 cM) on chromosome 1. The second major effect QTL for shoot dry weight QTL *qSDW2* increased the trait by more than 11% with allele from Madina Koyo. This QTL was mapped to 55.6 cM, only 1 cM away from a shoot length QTL *qSL2.3* (54.6 cM) on chromosome 2, demonstrating a clear consistency between QTLs of these two traits. Shoot length has been reported to correlate positively with shoot dry weight (2012; Souleymane *et al*., 2016 and Bizimana *et al*., 2017). The close mapping of these two QTLs is in agreement with the observtion made by Veldboom *et al*. (1994) and Xiao *et al*. (1996) that, correlated traits have QTLs mapped often to the same chromosomal location. Since longer shoots will result in higher dry weights and both are related to biomass, it is plausible that either there exist a single QTL at these regions on chromosomes 1 and 2 that control shoot biomass in rice under salt stress or there are two tightly linked genes, acting together to dictate shoot biomass. Fine mapping within this region will bring a resolve to whether there is a pleiotropic effect or there actually exist two genes that are tightly linked.

Thirty-three (33) out of the 46 identified QTLs were linked to shoot related traits (either shoot length or shoot dry weight) whiles only 5 were related to root related trait (root dry weight; no QTL was identified for root length). These findings corroborate with other reports that, shoot growth is severely affected under salt stress than roots (Läuchli and Grattan 2007; Ahmed *et al*., 2012; Pradheeban *et al*., 2015; Sakina *et al*., 2016; and Bizimana *et al*., 2017). Under salt stress, the rice plant has been observed to reduce its leaf surface area as opposed to their roots (Munns and Tester 2008). The authors likened this phenomenon to an adaptation mechanism where plant cells

mitigate initial osmotic stress by maintaining as much water as possible. They recounted that, the osmotic imbalance caused by salt stress affects the rate at which growing leaves expand while new leaves and lateral buds emerge more slowly or in the extreme, remain quiescent. In older leaves, where no expansion occurs, dilution of accumulated salts ceases. This leads to a considerable build-up of salts in leaves and eventually results in their death. When this is not compensated for by development of new leaves, the photosynthetic capacity of plants is critically reduced, causing further reduction in shoot growth relative to roots.

CHAPTER SIX

6.1 General Discussion

Soil salt stress is a widespread problem that limits the productivity of most crops. In rice, the effect of salt stress is most profound at the early seedling and at the reproductive stages, where depending on the severity, total crop failure occurs (Munns *et al*., 2006; Asch *et al*., 2000). To ameliorate this and many other constraints to rice cropping, plant breeding has been proposed as the best option (Ashraf and Wu, 1994; Silvey, 1981; Ashraf *et al*., 1986; Watt, 1983; Ray and Islam 2008; Osei *et al*., 2014). However, the quest to breed for cultivars with better adaptability to salt stress is faced with several challenges, including the fact that tolerance to the stress at these two growth stages is not correlated. This means control mechanism at the seedling stage is completely different from that which governs reproductive stage tolerance. Hence breeding efforts should aim at pyramiding the two mechanisms into one to ensure tolerance throughout the crop‟s cycle. Previous efforts have made use of almost same pool of donor QTLs/genes particularly from „Pokkali‟, „Nona Bokra‟ and their derivatives. This has rather narrowed the genepool where similar sets of QTLs are deployed. In this study, Madina Koyo, a newly identified salt stress donor was hybridized with Sahel 317, a popular variety in Senegal and the resulting breeding lines evaluated under field conditions for tolerance to salt stress at the reproductive stage and in hydroponic culture for tolerance at the seedling stage where regions controlling tolerance were mapped.

Salt stress caused a drastic decrease in growth and productivity at the seedling and reproductive stages in this study. Average reduction of 63.4% in fitness related traits (shoot and root lengths and dry weights) at the seedling level was observed in this study. Similar reduction in fitness related traits such as leaf rolling, yellowing and

drying, reduction in shoot and root growth, thin and weak shoots leading to general stunted growth and eventual death of seedlings have been previously documented (Krishnamurthy *et al*., 2016; Al-Amin *et al*., 2013; Islam, 2004; Niones, 2004; Bonilla *et al*., 2002). Average reduction in grain yield was observed to be as high as 72% among the test entries under reproductive stage evaluation, a result that validates the findings of Zeng and Shannon, (2000), Nakhoda *et al*. (2012), Bimpong *et al*. (2014) and Bimpong *et al*. (2016). Khatun and Flowers (1995) and Abdullah *et al*. (2001) in their studies, attributed the drastic salt stress-induced reduction in grain yield to a decrease in either pollen viability or receptivity of the stigmatic surface, or both. Another cause of this decrease in yield could be due to poor photosynthetic rates resulting from several salt stress-induced factors such as: reduced leaf area, chlorophyll due to leaf burning, stomatal closure and conductance, among others (Flowers and Yeo, 1981; Dionisio-Sese and Tobita, 2000).

Under hydroponic and field conditions, some progenies performed better than the two parents for all traits suggesting that segregation was transgressive. Transgressive segregation has been suggested to be common among morphological traits in plants and its occurrence has been attributed to the complementary gene action of additive alleles that are dispersed between the parental lines (Rieseberg *et al*. 1999). In rice, transgressive segregation under saline conditions has been reported in different populations such as F_2 (Sabouri *et al*., 2009; Rana *et al*., 2009; Mohammadi *et al*., 2014), F_3 (Kaushik *et al*., 2003), F_6 (Bizimana *et al*., 2017) BC_3F_2 (Bimpong *et al*., 2016) and RIL (Thomson *et al*., 2010; Wang *et al*., 2012; Tiwari *et al*., 2016; Rhaman *et al*., 2017) populations.

A total of 46 $F_{3:4}$ progenies were identified to have better adaptation to salt stress at the early seedling stage (based on SES score) whiles 45 $F_{2:4}$ were adjudged to be

tolerant at the reproductive stage (based on grain yield). Out of the combined 91, only one progeny, ARS1181-1-6-27, was observed to be tolerant to salt stress at both growth stages. Another 4 lines (ARS1181-1-7-6, ARS1181-1-6-6, ARS1181-1-8-26 and ARS1181-1-10-1) combined tolerance at the seedling stage with better yield stability under stress and control conditions. Similar findings have been reported by Bimpong *et al*. (2016) when they identified 16 *Saltol* introgression lines with minimal yield losses. The 5 tolerant progenies identified in this study could be evaluated further with the objective of varietal release *per se* or be used as parents in forward breeding efforts.

Heritability act as predictive instrument in expressing the reliability of phenotypic value (Kumar *et al*., 2016) and also serves as an index of trait transmission from parents to progenies by predicting the amount of the phenotypic variance that is due to genotypic or genetic effects. In this study, heritability estimates were generally high for traits measured during the reproductive stage screening as well as the early seedling stage evaluation. However, heritability was higher under control compared to stress conditions. Earlier, Mohammadi *et al*. (2014) had reported a similar decrease in heritability under stress compared to control conditions. Contrary, Salam *et al*. (2011) observed higher heritability estimates under stress conditions compared to control. The high heritability observed for shoot and root lengths as well as their dry weights in this study, suggests the likelihood to improve genetic gain in breeding for salt stress tolerance in rice when selection is based on these traits.

QTL mapping has been implemented in many breeding programs to discover genes underlying quantitative traits. However, many of these reported QTLs covered large chromosome intervals, thus, limiting the application of flanking markers in predicting

the phenotype of the plant. In this study, Genotype by Sequencing approach was used to develop a high-density genetic linkage map of rice for identification of QTLs for traits related to salt stress tolerance, with a SNP at every 0.46 cM on the average. A total of 46 QTLs were detected in this study for all traits studied except for root length. All 4 traits for which QTLs were detected were governed by more than one QTL which were generally contributed by both parents with the exception of SES QTLs which were largely governed by the donor parent Madina Koyo allele (7 out of 8 QTLs). Each trait for which QTLs were identified, mapping occurred on different chromosomes. This suggests the presence of be epistatic interactions among trait-specific QTLs with all loci acting together to express the trait. Similar observation has been reported by (Bizimana *et al*., 2017; Rahman *et al*., 2017; De Leon *et al*., 2017). In some instances, the same region on a particular chromosome controlled more than one QTL e.g. around 25.7-27,4 cM region on chromosome 1 *qSL1.1* (shoot length) and *qSDW1.1* (shoot dry weight). It is probable that, this region on chromosome 1 may either have a pleiotropic effect or there might be the presence of several tightly linked genes acting together to express different effects. Improving salt tolerance in rice cultivars through the introgression of this locus is likely to yield much progress because, this region seems to control three important traits. Similar pleiotropic or tightly linked gene effects had earlier been reported though on different chromosomes (Sabouri and Sabouri, 2008; Bizimana *et al*. (2017). These distribution of trait-specific QTLs on different chromosomes and the colocalization of QTLs goes to buttress the point that salt tolerance in rice is a complex quantitative trait hence its mitigation requires the pyramiding of several different traits acting together.

Generally, traits measured under reproductive stage evaluation were poorly correlated except for number of effective tillers and grain yield. A positive correlation between

these two traits confirms that, number of effective tillers is a component of grain yield and that, an increase in number of tillers will cause an increase in grain yield of that genotype. Hence, an indirect selection of yield could be made by selecting directly for number of effective tillers in salt stress breeding programs. Grain yield and tiller number were also found to be positively correlated under salt stress by Tiwari *et al*. (2016) and Zeng *et al*. (2002). Conversely, Mishra *et al*. (2014) observed a negative association between grain yield and tiller number. Contrary to the weak correlation observed during the reproductive stage evaluation, correlation among traits were relatively stronger during seedling stage evaluation. A very high and positive association was observed between SES and ion leakage. De

Leon *et al*. (2015) recounted similar positive and significant association between SES and ion leakage when they evaluated some American rice germplasm for tolerance to salt stress. The implication is that, these two traits could be used interchangeably. Considering that SES has been argued to be subjective (De Leon *et al*., 2015), ion leakage may be used to enforce scoring-based selection. SES correlated negatively with root length, root dry weight and shoot dry weight in this study. Similar significant and negative correlation between SES and root length, root dry weight and shoot dry weight had earlier been reported by Barua *et al*. (2015) and De Leon *et al*. (2016). The negative association implies that progenies with longest and much dense root system and heaviest shoot weights had the least score, thus, were the most tolerant. Salt stress is known to limit the amount of water absorption through the roots, leading to accumulation of concentrations of salt which eventually inhibit cell growth (Bimpong *et al* 2016; Munn and Tester 2008). Therefore, only tolerant genotypes (with lower SES) will be able to maintain healthy cell growth to produce enough biomass as observed in this study. The implication for breeding is that, selecting for progenies

with least SES score will mean indirectly selecting for progenies with longer and dense root systems capable of exploring deep and wide into the soil for water (during initial osmotic stress caused by salt stress) and nutrients (in the face of deficiency or toxicity on the near surface).

Altogether, 46 QTLs were identified in this study for all traits except root length. Of these, 6 (*qSDW1.1, qSDW2, qSL1.1, qSL2.2, qSL2.3* and *qSL2.4*) were major effect QTLs with phenotypic contributions ranging from 11% - 99%. The *qSDW1.1* on chromosome 1, with allele contribution from the recipient parent Sahel 317 at 25.7 cM, could improve the weight of shoots by 30 folds. A major effect QTL (*qSL1.1*) that increased shoot length, hence seedling vigour under stress by almost 99% was identified on chromosome 1 at 26.4 cM. The increase in this QTL was influenced by alleles from the donor parent Madina Koyo. In a related study, *qSHL1.38* explaining more than 50% of the variation in shoot length was mapped at 168 cM on chromosome 1 by De Leon *et al*. (2016). Comparison with other studies suggests that most of these QTLs are new. These QTLs present a new opportunity to improve adaptation to salt stress and bring diversity to the genepool of QTLs available for deployment which could be useful in salt stress breeding programs.

None of the 8 SES QTLs had a major effect. Only one was mapped to chromosome 1 but was about 23 Mb away from previously identified major effect QTL *Saltol* and was not close. Neither was any of the QTLs identified for the other traits mapped in this region. This suggests that tolerance genes from new donor Madina Koyo is controlled by a different QTL acting from a different locus (or loci). Similarly, De Leon *et al*. (2017) did not detect any significant QTL near or around *Saltol* or *qSKC1*

region in spite of SSR and SNP markers availability at the locus. Using a different salt stress donor called Hasawi, both Bimpong *et al*. (2014) and Bizimana *et al*. (2017) reported similar results where no SES QTLs was detected on chromosome 1. In the study of Thomson *et al*. (2010), neither of the two identified SES QTLs were mapped to chromosome 1 but to chromosomes 4 and 9.

A combined total of 33 QTLs identified in this study were shoot related traits (either shoot length or shoot dry weight) whiles only 5 were related to root related trait (root dry weight; no QTL was identified for root length). These findings validate other reports that the shoot growth is severely affected with the salt stress than roots (Läuchli and Grattan 2007; Ahmed *et al*., 2012; Pradheeban *et al*., 2015; Sakina *et al*., 2016; and Bizimana *et al*., 2017). Under salt stress, the rice plant has been observed to reduce its leaf surface area as opposed to their roots (Munns and Tester 2008). They opined that, this phenomenon is an adaptation mechanism towards the mitigation of initial osmotic stress by maintaining as much water as possible in cells. They recounted that, the osmotic imbalance caused by salt stress affects the rate at which growing leaves expand while new leaves and lateral buds emerge more slowly or in the extreme, remain quiescent. In older leaves, where no expansion occurs, dilution of accumulated salts ceases. This leads to a considerable build-up of salts in leaves and eventually results in their death. When this is not compensated for by development of new leaves, the photosynthetic capacity of plants is critically reduced, causing further reduction in shoot growth relative to roots.

CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

Salt stress greatly reduced the productivity of the rice progenies evaluated at the early seedling and reproductive stages in this study. The combined single tiller approach and augmented design used in this study was effective in evaluating the high numbers while ensuring that the same genotype (seed) was effectively evaluated under control and stress though they are segregating. Heritability for all studied traits were high suggesting that, much progress will be made in breeding for tolerance to salt stress through phenotypic selection. Grain yield positively associated with tiller number, confirming that, increased tiller number will increase grain yield of a progeny. Thus, selecting for higher tiller number will translate into direct selection for grain yield under salt stress conditions. A total of 45 $F_{2:3}$ progenies (representing 5% of the total progenies studied) were identified to have better or comparable grain yield to the donor parent Madina Koyo. Some of these progenies had yield stability as high as 99.6%.

Though salt stress reduced progeny performance for fitness related traits, reduction was not uniform across the studied genotypes. A total of 46 progenies representing 15% of the population had better or comparable SES with regards to the tolerant check FL478. These progenies, if selected as parents, will have twice as much better SES less than the entire population suggesting the potential to improve tolerance to salt stress through phenotypic selection. There exist a strong positive association between SES and ion leakage which suggests these traits could be used interchangeably. Correlation between SES and shoot dry weight, root dry weight and root length was negative, confirming that progenies with least SES (tolerant progenies) had the denser and deeper root systems as well as high biomass.

Altogether, 1 progeny, ARS1181-1-6-27, was observed to be tolerant to salt stress at both growth stages while 4 other lines (ARS1181-1-7-6, ARS1181-1-6-6, ARS11811-8-26 and ARS1181-1-10-1) combined tolerance at the seedling stage with better yield stability. These four lines could be evaluated further and compared with local cultivars for yield and grain quality properties with the objective of releasing them as new varieties. Another approach would be to use these four lines as parents in forward breeding for biotic and abiotic stress tolerance in rice.

Composite interval mapping identified 46 QTLs for four of the studied traits namely SES, shoot length, shoot dry weight and root dry weight. Six QTLs (*qSDW1.1*, *qSDW2*, *qSL1.1*, *qSL2.2*, *qSL2.3* and *qSL2.4*) were of major effect explaining between 11% and 99% of the variation in shoot length and shoot dry weight respectively. These major effect QTLs could greatly impart breeding for tolerance to salt stress. Though new QTLs were identified for SES, all had minor effects. Madina Koyo seems to dictate tolerance to salt stress at the early seedling from a locus different from the *Saltol* region because none of the QTLs (especially for SES) was mapped to the *Saltol* locus on chromosome 1. Some loci on chromosomes 1 and 2 behaved as though they had pleiotropic effect for SES, shoot length and shoot dry weight. It could also be that the genes within these regions were tightly linked and acted together to control their respective traits. Altogether, 33 QTLs were detected for shoot related traits while only 5 were mapped for root related traits. This validates earlier reports that shoot related traits are more important in salt stress studies.

7.2 Recommendations

i. During early generation progeny testing, single tiller approach should be used to evaluate segregating lines under stress conditions whiles raising enough seeds for next generation testing.

ii. The 45 $F_{2:3}$ progenies identified to have better yield stability should be further evaluated under replicated trial to confirm their performance under stress conditions at the reproductive stage.

iii. Results for the 46 salt tolerant $F_{3:4}$ progenies at the early seedling stage should be validated by screening them again for tolerance at the seedling stage.

iv. The 5 progenies, ARS1181-1-6-27, ARS1181-1-7-6, ARS1181-1-6-6, ARS1181-1-8-26 and ARS1181-1-10-1 that combined tolerance at the early seedling and the reproductive stage should be fixed and evaluated further in multi-environmental trials with the intention of varietal release or could be used as parents in forward breeding programs to quickly improve genetic gain of future breeding efforts.

v. Fine mapping for newly identified major effect QTLs *qSDW1.1* and *qSL1.1* should be conducted and annotation and expression of their genes done to understand the mechanism(s) involved in their effect. This will permit deployment of these important QTLs in breeding programs aimed at improving tolerance to salt stress in rice.

vi. The locus on chromosome 1 habouring QTLs for shoot length & shoot dry weight and that on chromosome 2 where QTLs for SES, shoot length and shoot dry weight colocalized should be fine mapped to ascertain these loci are in pleiotropy or there are indeed different QTLs.

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Appendix

Appendix 1. Top 5% progenies for Grain Yield under control conditions

Designation	Block	Grain Yield (g)	Days to Flowering (Days)	No. of Tillers	Plant Height (cm)
ARS1181-1-8-118	10	160.51	77	29	167.20
ARS1181-1-8-181	2	106.48	84	20	145.85
ARS1181-1-1-6	1	105.07	77	22	157.95
ARS1181-1-14-14	8	104.09	79	21	102.82
ARS1181-1-5-46	7	103.25	85	30	171.82
ARS1181-1-8-39	4	101.17	66	25	161.32
ARS1181-1-8-120	7	100.06	78	31	154.82
ARS1181-1-3-76	1	99.55	74	22	170.95
ARS1181-1-3-12	1	97.19	67	19	95.95
ARS1181-1-7-133	10	92.84	84	24	155.20
ARS1181-1-10-19	8	92.05	78	14	147.82
ARS1181-1-4-47	12	90.7	82	26	112.45
ARS1181-1-4-23	13	90.08	82	22	110.14
ARS1181-1-6-78	7	87.92	82	23	189.82
ARS1181-1-8-69	3	86.47	78	18	166.57
ARS1181-1-13-55	3	85.67	85	26	82.57
ARS1181-1-14-3	13	85.44	74	25	99.14
ARS1181-1-3-70	4	85.36	77	17	183.32
ARS1181-1-4-1	12	84.49	81	29	103.45
ARS1181-1-5-44	11	83.02	81	19	175.70
ARS1181-1-3-10	2	82.82	74	15	80.85
ARS1181-1-8-204	13	82.02	77	11	130.14
ARS1181-1-4-42	6	81.66	82	25	108.82
ARS1181-1-4-8	8	81.39	81	38	94.82
ARS1181-1-8-165	11	80.85	80	27	149.70
ARS1181-1-9-66	13	78.88	81	29	141.14
ARS1181-1-13-11	5	78.79	81	23	89.07
ARS1181-1-13-15	9	77.72	74	18	169.57
ARS1181-1-3-65	7	77.46	75	14	169.82
ARS1181-1-9-40	2	77.06	77	32	122.85
ARS1181-1-3-35	13	76.82	66	19	111.14
ARS1181-1-1-8	12	76.07	77	30	159.45
ARS1181-1-7-20	7	75.75	75	14	159.82
ARS1181-1-4-6	2	75.57	84	30	104.85
ARS1181-1-7-41	1	74.02	74	23	175.95
ARS1181-1-7-135	4	73.98	84	30	157.32
ARS1181-1-5-1	1	73.96	77	21	152.95
ARS1181-1-8-27	6	73.07	66	23	171.82
ARS1181-1-6-15	13	71.74	81	18	191.14

ARS1181-1-4-56	9	71.43	82	20	105.57
ARS1181-1-1-33	2	71.35	84	15	154.85
ARS1181-1-13-62	8	71.34	84	23	93.82
ARS1181-1-7-26	1	71.22	74	13	155.95
ARS1181-1-5-38	5	71.15	81	27	191.07
Mean of top 5%		85.63	78.23	22.73	140.85
Mean of all progenies		29.86	77	23.84	
Checks					
Sahel317		54.21	77	28	111.93
Madina Koyo		41.09	72	21	122.21
Sahel 108		61.04	72	20	84.21
Sahel 134		40.51	68	17	93.93
SMK3		59.45	65	25	152.57
SMK87		39.23	65	23	130.77
FL478		25.07	75	15	95.57
NERICA-L9		34.69	87	18	101.64
LSD					
Two Control Treatments		2.56	0.59	1.16	1.83
Two Augmented Treatments (Same Block)		9.57	2.21	4.35	6.85
Two Augmented Treatments (Different Blocks)		10.15	2.34	4.62	7.27
An Augmented Treatment and A Control Treatment		7.43	1.71	3.38	5.32

Appendix 2. Top 5% progenies for Grain Yield under stress conditions

Designation	**Block**	**Grain Yield (g)**	**No. of Days to Flowering**	**No. Tillers**	**Plant Height (cm)**

			(Days)		
ARS1181-1-13-23	2	30.15	77	14	47.22
ARS1181-1-14-36	2	30.15	82	12	48.22
ARS1181-1-4-59	8	29.18	81	19	87.97
ARS1181-1-14-24	6	28.55	82	17	64.47
ARS1181-1-4-7	13	28.03	81	23	79.10
ARS1181-1-3-8	10	27.98	59	11	88.10
ARS1181-1-7-4	13	27.83	100	16	67.10
ARS1181-1-4-10	10	27.73	77	17	64.22
ARS1181-1-7-263	2	27.15	67	16	91.22
ARS1181-1-3-5	3	26.85	58	60	93.47
ARS1181-1-1-5	8	26.48	75	70	88.97
ARS1181-1-4-12	7	26.23	77	18	62.72
ARS1181-1-1-15	4	26	81	16	63.35
ARS1181-1-3-30	2	25.95	59	15	68.22
ARS1181-1-8-3	5	25.75	67	70	87.47
ARS1181-1-6-105	5	25.55	88	11	92.47
ARS1181-1-6-27	9	25.53	88	21	72.47
ARS1181-1-3-22	5	25.45	67	17	72.47
ARS1181-1-15-8	5	25.25	77	13	53.47
ARS1181-1-8-33	9	25.23	71	60	88.47
ARS1181-1-9-11	8	25.18	74	11	95.97
ARS1181-1-5-38	5	24.85	88	24	118.47
ARS1181-1-7-238	7	24.53	59	60	40.72
ARS1181-1-6-31	12	24.48	88	22	96.85
ARS1181-1-8-28	4	24.4	59	13	83.35
ARS1181-1-6-11	4	24.3	88	17	105.35
ARS1181-1-4-64	8	24.28	73	21	86.97
ARS1181-1-6-16	13	24.23	88	14	98.10
ARS1181-1-4-3	1	23.73	81	18	70.10
ARS1181-1-3-75	3	23.53	78	16	102.35
ARS1181-1-4-15	11	23.23	84	19	62.60
ARS1181-1-4-13	5	23.05	79	18	66.47
ARS1181-1-6-79	6	22.95	88	18	86.47
ARS1181-1-8-2	14	22.78	69	22	106.1
ARS1181-1-8-18	10	22.73	67	80	97.22
ARS1181-1-4-65	2	22.45	84	19	85.22
ARS1181-1-8-68	2	22.25	65	13	89.22
ARS1181-1-6-15	13	22.13	88	16	50.10
ARS1181-1-6-45	10	21.93	88	40	96.22
ARS1181-1-9-3	5	21.75	74	10	98.47
ARS1181-1-2-74	6	21.65	70	13	66.47
ARS1181-1-8-181	2	21.55	77	12	94.22
ARS1181-1-6-62	11	21.53	88	20	74.60
ARS1181-1-3-15	9	21.28	53	10	68.85

Mean	24.9	76	14.7	80.04
Mean of all progenies	8.33	78.1	22.56	
Checks				
Sahel317	5.00	75	17	101.0
Madina Koyo	21.57	70	15	96.36
Sahel 108	4.50	70	18	85.79
Sahel 134	5.07	67	14	81.00
SMK3	1.57	63	13	134.43
SMK87	2.07	63	18	114.07
FL478	13.50	74	12	84.86
NERICA-L9	22.14	85	17	86.29
LSD				
Two Control Treatments	2.56	0.59	1.16	1.83
Two Augmented Treatments (Same Block)	9.57	2.21	4.35	6.85
Two Augmented Treatments (Different Blocks)	10.15	2.34	4.62	7.27
An Augmented Treatment and A Control Treatment	7.43	1.71	3.38	5.32

Appendix 3. Stability of top 5% progenies for grain yield per hill

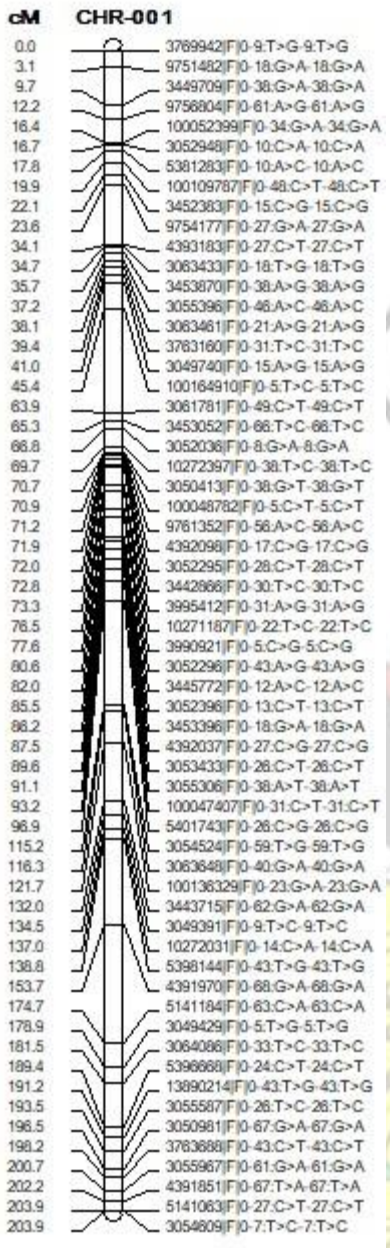
Designation	Grain Yield (g)_Control	Grain Yield (g)_Stress	Reduction (%)	Stability (%)
ARS1181-1-10-103	11.25	11.2	0.39	99.61
ARS1181-1-6-104	15	14.83	1.14	98.86
ARS1181-1-11-9	21.24	20.55	3.25	96.75
ARS1181-1-5-41	16.36	15.78	3.55	96.45
ARS1181-1-7-16	16.75	16.05	4.14	95.86
ARS1181-1-11-4	15.92	15.08	5.28	94.72
ARS1181-1-7-23	14.19	13.38	5.69	94.31
ARS1181-1-12-4	14.9	13.93	6.52	93.48
ARS1181-1-8-20	17.24	16.05	6.86	93.14
ARS1181-1-10-8	14.93	13.75	7.85	92.15
ARS1181-1-7-39	16.56	15.23	8.02	91.98
ARS1181-1-3-31	19.75	18.15	8.07	91.93
ARS1181-1-7-6	18.26	16.78	8.11	91.89
ARS1181-1-2-42	17.32	15.83	8.61	91.39
ARS1181-1-3-9	21.58	19.63	9.04	90.96
ARS1181-1-6-88	17.35	15.73	9.34	90.66
ARS1181-1-11-7	18.38	16.53	10.07	89.93
ARS1181-1-9-98	15.18	13.63	10.21	89.79
ARS1181-1-7-263	30.59	27.15	11.22	88.78
ARS1181-1-8-17	21.79	19.33	11.29	88.71
ARS1181-1-4-7	31.76	28.03	11.76	88.24
ARS1181-1-6-6	18.24	15.98	12.38	87.62
ARS1181-1-6-105	29.18	25.55	12.44	87.56
ARS1181-1-3-11	17.46	15.28	12.49	87.51
ARS1181-1-8-26	17.96	15.43	14.08	85.92
ARS1181-1-3-8	33.01	27.98	15.24	84.76
ARS1181-1-7-239	13.48	11.28	16.31	83.69
ARS1181-1-10-105	12.22	10.15	16.89	83.11
ARS1181-1-6-85	15.47	12.83	17.07	82.93
ARS1181-1-8-25	19.69	16.18	17.82	82.18
ARS1181-1-9-90	16.08	12.95	19.43	80.57
ARS1181-1-6-45	27.66	21.93	20.72	79.28
ARS1181-1-10-1	15.6	12.28	21.29	78.71
ARS1181-1-14-27	26.39	20.63	21.83	78.17
ARS1181-1-7-25	22.65	17.68	21.94	78.06
ARS1181-1-3-21	21.32	16.55	22.34	77.66
ARS1181-1-9-110	22.99	17.63	23.33	76.67
ARS1181-1-6-12	25.65	19.6	23.57	76.43
ARS1181-1-7-17	27.35	20.83	23.84	76.16
ARS1181-1-6-1	25.91	19.63	24.24	75.76
ARS1181-1-7-249	17.97	13.6	24.29	75.71
ARS1181-1-6-83	17.08	12.88	24.59	75.41

ARS1181-1-7-254	28.51	21.18	25.71	74.29
ARS1181-1-6-44	17.08	12.63	26.06	73.94
ARS1181-1-7-244	20.06	14.83	26.08	73.92
Mean	19.9	16.94	14.1	85.9
Mean for all progenies	29.86	8.33	72.1	27.9
Checks				
Sahel317	54.21	5	90.78	9.22
Madina Koyo	41.09	21.57	47.51	52.49
Sahel 108	61.04	4.5	92.63	7.37
Sahel 134	40.51	5.07	87.48	12.52
SMK3	59.45	1.57	97.36	2.64
SMK87	39.23	2.07	94.72	5.28
FL478	25.07	13.5	46.15	53.85
NERICA-L9	34.69	22.14	36.18	63.82

Appendix 4. P-values and odd ratios of Progenies that were either better or comparable to Madina Koyo and FL478

	SES Score			Genotypes vs Madina Koyo		Genotypes vs Madina FL478	
Genotype	Rep1	Rep2	Mean	P-value	Odds ratio	P-value	Odds ratio
ARS1181-1-9-6-B-6	1	1	1	<0.0001	817.61	<0.0001	442.42
ARS1181-1-2-11-B-11	1	1	1	<0.0001	639.84	<0.0001	156.81
ARS1181-1-2-13-B-13	1	1	1	<0.0001	639.84	<0.0001	122.71
ARS1181-1-7-2-B-2	1	1	1	<0.0001	553.94	<0.0001	122.71
ARS1181-1-9-30-B-30	1	1	1	<0.0001	515.21	<0.0001	106.24
ARS1181-1-6-9-B-9	1	1	1	<0.0001	493.8	<0.0001	98.81
ARS1181-1-12-3-B-3	3	1	2	<0.0001	489.24	0.01	94.71
ARS1181-1-12-14-B-14	1	3	2	<0.0001	469.16	0.01	93.83
ARS1181-1-13-1-B-1	1	3	2	<0.0001	371.8	0.01	71.31
ARS1181-1-13-18-B-18	1	3	2	<0.0001	371.8	0.01	89.98
ARS1181-1-1-12-B-12	3	1	2	<0.0001	353.48	0.01	71.31
ARS1181-1-3-17-B-17	3	1	2	<0.0001	332.31	0.01	67.79
ARS1181-1-4-30-B-30	1	3	2	<0.0001	332.31	0.02	63.73
ARS1181-1-4-38-B-38	1	3	2	<0.0001	318.66	0.02	63.73
ARS1181-1-7-6-B-6	3	1	2	<0.0001	312.2	0.02	61.12
ARS1181-1-8-26-B-26	3	1	2	<0.0001	312.2	0.02	59.88
ARS1181-1-4-14-B-14	3	1	2	<0.0001	300.78	0.02	57.69
ARS1181-1-6-6-B-6	1	3	2	<0.0001	300.78	0.02	57.69
ARS1181-1-8-35-B-35	1	3	2	<0.0001	300.78	0.02	59.88
ARS1181-1-8-4-B-4	3	1	2	<0.0001	299.38	0.02	57.69
ARS1181-1-8-16-B-16	3	1	2	<0.0001	297.02	0.02	57.42
ARS1181-1-2-4-B-4	3	3	3	<0.0001	284.68	0.02	54.6
ARS1181-1-10-1-B-1	3	3	3	<0.0001	284.68	0.02	54.6
ARS1181-1-2-3-B-3	3	3	3	<0.0001	284.68	0.02	56.97
ARS1181-1-10-11-B-11	3	3	3	<0.0001	235.38	0.02	54.6
ARS1181-1-12-8-B-8	3	3	3	<0.0001	225.6	0.03	43.27
ARS1181-1-10-14-B-14	3	3	3	<0.0001	225.6	0.03	45.14
ARS1181-1-14-1-B-1	3	3	3	<0.0001	215.31	0.03	43.27
ARS1181-1-14-2-B-2	3	3	3	<0.0001	215.31	0.03	41.29
ARS1181-1-1-10-B-10	3	3	3	<0.0001	203.78	0.03	41.29
ARS1181-1-2-7-B-7	3	3	3	<0.0001	201.74	0.03	39.08
ARS1181-1-4-42-B-42	3	3	3	<0.0001	201.74	0.03	38.69
ARS1181-1-4-44-B-44	3	3	3	<0.0001	201.74	0.03	38.69
ARS1181-1-2-10-B-10	3	3	3	<0.0001	201.74	0.03	38.69

ARS1181-1-7-3-B-3	3	3	3	<0.0001	193.36	0.03	37.08
ARS1181-1-6-33-B-33	3	3	3	<0.0001	193.36	0.03	37.08
ARS1181-1-8-12-B-12	3	3	3	<0.0001	190.16	0.04	36.47
ARS1181-1-8-9-B-9	3	3	3	<0.0001	190.16	0.03	37.08
ARS1181-1-10-6-B-6	3	3	3	<0.0001	189.54	0.04	36.47
ARS1181-1-12-6-B-6	3	3	3	<0.0001	182.36	0.04	34.97
ARS1181-1-12-17-B-17	3	3	3	<0.0001	182.36	0.04	36.35
ARS1181-1-13-5-B-5	3	3	3	<0.0001	181.66	0.04	34.84
ARS1181-1-11-3-B-3	3	3	3	<0.0001	181.66	0.04	34.97
ARS1181-1-1-6-B-6	3	3	3	<0.0001	179.98	0.04	34.84
ARS1181-1-7-9-B-9	3	3	3	<0.0001	172.59	0.04	33.1
ARS1181-1-6-27-B-27	3	3	3	<0.0001	172.59	0.04	34.52
Mean			2.4				
FL478			2.4				
Madina Koyo	1	3	4				
Sahel 317	9	7	8				
IR 29			8.4				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12.2 9756804|F|0-61:A>G-61:A>G
16.4 100052399|F|0-34:G>A-34:G>A
16.7 3052948|F|0-10:C>A-10:C>A
17.8 5381283|F|0-10:A>C-10:A>C
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37.2 3055396|F|0-46:A>C-46:A>C
38.1 3063461|F|0-21:A>G-21:A>G
39.4 3783160|F|0-31:T>C-31:T>C
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45.4 100164910|F|0-5:T>C-5:T>C
63.9 3061781|F|0-49:C>T-49:C>T
65.3 3453052|F|0-66:T>C-66:T>C
66.8 3052036|F|0-8:G>A-8:G>A
69.7 10272397|F|0-38:T>C-38:T>C
70.7 3050413|F|0-38:G>T-38:G>T
70.9 100048782|F|0-5:C>T-5:C>T
71.2 9761352|F|0-56:A>C-56:A>C
71.9 4392098|F|0-17:C>G-17:C>G
72.0 3052295|F|0-28:C>T-28:C>T
72.8 3442866|F|0-30:T>C-30:T>C
73.3 3995412|F|0-31:A>G-31:A>G
76.5 10271187|F|0-22:T>C-22:T>C
77.6 3990921|F|0-5:C>G-5:C>G
80.6 3052296|F|0-43:A>G-43:A>G
82.0 3445772|F|0-12:A>C-12:A>C
85.5 3052396|F|0-13:C>T-13:C>T
86.2 3453396|F|0-18:G>A-18:G>A
87.5 4392037|F|0-27:C>G-27:C>G
89.6 3053433|F|0-26:C>T-26:C>T
91.1 3055306|F|0-38:A>T-38:A>T
93.2 100047407|F|0-31:C>T-31:C>T
96.9 5401743|F|0-26:C>G-26:C>G
115.2 3054524|F|0-59:T>G-59:T>G
116.3 3063648|F|0-40:G>A-40:G>A
121.7 100136329|F|0-23:G>A-23:G>A
132.0 3443715|F|0-62:G>A-62:G>A
134.5 3049391|F|0-9:T>C-9:T>C
137.0 10272031|F|0-14:C>A-14:C>A
138.8 5398144|F|0-43:T>G-43:T>G
153.7 4391970|F|0-68:G>A-68:G>A
174.7 5141184|F|0-63:C>A-63:C>A
178.9 3049429|F|0-5:T>G-5:T>G
181.5 3064086|F|0-33:T>C-33:T>C
189.4 5396668|F|0-24:C>T-24:C>T
191.2 13890214|F|0-43:T>G-43:T>G
193.5 3055587|F|0-26:T>C-26:T>C
196.5 3050981|F|0-67:G>A-67:G>A
196.2 3783688|F|0-43:C>T-43:C>T
200.7 3055967|F|0-61:G>A-61:G>A
202.2 4391851|F|0-67:T>A-67:T>A
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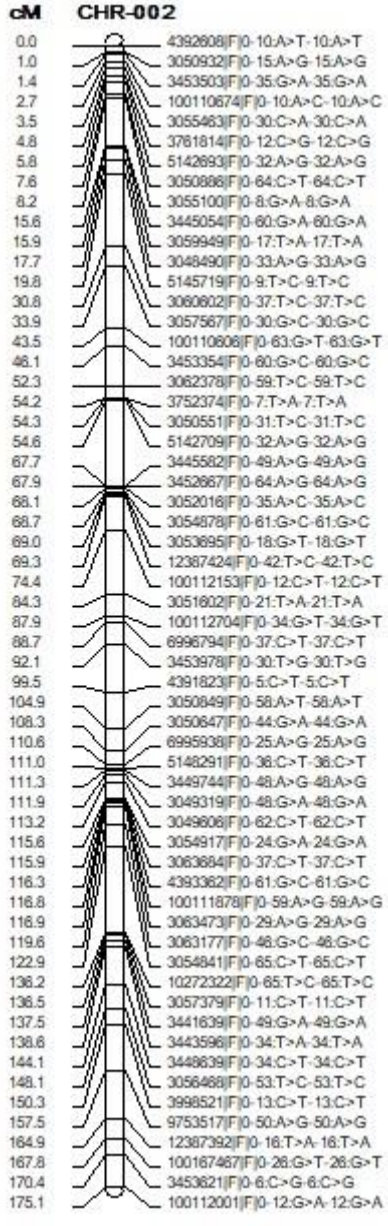
203.9 3054809|F|0-7:T>C-7:T>C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2.7 100110674|F|0-10:A>C-10:A>C
3.5 3055483|F|0-30:C>A-30:C>A
4.8 3761814|F|0-12:C>G-12:C>G
5.8 5142693|F|0-32:A>G-32:A>G
7.6 3050886|F|0-64:C>T-64:C>T
8.2 3055100|F|0-8:G>A-8:G>A
15.6 3445054|F|0-60:G>A-60:G>A
15.9 3059949|F|0-17:T>A-17:T>A
17.7 3048490|F|0-33:A>G-33:A>G
19.8 5145719|F|0-9:T>C-9:T>C
30.8 3060602|F|0-37:T>C-37:T>C
33.9 3057567|F|0-30:G>C-30:G>C
43.5 100110606|F|0-63:G>T-63:G>T
46.1 3453354|F|0-60:G>C-60:G>C
52.3 3062378|F|0-59:T>C-59:T>C
54.2 3752374|F|0-7:T>A-7:T>A
54.3 3050551|F|0-31:T>C-31:T>C
54.6 5142709|F|0-32:A>G-32:A>G
67.7 3445582|F|0-49:A>G-49:A>G
67.9 3452667|F|0-64:A>G-64:A>G
68.1 3052016|F|0-35:A>C-35:A>C
68.7 3054878|F|0-61:G>C-61:G>C
69.0 3053695|F|0-18:G>T-18:G>T
69.3 12387424|F|0-42:T>C-42:T>C
74.4 100112153|F|0-12:C>T-12:C>T
84.3 3051602|F|0-21:T>A-21:T>A
87.9 100112704|F|0-34:G>T-34:G>T
88.7 6996794|F|0-37:C>T-37:C>T
92.1 3453978|F|0-30:T>G-30:T>G
99.5 4391823|F|0-5:C>T-5:C>T
104.9 3050849|F|0-58:A>T-58:A>T
108.3 3050647|F|0-44:G>A-44:G>A
110.6 6995938|F|0-25:A>G-25:A>G
111.0 5148291|F|0-36:C>T-36:C>T
111.3 3449744|F|0-48:A>G-48:A>G
111.9 3049319|F|0-48:G>A-48:G>A
113.2 3049606|F|0-62:C>T-62:C>T
115.6 3054917|F|0-24:G>A-24:G>A
115.9 3063684|F|0-37:C>T-37:C>T
116.3 4393362|F|0-61:G>C-61:G>C
116.8 100111878|F|0-59:A>G-59:A>G
116.9 3063473|F|0-29:A>G-29:A>G
119.6 3063177|F|0-48:G>C-48:G>C
122.9 3054841|F|0-65:C>T-65:C>T
136.2 10272322|F|0-65:T>C-65:T>C
136.5 3057379|F|0-11:C>T-11:C>T
137.5 3441639|F|0-49:G>A-49:G>A
138.6 3443596|F|0-34:T>A-34:T>A
144.1 3449839|F|0-34:C>T-34:C>T
148.1 3056468|F|0-53:T>C-53:T>C
150.3 3998521|F|0-13:C>T-13:C>T
157.5 9753517|F|0-50:A>G-50:A>G
164.9 12387392|F|0-16:T>A-16:T>A
167.8 100167467|F|0-26:G>T-26:G>T
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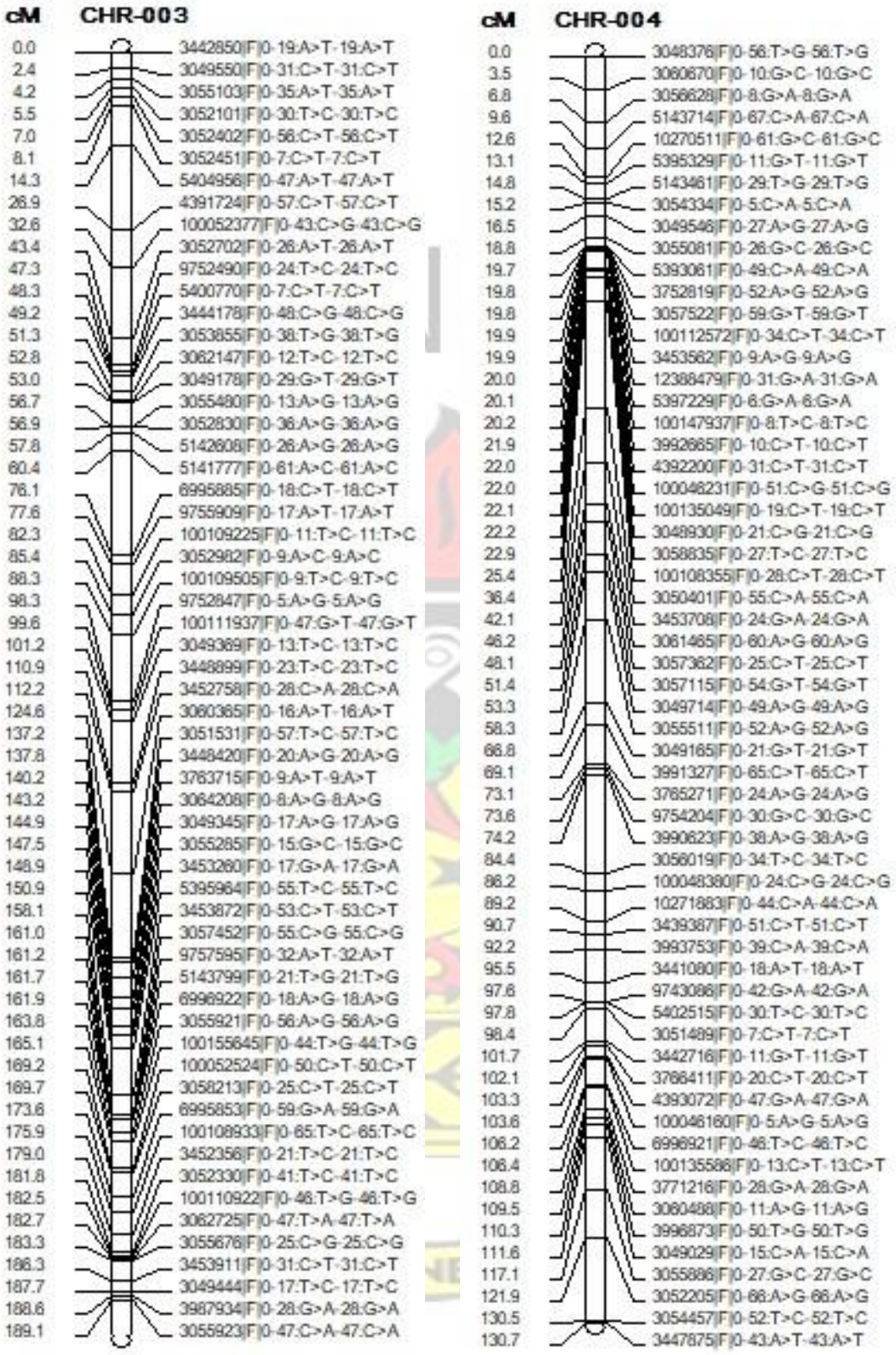
175.1 100112001|F|0-12:G>A-12:G>A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7.0 3052402|F|0-56:C>T-56:C>T
8.1 3052451|F|0-7:C>T-7:C>T
14.3 5404956|F|0-47:A>T-47:A>T
26.9 4391724|F|0-57:C>T-57:C>T
32.6 100052377|F|0-43:C>G-43:C>G
43.4 3052702|F|0-26:A>T-26:A>T
47.3 9752490|F|0-24:T>C-24:T>C
48.3 5400770|F|0-7:C>T-7:C>T
49.2 3444178|F|0-48:C>G-48:C>G
51.3 3053855|F|0-38:T>G-38:T>G
52.8 3062147|F|0-12:T>C-12:T>C
53.0 3049178|F|0-29:G>T-29:G>T
56.7 3055480|F|0-13:A>G-13:A>G
56.9 3052830|F|0-36:A>G-36:A>G
57.8 5142606|F|0-26:A>G-26:A>G
60.4 5141777|F|0-61:A>C-61:A>C
76.1 6995885|F|0-18:C>T-18:C>T
77.6 9755909|F|0-17:A>T-17:A>T
82.3 100109225|F|0-11:T>C-11:T>C
85.4 3052982|F|0-9:A>C-9:A>C
88.3 100109505|F|0-9:T>C-9:T>C
98.3 9752847|F|0-5:A>G-5:A>G
99.6 100111937|F|0-47:G>T-47:G>T
101.2 3049369|F|0-13:T>C-13:T>C
110.9 3448899|F|0-23:T>C-23:T>C
112.2 3452758|F|0-28:C>A-28:C>A
124.6 3060365|F|0-16:A>T-16:A>T
137.2 3051531|F|0-57:T>C-57:T>C
137.8 3448420|F|0-20:A>G-20:A>G
140.2 3763715|F|0-9:A>T-9:A>T
143.2 3064208|F|0-8:A>G-8:A>G
144.9 3049345|F|0-17:A>G-17:A>G
147.5 3055285|F|0-15:G>C-15:G>C
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150.9 5395964|F|0-55:T>C-55:T>C
158.1 3453872|F|0-53:C>T-53:C>T
161.0 3057452|F|0-55:C>G-55:C>G
161.2 9757595|F|0-32:A>T-32:A>T
161.7 5143799|F|0-21:T>G-21:T>G
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165.1 100155645|F|0-44:T>G-44:T>G
169.2 100052524|F|0-50:C>T-50:C>T
169.7 3058213|F|0-25:C>T-25:C>T
173.6 6995853|F|0-59:G>A-59:G>A
175.9 100108933|F|0-65:T>C-65:T>C
179.0 3452356|F|0-21:T>C-21:T>C
181.8 3052330|F|0-41:T>C-41:T>C
182.5 100110922|F|0-46:T>G-46:T>G
182.7 3062725|F|0-47:T>A-47:T>A
183.3 3055676|F|0-25:C>G-25:C>G
186.3 3453911|F|0-31:C>T-31:C>T
187.7 3049444|F|0-17:T>C-17:T>C
188.6 3967934|F|0-28:G>A-28:G>A
189.1 3055923|F|0-47:C>A-47:C>A
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3.5 3060670|F|0-10:G>C-10:G>C
6.8 3056628|F|0-8:G>A-8:G>A
9.6 5143714|F|0-67:C>A-67:C>A
12.6 10270511|F|0-61:G>C-61:G>C
13.1 5395329|F|0-11:G>T-11:G>T
14.8 5143461|F|0-29:T>G-29:T>G
15.2 3054334|F|0-5:C>A-5:C>A
16.5 3049546|F|0-27:A>G-27:A>G
18.8 3055081|F|0-26:G>C-26:G>C
19.7 5393061|F|0-49:C>A-49:C>A
19.8 3752819|F|0-52:A>G-52:A>G
19.8 3057522|F|0-59:G>T-59:G>T
19.9 100112572|F|0-34:C>T-34:C>T
19.9 3453562|F|0-9:A>G-9:A>G
20.0 12388479|F|0-31:G>A-31:G>A
20.1 5397229|F|0-6:G>A-6:G>A
20.2 100147937|F|0-8:T>C-8:T>C
21.9 3992665|F|0-10:C>T-10:C>T
22.0 4392200|F|0-31:C>T-31:C>T
22.0 100048231|F|0-51:C>G-51:C>G
22.1 100135049|F|0-19:C>T-19:C>T
22.2 3048930|F|0-21:C>G-21:C>G
22.9 3058835|F|0-27:T>C-27:T>C
25.4 100108355|F|0-28:C>T-28:C>T
36.4 3050401|F|0-55:C>A-55:C>A
42.1 3453708|F|0-24:G>A-24:G>A
46.2 3061465|F|0-60:A>G-60:A>G
48.1 3057362|F|0-25:C>T-25:C>T
51.4 3057115|F|0-54:G>T-54:G>T
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58.3 3055511|F|0-52:A>G-52:A>G
66.8 3049165|F|0-21:G>T-21:G>T
69.1 3991327|F|0-65:C>T-65:C>T
73.1 3765271|F|0-24:A>G-24:A>G
73.6 9754204|F|0-30:G>C-30:G>C
74.2 3990623|F|0-38:A>G-38:A>G
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86.2 100048380|F|0-24:C>G-24:C>G
89.2 10271883|F|0-44:C>A-44:C>A
90.7 3439387|F|0-51:C>T-51:C>T
92.2 3993753|F|0-39:C>A-39:C>A
95.5 3441080|F|0-18:A>T-18:A>T
97.6 9743086|F|0-42:G>A-42:G>A
97.8 5402515|F|0-30:T>C-30:T>C
98.4 3051489|F|0-7:C>T-7:C>T
101.7 3442716|F|0-11:G>T-11:G>T
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111.6 3049029|F|0-15:C>A-15:C>A
117.1 3055886|F|0-27:G>C-27:G>C
121.9 3052205|F|0-66:A>G-66:A>G
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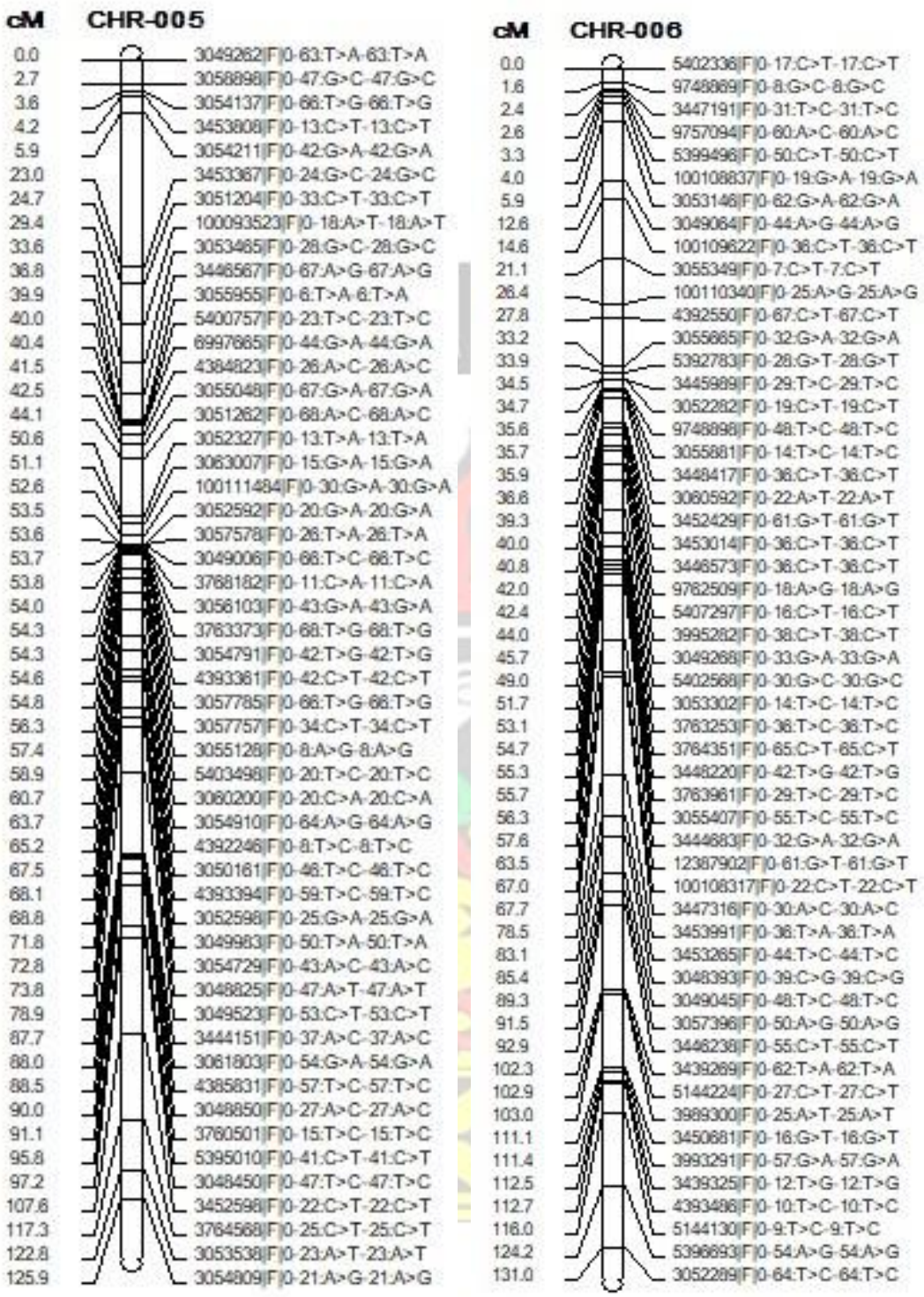
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29.4 100093523|F|0-18:A>T-18:A>T
33.6 3053465|F|0-28:G>C-28:G>C
36.8 3446567|F|0-67:A>G-67:A>G
39.9 3055955|F|0-6:T>A-6:T>A
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42.5 3055048|F|0-67:G>A-67:G>A
44.1 3051262|F|0-68:A>C-68:A>C
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53.7 3049006|F|0-66:T>C-66:T>C
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54.0 3056103|F|0-43:G>A-43:G>A
54.3 3763373|F|0-68:T>G-68:T>G
54.3 3054791|F|0-42:T>G-42:T>G
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68.8 3052596|F|0-25:G>A-25:G>A
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73.8 3048825|F|0-47:A>T-47:A>T
78.9 3049523|F|0-53:C>T-53:C>T
87.7 3444151|F|0-37:A>C-37:A>C
88.0 3061803|F|0-54:G>A-54:G>A
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97.2 3048450|F|0-47:T>C-47:T>C
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117.3 3764568|F|0-25:C>T-25:C>T
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125.9 3054809|F|0-21:A>G-21:A>G
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3.3 5399496|F|0-50:C>T-50:C>T
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5.9 3053146|F|0-62:G>A-62:G>A
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21.1 3055349|F|0-7:C>T-7:C>T
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35.6 9748898|F|0-48:T>C-48:T>C
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55.3 3448220|F|0-42:T>G-42:T>G
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78.5 3453991|F|0-36:T>A-36:T>A
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89.3 3049045|F|0-48:T>C-48:T>C
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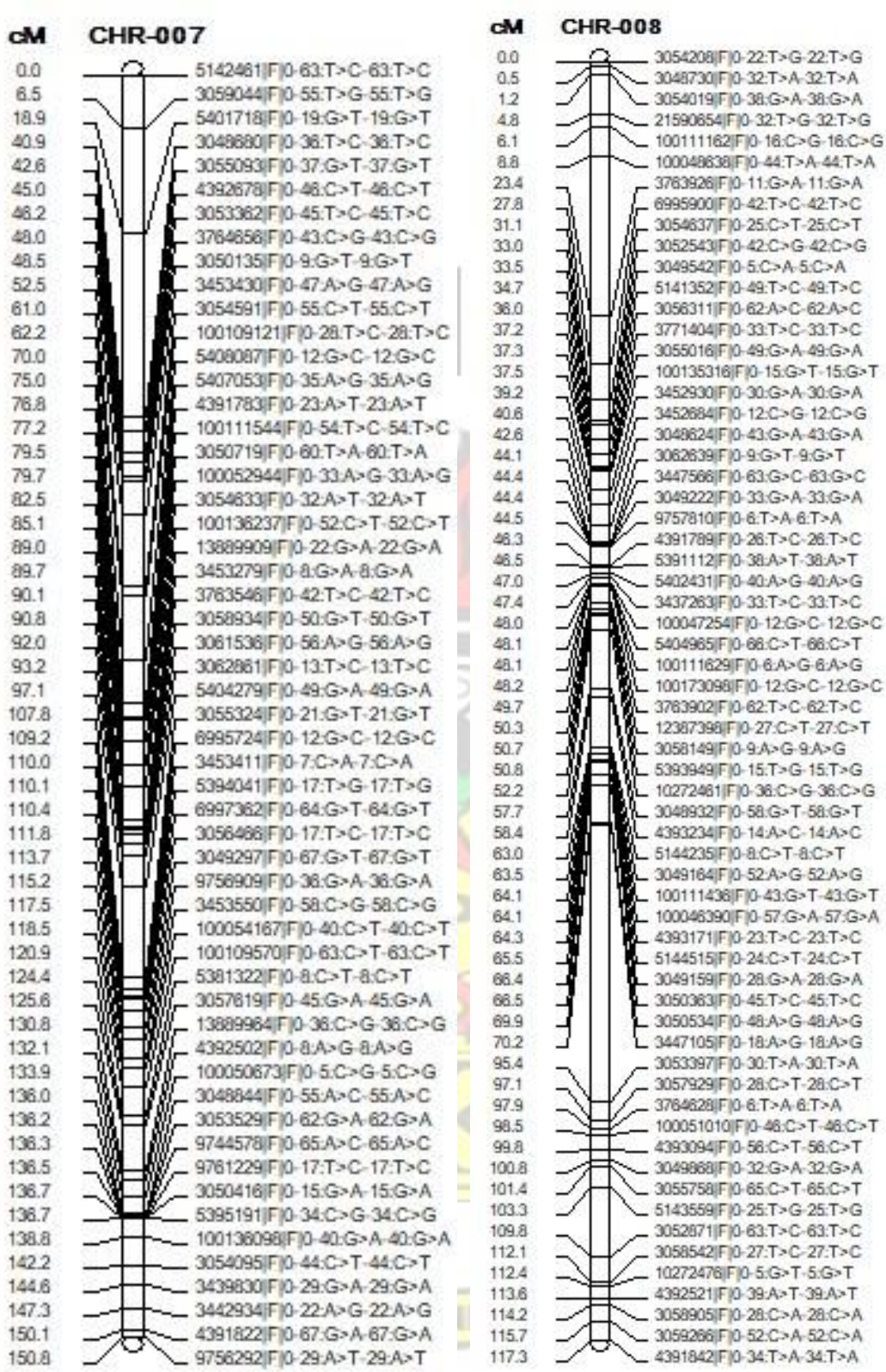
92.9 3446238|F|0-55:C>T-55:C>T
102.3 3439269|F|0-62:T>A-62:T>A
102.9 5144224|F|0-27:C>T-27:C>T
103.0 3989300|F|0-25:A>T-25:A>T
111.1 3450681|F|0-16:G>T-16:G>T
111.4 3993291|F|0-57:G>A-57:G>A
112.5 3439325|F|0-12:T>G-12:T>G
112.7 4393486|F|0-10:T>C-10:T>C
116.0 5144130|F|0-9:T>C-9:T>C
124.2 5396693|F|0-54:A>G-54:A>G
131.0 3052289|F|0-64:T>C-64:T>C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18.9 5401718|F|0-19:G>T-19:G>T
40.9 3048680|F|0-36:T>C-36:T>C
42.6 3055093|F|0-37:G>T-37:G>T
45.0 4392678|F|0-46:C>T-46:C>T
46.2 3053362|F|0-45:T>C-45:T>C
48.0 3764656|F|0-43:C>G-43:C>G
48.5 3050135|F|0-9:G>T-9:G>T
52.5 3453430|F|0-47:A>G-47:A>G
61.0 3054591|F|0-55:C>T-55:C>T
62.2 100109121|F|0-28:T>C-28:T>C
70.0 5408087|F|0-12:G>C-12:G>C
75.0 5407053|F|0-35:A>G-35:A>G
76.8 4391783|F|0-23:A>T-23:A>T
77.2 100111544|F|0-54:T>C-54:T>C
79.5 3050719|F|0-60:T>A-60:T>A
79.7 100052944|F|0-33:A>G-33:A>G
82.5 3054633|F|0-32:A>T-32:A>T
85.1 100136237|F|0-52:C>T-52:C>T
89.0 13889909|F|0-22:G>A-22:G>A
89.7 3453279|F|0-8:G>A-8:G>A
90.1 3763546|F|0-42:T>C-42:T>C
90.8 3058934|F|0-50:G>T-50:G>T
92.0 3061536|F|0-56:A>G-56:A>G
93.2 3062861|F|0-13:T>C-13:T>C
97.1 5404279|F|0-49:G>A-49:G>A
107.8 3055324|F|0-21:G>T-21:G>T
109.2 6995724|F|0-12:G>C-12:G>C
110.0 3453411|F|0-7:C>A-7:C>A
110.1 5394041|F|0-17:T>G-17:T>G
110.4 6997362|F|0-64:G>T-64:G>T
111.8 3056466|F|0-17:T>C-17:T>C
113.7 3049297|F|0-67:G>T-67:G>T
115.2 9756909|F|0-36:G>A-36:G>A
117.5 3453550|F|0-58:C>G-58:C>G
118.5 100054167|F|0-40:C>T-40:C>T
120.9 100109570|F|0-63:C>T-63:C>T
124.4 5381322|F|0-8:C>T-8:C>T
125.6 3057619|F|0-45:G>A-45:G>A
130.8 13889964|F|0-36:C>G-36:C>G
132.1 4392502|F|0-8:A>G-8:A>G
133.9 100050673|F|0-5:C>G-5:C>G
136.0 3048844|F|0-55:A>C-55:A>C
136.2 3053529|F|0-62:G>A-62:G>A
136.3 9744578|F|0-65:A>C-65:A>C
136.5 9761229|F|0-17:T>C-17:T>C
136.7 3050416|F|0-15:G>A-15:G>A
136.7 5395191|F|0-34:C>G-34:C>G
138.8 100136098|F|0-40:G>A-40:G>A
142.2 3054095|F|0-44:C>T-44:C>T
144.6 3439830|F|0-29:G>A-29:G>A
147.3 3442934|F|0-22:A>G-22:A>G
150.1 4391822|F|0-67:G>A-67:G>A
150.8 9756292|F|0-29:A>T-29:A>T
cM
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1.2 3054019|F|0-38:G>A-38:G>A
4.8 21590654|F|0-32:T>G-32:T>G
6.1 100111162|F|0-16:C>G-16:C>G
8.8 100048638|F|0-44:T>A-44:T>A
23.4 3763926|F|0-11:G>A-11:G>A
27.8 6995900|F|0-42:T>C-42:T>C
31.1 3054637|F|0-25:C>T-25:C>T
33.0 3052543|F|0-42:C>G-42:C>G
33.5 3049542|F|0-5:C>A-5:C>A
34.7 5141352|F|0-49:T>C-49:T>C
36.0 3056311|F|0-62:A>C-62:A>C
37.2 3771404|F|0-33:T>C-33:T>C
37.3 3055016|F|0-49:G>A-49:G>A
37.5 100135316|F|0-15:G>T-15:G>T
39.2 3452930|F|0-30:G>A-30:G>A
40.6 3452684|F|0-12:C>G-12:C>G
42.6 3048624|F|0-43:G>A-43:G>A
44.1 3062639|F|0-9:G>T-9:G>T
44.4 3447586|F|0-63:G>C-63:G>C
44.4 3049222|F|0-33:G>A-33:G>A
44.5 9757810|F|0-6:T>A-6:T>A
46.3 4391789|F|0-26:T>C-26:T>C
46.5 5391112|F|0-38:A>T-38:A>T
47.0 5402431|F|0-40:A>G-40:A>G
47.4 3437263|F|0-33:T>C-33:T>C
48.0 100047254|F|0-12:G>C-12:G>C
48.1 5404965|F|0-66:C>T-66:C>T
48.1 100111629|F|0-6:A>G-6:A>G
48.2 100173098|F|0-12:G>C-12:G>C
49.7 3763902|F|0-62:T>C-62:T>C
50.3 12367398|F|0-27:C>T-27:C>T
50.7 3058149|F|0-9:A>G-9:A>G
50.8 5393949|F|0-15:T>G-15:T>G
52.2 10272461|F|0-36:C>G-36:C>G
57.7 3048932|F|0-58:G>T-58:G>T
58.4 4393234|F|0-14:A>C-14:A>C
63.0 5144235|F|0-8:C>T-8:C>T
63.5 3049164|F|0-52:A>G-52:A>G
64.1 100111436|F|0-43:G>T-43:G>T
64.1 100046390|F|0-57:G>A-57:G>A
64.3 4393171|F|0-23:T>C-23:T>C
65.5 5144515|F|0-24:C>T-24:C>T
66.4 3049159|F|0-28:G>A-28:G>A
66.5 3050363|F|0-45:T>C-45:T>C
69.9 3050534|F|0-48:A>G-48:A>G
70.2 3447105|F|0-18:A>G-18:A>G
95.4 3053397|F|0-30:T>A-30:T>A
97.1 3057929|F|0-28:C>T-28:C>T
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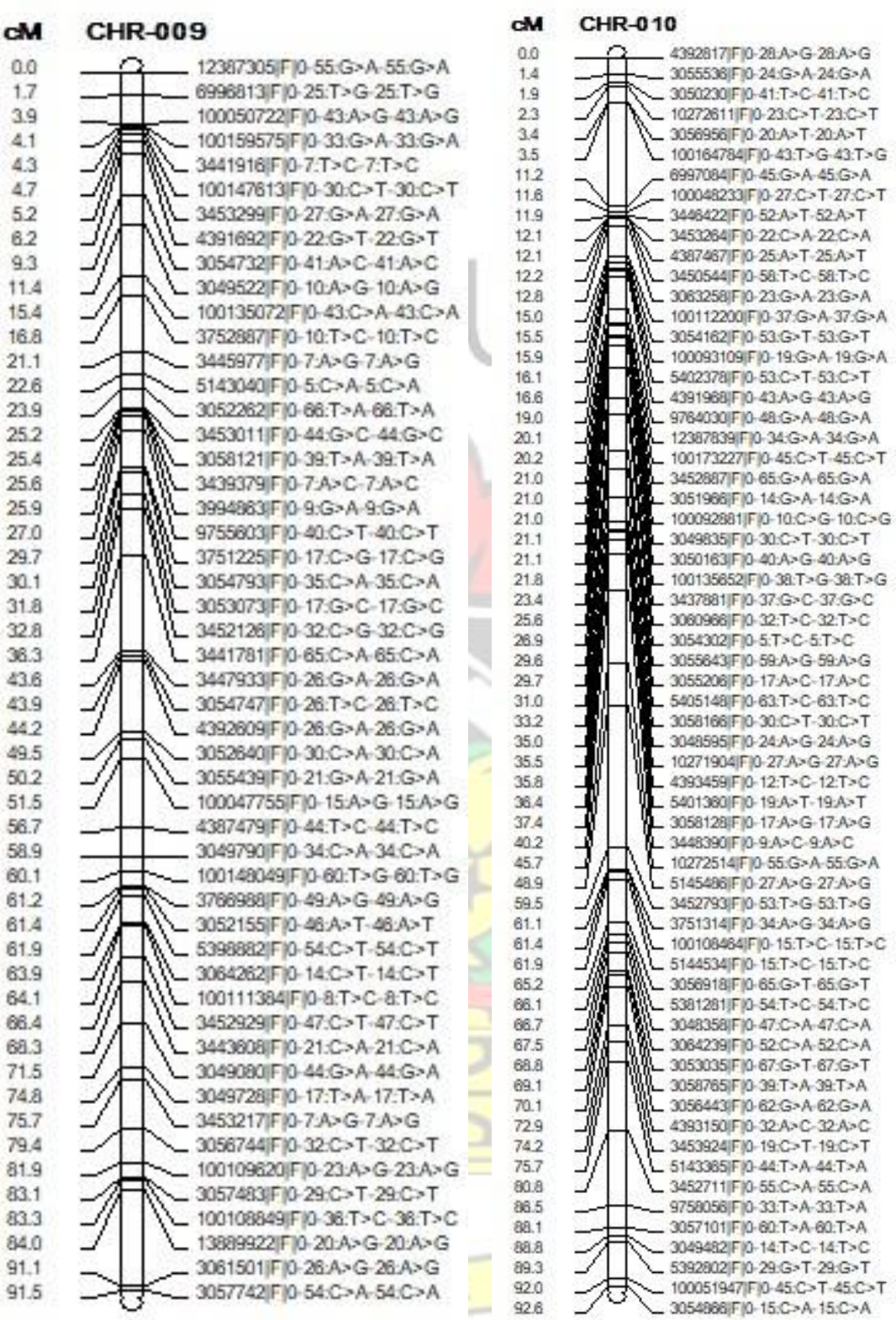
98.5 100051010|F|0-46:C>T-46:C>T
99.8 4393094|F|0-56:C>T-56:C>T
100.8 3049868|F|0-32:G>A-32:G>A
101.4 3055758|F|0-65:C>T-65:C>T
103.3 5143559|F|0-25:T>G-25:T>G
109.8 3052871|F|0-63:T>C-63:T>C
112.1 3058542|F|0-27:T>C-27:T>C
112.4 10272476|F|0-5:G>T-5:G>T
113.6 4392521|F|0-39:A>T-39:A>T
114.2 3058905|F|0-28:C>A-28:C>A
115.7 3059266|F|0-52:C>A-52:C>A
117.3 4391842|F|0-34:T>A-34:T>A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4.3 3441916|F|0-7:T>C-7:T>C
4.7 100147613|F|0-30:C>T-30:C>T
5.2 3453299|F|0-27:G>A-27:G>A
6.2 4391692|F|0-22:G>T-22:G>T
9.3 3054732|F|0-41:A>C-41:A>C
11.4 3049522|F|0-10:A>G-10:A>G
15.4 100135072|F|0-43:C>A-43:C>A
16.8 3752887|F|0-10:T>C-10:T>C
21.1 3445977|F|0-7:A>G-7:A>G
22.6 5143040|F|0-5:C>A-5:C>A
23.9 3052262|F|0-66:T>A-66:T>A
25.2 3453011|F|0-44:G>C-44:G>C
25.4 3058121|F|0-39:T>A-39:T>A
25.6 3439379|F|0-7:A>C-7:A>C
25.9 3994863|F|0-9:G>A-9:G>A
27.0 9755803|F|0-40:C>T-40:C>T
29.7 3751225|F|0-17:C>G-17:C>G
30.1 3054793|F|0-35:C>A-35:C>A
31.8 3053073|F|0-17:G>C-17:G>C
32.8 3452126|F|0-32:C>G-32:C>G
36.3 3441781|F|0-65:C>A-65:C>A
43.6 3447933|F|0-26:G>A-26:G>A
43.9 3054747|F|0-26:T>C-26:T>C
44.2 4392609|F|0-26:G>A-26:G>A
49.5 3052640|F|0-30:C>A-30:C>A
50.2 3055439|F|0-21:G>A-21:G>A
51.5 100047755|F|0-15:A>G-15:A>G
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58.9 3049790|F|0-34:C>A-34:C>A
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61.4 3052155|F|0-46:A>T-46:A>T
61.9 5398882|F|0-54:C>T-54:C>T
63.9 3064262|F|0-14:C>T-14:C>T
64.1 100111384|F|0-8:T>C-8:T>C
66.4 3452929|F|0-47:C>T-47:C>T
68.3 3443608|F|0-21:C>A-21:C>A
71.5 3049080|F|0-44:G>A-44:G>A
74.8 3049728|F|0-17:T>A-17:T>A
75.7 3453217|F|0-7:A>G-7:A>G
79.4 3056744|F|0-32:C>T-32:C>T
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83.1 3057483|F|0-29:C>T-29:C>T
83.3 100108849|F|0-36:T>C-36:T>C
84.0 13889922|F|0-20:A>G-20:A>G
91.1 3061501|F|0-26:A>G-26:A>G
91.5 3057742|F|0-54:C>A-54:C>A
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CHR-010
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1.9 3050230|F|0-41:T>C-41:T>C
2.3 10272611|F|0-23:C>T-23:C>T
3.4 3056956|F|0-20:A>T-20:A>T
3.5 100164784|F|0-43:T>G-43:T>G
11.2 6997084|F|0-45:G>A-45:G>A
11.6 100048233|F|0-27:C>T-27:C>T
11.9 3448422|F|0-52:A>T-52:A>T
12.1 3453264|F|0-22:C>A-22:C>A
12.1 4387467|F|0-25:A>T-25:A>T
12.2 3450544|F|0-58:T>C-58:T>C
12.8 3063258|F|0-23:G>A-23:G>A
15.0 100112200|F|0-37:G>A-37:G>A
15.5 3054162|F|0-53:G>T-53:G>T
15.9 100093109|F|0-19:G>A-19:G>A
16.1 5402378|F|0-53:C>T-53:C>T
16.6 4391968|F|0-43:A>G-43:A>G
19.0 9764030|F|0-48:G>A-48:G>A
20.1 12387839|F|0-34:G>A-34:G>A
20.2 100173227|F|0-45:C>T-45:C>T
21.0 3452887|F|0-65:G>A-65:G>A
21.0 3051966|F|0-14:G>A-14:G>A
21.0 100092881|F|0-10:C>G-10:C>G
21.1 3049835|F|0-30:C>T-30:C>T
21.1 3050163|F|0-40:A>G-40:A>G
21.8 100135652|F|0-38:T>G-38:T>G
23.4 3437881|F|0-37:G>C-37:G>C
25.6 3060966|F|0-32:T>C-32:T>C
26.9 3054302|F|0-5:T>C-5:T>C
29.6 3055643|F|0-59:A>G-59:A>G
29.7 3055206|F|0-17:A>C-17:A>C
31.0 5405148|F|0-63:T>C-63:T>C
33.2 3058166|F|0-30:C>T-30:C>T
35.0 3048595|F|0-24:A>G-24:A>G
35.5 10271904|F|0-27:A>G-27:A>G
35.8 4393459|F|0-12:T>C-12:T>C
36.4 5401360|F|0-19:A>T-19:A>T
37.4 3058128|F|0-17:A>G-17:A>G
40.2 3448390|F|0-9:A>C-9:A>C
45.7 10272514|F|0-55:G>A-55:G>A
48.9 5145486|F|0-27:A>G-27:A>G
59.5 3452793|F|0-53:T>G-53:T>G
61.1 3751314|F|0-34:A>G-34:A>G
61.4 100108464|F|0-15:T>C-15:T>C
61.9 5144534|F|0-15:T>C-15:T>C
65.2 3056918|F|0-65:G>T-65:G>T
66.1 5381281|F|0-54:T>C-54:T>C
66.7 3048358|F|0-47:C>A-47:C>A
67.5 3064239|F|0-52:C>A-52:C>A
68.8 3053035|F|0-67:G>T-67:G>T
69.1 3058765|F|0-39:T>A-39:T>A
70.1 3056443|F|0-62:G>A-62:G>A
72.9 4393150|F|0-32:A>C-32:A>C
74.2 3453924|F|0-19:C>T-19:C>T
75.7 5143365|F|0-44:T>A-44:T>A
80.8 3452711|F|0-55:C>A-55:C>A
86.5 9758056|F|0-33:T>A-33:T>A
88.1 3057101|F|0-60:T>A-60:T>A
88.8 3049482|F|0-14:T>C-14:T>C
89.3 5392802|F|0-29:G>T-29:G>T
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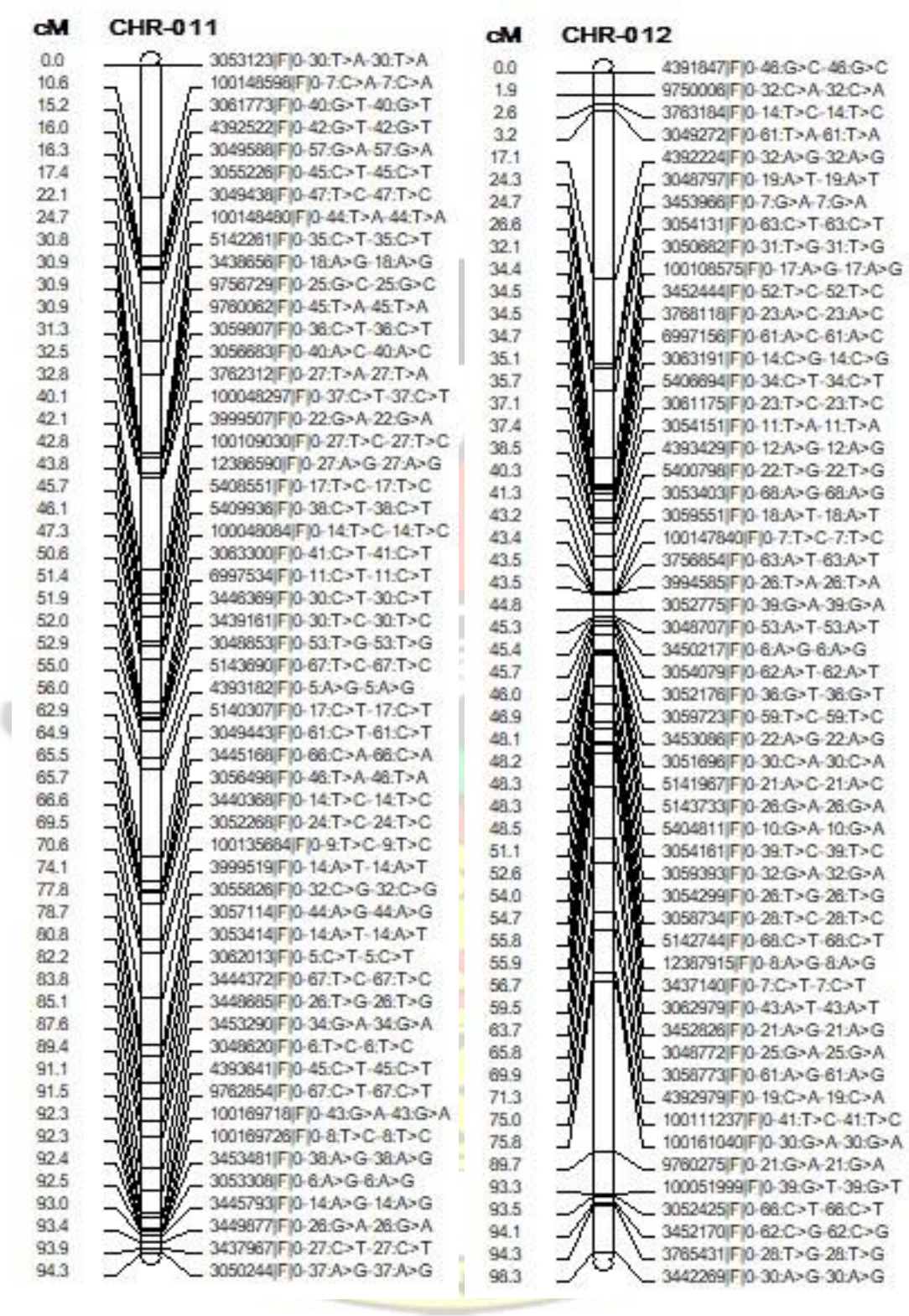
92.6 3054866|F|0-15:C>A-15:C>A



Appendix 5. Framework linkage map constructed with 3698 SNP markers from Sahel317/Madina Koyo