GENETIC STUDIES OF SALINITY TOLERANCE OF MANGROVE RICE VARIETIES IN SIERRA LEONE

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DECLARATION

I, Vangahun, Juliana Mariama, hereby declare that the thesis herein is my own work, produced from research under supervision, and that neither part nor whole has been presented elsewhere for the award of a degree. Any reference to work of other researcher(s) has been correctly cited,

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ABSTRACT

Rice is a major food crop in Sierra Leone with a high per capita consumption of 104 kg annually. Average yield (t/ha) is low, leading to a yield gap of 350,000 metric tons, which is met through import. A good number of rice farmers in Sierra Leone are cultivating on mangrove swamps, which are highly associated with salinity, resulting in the low average yield (tonnes per hectare). Salinity is a major abiotic stress devastating rice production in saline soils, including the mangrove ecology. Rice has been listed as the most salt sensitive cereal crop with a threshold of 3 dsm-1 beyond which yield begins to decline for most cultivated species. Understanding farmer's knowledge on local rice varieties / land races and incorporation of molecular tools may enhance breeding for tolerance to salt stress in mangrove rice.

The present study was undertaken to: (1) evaluate production constraints and varietal preferences of rice farmers in the mangrove ecology of Sierra Leone; (2) characterize the genetic diversity and population structure of mangrove rice landraces; (3) identify salinity tolerant QTL using DarT markers; and (4) estimate the relative efficiency of selection for tolerance to salinity in rice. A focus group discussion (FGD) and semi structured questionnaire were used in this study to sample responses from farmers. A total of 90 rice accessions collected from three districts (Kambia, Port Loko, and Moyamba) in Sierra Leone were evaluated in a completely randomised (CRD) design under screen house conditions in two replications. A cross between salt tolerance line (Nerica L9) and susceptible line (Sahel 317) was performed and a total of 462 F3:4 progenies were generated. The progenies, together with the two parents, were evaluated for tolerance to salinity in two replications under field conditions in Mbe, AfricaRice, Bouake. The 462 F3:4 progenies were genotyped for tolerance to Salinity using 13,432 SNP markers. Selection index and morphological characters were determined using BC_1F_3 populations.

The present study identified major production constraints such as high cost of labour, salinity, lack of credit, lack of access to improved seeds and fertilizer, crab damage pest and diseases as most important. High yield, tall plant height, late maturity, good taste and high tillering ability were the top five traits farmers considered in selecting a variety. The cluster analysis found four distinct groups within the 90 germplasm which were also confirmed by the molecular analysis. The present study identified two major QTL (qDTF1.1 and qDTF1.2.) for time to 50% flowering on chromosome one, One major QTL (qDTF2) for the same trait on chromosome two. Two major QTL (qPH1.1 and qPH1.2) for plant height on chromosome one. Two QTL on chromosome 1 (qPN1) and chromosome two (qPN2). The study found that salt tolerance indices STI, DMP and TSI had discriminated among rice populations for yield under saline field conditions. Five agronomic traits including days to 50% flowering, plant height, number of tillers, number of panicle and filled-grain showed strong and positive correlations with grain weight hence are good selection indices under salt stress. The findings highlighted above could provide useful information for breeding for salt tolerance in mangrove rice in Sierra Leone.

DEDICATION

This piece of work is dedicated to my very supportive husband Mr. Sorie Sulaiman Sesay, and my Dad, Mr. M.S Vangahun (of Blessed Memory), for their contributions to my academic successes.

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To God be all the glory; great things He has done.

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LIST OF ABBREVIATIONS

Africarice	Africa Rice Centre
AFLP	Amplified Fragment Length Polymorphism
CSSL	Chromosome Segment Substitution
Ece	Electrical Conductivity of Saturated Extract
FBO	Farmer Based Organization
FGD	Focus Group Discussion
MABC	Marker Assisted Back-crossing
MAS	Marker Assisted Selection
MPI	Mean productivity Index
NGOs	Non-Governmental Organizations
PIC	Polymorphic Information Content
PGPB	Plant Growth Promoting Rhizobacteria
PRA	Participatory Rural Appraisal
QACs	Quaternary Ammonium Compound
RAPD	Random Amplified Polymorphism DNA
RARC	Rokupr Agricultural Research Center
RFLP	Restriction Fragment length Polymorphism
RGR	Relative Growth Rate
RIL	Recombinant Inbred Line
ROS	Relative Oxygen Species
RWC	Relative Water Content
SES	Standard Evaluation System
SNP	Single Nucleotide Polymorphism

SSLP	Single Sequence Length Polymorphism
SSR	Simple Sequence Repeat
SSI	Stress Susceptibility Index
STI	Stress Tolerance Index
TOL	Tolerance Index
WARDA	West Africa Rice Development Association
YI	Yield Stability Index

CHAPTER ONE

1.0 GENERAL INTRODUCTION

Rice (*Oryza sativa* L) is the most important staple food crop for more than half of the global population mostly in developing countries (Zhang and Xie, 2014). It occupies 9% of the earth's biosphere and provide 21% and 15% of the world per capita energy and per capita protein respectively (Sabar *et al.*, 2019). Although tremendous efforts have been made to increase world rice production, it demands still surpasses the supply (Sasaki *et al*., 2000; Zhang and Xie, 2014). The population of the world is projected to increase to 2.3 billion people by 2050 (FAO, 2009). Khush (2005), estimated that rice production will have to increase by 40% by the year 2030 to meet global needs. Considerable improvement of grain yield is vital to meet the growing demand for rice. Though rice is adapted to wide range of agro-climatic conditions, its productivity is affected by numerous biotic and abiotic stresses (Zhang & Xie, 2014).

Rice provides about 50-80% of the daily calorie intake, accounting for about three billion people globally (Muthayya *et al.*, 2014). Rice has become the most popular source of food in Africa due to change in consumer preference and simplicity of cooking (Nwanze *et al.,* 2006; Seck *et al.,* 2013), leading to a tremendous increase in cereal consumption in Africa from 9.3% to 15.2% in 1961 and 2007 respectively. Despite this increase, only 54% of local cereal consumption is produced in Africa. Total rice consumption in Africa totals 11.6 million tonnes of milled rice per year, of which 3.3 million tonnes (33.6%) is achieved through importation (FAO, 1998). Rice Utilization in Sub- Saharan Africa has made a tremendous yearly increase of 6%, which is the highest in the world (Africarice, 2010). The rate of increase in the consumption of rice in Africa has not been matched by corresponding increases in production and the demand-supply gap is widening (FAO, 2012). Africa accounts for 36% of global imports in 2015, and presently spend more than 4 billion US$ on rice importation (FAO, 2018). This places a heavy demand on scare foreign currency reserves of countries in the region, which are among the poorest in the world.

In Sierra Leone, the dependency rate on rice is very high, representing 104 kg of total cereal consumption per year (Baggie *et al.,* 2018). Annual rice consumption in the country is estimated at 530,000 metric tons with a total 200,000 metric tons produce each year (FAO, 2004). The yield gap of 350,000 metric tons is achieved only through heavy importation of foreign rice. It is the single largest component of the country's agricultural sector both in terms of the population engaged in its production and its contribution to the total value (FAO, 2004). Arable land under cultivation in Sierra Leone is about 5,400,000 ha, of which mangrove swamps cultivation accounts for 25,000 ha of the total arable land (Agyen-Sampong, 1994). Reports of the Ministry of Agriculture, Forestry and Food Security suggest that mangrove rice account for 12% of the total rice production in the country (PEMSD/MAFFS, 2014). Mangrove ecology is associated by series of environmental stresses such as soil salinity, acidity, flooding from major rivers, mineral toxicity like aluminium, disease, extreme temperature and drought (Giri *et al.,* 2011). Moreover, abiotic stresses are a major cause of yield losses responsible for over 50% reduction in crop yield (Rahman *et al.,* 2019) with salinity causing major losses.

Salinity is a critical abiotic stress devastating rice production in saline soils including the mangrove ecology (Bimpong *et al.,* 2013, Rahman, *et al.,* 2019). Rice is the most salt-sensitive cereal crop having a tolerance limit of 3 dsm-^{1} beyond which yield begin to decline for most cultivated species (Hoang *et al.,* 2015, Rahman *et al.,* 2017). Salt stress can adversely affect seedling establishment, growth rates, tillering, spikelet formation, and cause significant crop loss worldwide (Dahanayaka *et al.,* 2017; Rahman *et al.,* 2019). The coastal rice farming lands are very fertile due to the daily deposit of silt by sea water, but the harmful effect of salinity diminishes the health and fertility of the soil. This results in low rice production, low income for farmers and consequently reduces farm labour employment in the Mangrove area (Gregorio, 1997). Mangrove rice farmers are faced with the dreadful effect of salinity in their rice field, which has led to the reduction in the cultivated area under mangrove. The key constraint encountered by mangrove rice farmers is salinity followed by the continuous use of local/ unimproved and low-yielding materials.

Knowledge of farmer's on local rice varieties / land races is important for breeders to develop strategies to incorporate them into novel crop as well as generation of new varieties and their adoption (Jenkins *et al*., 2019). Most of the farmer's varieties are not uniform and are susceptible to salt stress. It is important therefore to screen for variability among farmers varieties, and to enhance the yield potential of these varieties for saline environment (Boyer, 1982).

Salinity tolerance in rice is controlled by polygenes. Therefore, information on the genetic control regulating the mechanism of salt tolerance is thus important in the process of developing new or improved varieties that are tolerant to salinity. Quantitative trait loci (QTL) map studies on salt-tolerance have the potential to enhance the improvement of plant against salt stress. Therefore, identification of QTL with strong association with salinity tolerance trait would speed up the breeding for salt tolerance through conventional breeding methods (Thomson 2009). Also, such QTLs would be more useful when they are tightly flanked by markers.

Previous work on screening for salinity have been purely based on conventional breeding techniques. Breeding rice varieties for mangrove ecology based on morphological screening alone is not sufficient due to it polygenic nature of inheritance and environmental effects (Parvaiz and Satyawati, 2008; Islam *et al.,* 2011).

The introduction of genomic tools and techniques is rapidly changing the approach to plant breeding. It speeds up the study of the genotype and its association with the phenotype, particularly for traits like salinity which is controlled by polygenes (Rahman *et al*., 2017). Molecular markers have proven to be very efficient in determining the genetic basis underlying salt stress tolerance in rice (Rena *at al.,* 2019). They also enhances the process of identifying QTL with major effects which can speed up breeding process. Single Nucleotide Polymorphism (SNP) is gaining popularity for identification of QTL for salinity and genetic diversity studies in rice (Park *et al.,* 2019; *Rana et al.,* 2019). Several works have been done on salinity related QTLs (Munns, 2005; Rahman *et al*., 2017). Earlier studies have discovered QTLs responsible for rice tolerance in salinity on chromosome 1, 5, 6 and 7 (Zhang *et al*., 1995; Gong *et al*., 1999).

In populations such as the mangrove rice accessions, where no much research has been done before, understanding of secondary traits such as tolerance indices may shed more light on genetic backgrounds of the materials hence contributes towards effective breeding for improvement against salt stress in the mangrove swamps of Sierra Leone.

Therefore, the objectives of the study were to:

1. evaluate production constraints and varietal preferences of farmers involved in rice production in the mangrove ecology in Sierra Leone;
2. characterize the genetic diversity and population structure of mangrove rice landraces;

3. identify and map salinity tolerance QTL using SNP markers; and
4. estimate the relative efficiency of selection for tolerance to salinity in rice.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. Origin, taxonomy and distribution of rice

Rice is a semi-aquatic, monocotyledon plant that is part of the grass family known as *Gramineae* or *Poaceae* (IRRI, 2010). The genus *Oryza* originated over 130 million years ago and was distributed as a wild grass in Gondwanaland (super continent), which was later divided as Asia, Africa, Americas, Australia and Antarctica. Out of the five continents, *Oryza* was not distributed in Antarctica (IRRI, 2010). Different species of rice are believed to have originated on different dates and in geographical areas. For instance, *Oryza sativa* was first cultivated in Southeast Asia, where it is said to have originated from (Civan *et al*., 2019). Oryza barthii is believed to be the wild ancestor of Oryza glaberrima and was domesticated by natives along the Niger River in Africa some 3000 years ago.

The genus *Oryza* is made up of about twenty-four (24) species, of which only two (*O. sativa* and *O. glaberrima*) are cultivated for human consumption (Esa *et al*., 2013). Of the 24 *Oryza* species, nine wild types are tetraploid, whereas the other 15 species including the two cultivated forms are diploid (Khush, 1997). Among the wild species, only *O. rufipogon* produces fertile F_1 with *O. sativa*, and as such is considered as to share ancestry with *O. sativa*, whereas *O. barthii* shares ancestry with *O. glaberrima* (Asaf *et al*., 2017). The two cultivated species are a descendant of a common ancestor that has an AA genome and is believed to be a classical illustration of parallel evolution (Wambugu *et al*., 2013). *Oryza sativa* has a superior yield and milling quality and cultivated across the globe except Antarctica, whereas *Oryza glaberrima* is more adapted to

African but grown on a relatively smaller scale in West Africa (Khush and Virk, 2000; Esa *et al*., 2013).

Wei and Hung (2019) classified *Oryza* into ten genome types including the AA, BB, CC, BBCC, CCDD, EE, FF, GG, HHJJ and HHKK genomes with *O. sativa*, *O. glaberrima* and six wild rice species belonging to the AA genome. Based on the chromosome count, *Oryza* is divided into four main sections (categories): japonica, indica, aromatic and glutinous (Asaf *et al*., 2017). *Oryza sativa* has been classified into indica and japonica subspecies based on its appearance and composition, with the genome measuring 430 mb across the 12 chromosomes (Goff *et al*., 2002). The japonica also known as Sinica variety is a "sticky and short-grained rice", whereas, indica is a long-grained, "non-sticky" with reduced amylopectin rice. Moreover, Japonica varieties possess shorter plants, leaf shape, lighter leaf color, strong cold tolerance, strong lodging resistance, are non-shattering, lower tiller number, slower germinating rate, more sensitive to rice blast, longer and denser glume pubescence and longer awns compared to indica varieties (Matsuo *et al.*, 1997; Feng *et al*., 2017).

2.2. Rice production and utilization

In 2017, the quantity of husked rice produced globally was above 482 million metric tons (Chauhan *et al*., 2017). The Asian countries are the highest producers of rice (Milovanovic and Smutka, 2017), accounting for 90% of the rice produced and consumed in the world (Hoang *et al*., 2015). China is the highest producer of paddy rice in the world, producing over 210 million metric tons in 2017 (Alldrick, 2017). Rice is gradually becoming a primary cereal food in Africa, but the continent accounts for only 3% of the total quantity of rice produced worldwide. Rice production in Africa increased from 9% in 1961 to 15% in 2007. In Africa, land area under rice production in 2003 was estimated at 10.23 million hectares with a total production of paddy rice projected at 15.08 million tons. This accounted for 6.11% and 3.3% of the world rice area and production, respectively.

The consumption of rice has experienced an increasing demand as a result of growing population in the world (Neumann *et al*., 2010). The total worldwide demand for milled rice was estimated at 477 million metric tons between the period of 2016 and 2017 (Shahbandeh, 2017), which increased to 503.9 million tons (1.05%) from 2017 to 2018 (FAO, 2018), and 509.1 million metric tons (1.07%) between 2018 and 2019 (FAO, 2019).

Rice has become the most popular source of energy intake in Africa (Nwanze *et al*., 2006). Countries in Sub Sahara Africa (SSA) account for total consumption of rice. It is projected that the total consumption of rice in SSA would increase from 27-28 million tons recorded presently to account 36 million tons in the year 2026 (USDA, 2017). A huge increase in the total rice consumption in Africa has been recorded from 16 metric tons in 2000 to 29 metric tons in 2006 and 12 metric tons in SSA has be reported (Seck *et al*., 2013). The rate of increase in the consumption of rice in Africa has not been matched by the corresponding increases in production and the demand /supply gap is widening. Africa imports 13.9 million tons of rice in 2016 (FAO, 2017). In Sierra Leone, rice, as the staple food, is the ultimate source of food for most of the population and is the highest employer (80%) of the labour force. The average per capita consumption of rice is over 100 kg, providing 50-80% of calories (FAO, 2004).

Rice is cooked by boiling, or it can be ground into a flour. It is eaten by itself and in a great variety of soups, side dishes and main dishes in Asia, middle eastern and many other countries. In West Africa, rice is mostly eaten as a main dish with cooked vegetables, stew, and soup and the grounded flour used for pastries, porridge, etc. Rice flour is used as an alternative to wheat flour in gluten-free baked goods. It is high in fiber, gluten-free, and low in saturated fat, cholesterol, and sodium.

Rice straw, rice husk, rice bran, rice germ and broken rice are the main rice by-products in the rice industry. Rice by-products may serve as important sources of raw material that could be used as ingredients of functional food and nutraceuticals. They have great potential to be converted into human food to improve food security in the country.

The bran and rice polish are also sometimes used as livestock feed; the bran is used in oil processing for both food and industrial utilization. The bran is also known to improve the quality of food, especially color, appearance, taste, and texture (Sharif *et al*., 2014). Many studies have also reported that rice bran also has the potential to prevent a range of chronic disease (Kin *et al*., 2012; Esa *et al*., 2013; Gul *et al*., 2015).

Broken rice is used in brewing, distilling and in the manufacture of starch and rice flour. Rice hulls are used as fuel, in the manufacturing of fertilizer, and an industrial chemical called furfural and packing materials. The straw is also used as feed, livestock bedding, roof thatching, garments, broom straw and mats. Studies have shown that rice bran, RBO, rice husk, and broken rice have potential health, animal, and alternative food uses and can be produced sustainably as they are considered renewables (De Godoy *et al*., 2013; Esa *et al*., 2013).

2. 3 Mangrove rice cultivation in West Africa

Mangrove rice holds a strategic position in the total regional rice production in West Africa and the mangrove swamps serve as one of the richest rice-growing ecologies in terms of available nutrients (Spaldling *et al*., 2010). Nineteen countries in West Africa are said to have mangrove swamps, accounting for 2.4 million hectares of mangrove in West African countries (Adefurin, and Zwart, 2016), and more areas are being brought under cultivation. Mangrove swamp rice accounts for about 10% of the total regional production. Aygen-Sampong (1982) reported that over 150,000 ha of the mangroves are still under-utilized in Sierra Leone, up to Senegal, and about 800,000 ha in Nigeria.

Mangrove rice cultivation is a system found within the tidal wetlands of the mangrove ecosystem which accounts for main system of rice production in West Africa (Adefurin and Zwart, 2016). This system contributes to food security in some West African countries (Ayegn-Sampong, 1984). Soils in the mangrove region are relatively more fertile compared to other rice ecologies like the upland, Inland valley swamps, irrigated, and other because of continuous deposits of silt from flooding. The fertility status is also retained from nutrient rich sediment that are carried into the land by sea water during the dry season. (Zinnah, 1993).

Sierra Leone is among the West African countries where mangrove rice cultivation is playing a significant role in food security (Agyen-Sampong, 1982). Mangrove swamp rice production is practiced in three districts (Kambia, Port Loko and Moyamba) and contributes about 12% of the total rice cultivated in the country (Gborie *et al*., 2014). Rice cultivation in mangrove swamps is controlled by natural mechanism and the soil is enriched through the daily deposit of silt from the ocean during the dry season and microbial activities within the root zone. It is highly dependent on rainfall to supply fresh water in the swamps, hence leaching out the excess salt concentration to non-harmful levels (Baggie *et al*., 2018).

Mangrove swamps are classified into two distinct categories base on the salt-free duration and the flooding types (Baggie *et al*., 2018). These include the tidal mangrove swamps as well the associated mangrove swamps. Associated mangroves are found between the tidal swamps and the uplands and tidal mangroves experience tidal flooding throughout the year (Aygen-Sampong, 1982). The tidal mangroves are further divided into three main groups based on their closeness to the Atlantic Ocean and duration of salt free period. Group one comprises of mangrove swamps which are characterized by salt-free period of less than four months. To escape salt stress, only early maturing varieties (< 4 months) are suitable for cultivation in these locations. Group two have a salt-free period of four to six months, which indicate that medium duration rice varieties (4-6 months) are suitable for cultivation in such areas. Areas under category three have the longest salt-free period of more than six months which allows the cultivation of long duration mangrove varieties (Baggie *et al*., 2018).

Planting season for mangrove rice farmers starts as early as April-August, starting from land preparation and transplanting, and harvesting is normally from October to January. Manual land preparation using hand hoes are predominant in mangrove swamp rice cultivation with no modern inputs applied (Baggie *et al*., 2018). Local farmers involved in mangrove swamp farming plant over-grown seedlings about eight to ten weeks, using more seedling per hill (6-8) as compared to most lowland ecologies. This is a special indigenous knowledge of farmers to combat crabs damage in their fields. The average yield of 2.5 tons/ha is relatively high compared to 1.5 tons/ha in other lowland ecologies. Diseases, weeds and pests do not cause severe damage to crops in the mangrove swamps which enhance higher rice yields than in other agro-ecologies in Sierra Leone (Agyen-Sampong, 1994). The soils in the mangrove swamps are mostly acid sulphate soils and the dominant grass species is *Paspalum vaginatum* SW locally called kerekere (Agyen- Sampong *et al*., 1988).

2.4 Phenotypic and genotypic characterization of rice cultivar

2.4.1 Morphological Characterization

Assessment of germplasm collections is important to ensure the basis of conserving and proper use of germplasm which provide a good platform to characterised morphological traits of rice (Riley *et al*., 1995). Traits like leaf width, seed length, one hundred grain weight, awing, secondary branching, panicle type, leaf pubescence have been used as morphological markers in distinguishing parental lines (Hartati *et al*., 2019).

Morphological characters can be described as those characters that can easily be seen and identified; this enhances the rapid evaluation of such variation compared with other characters. Characterization can provide essential data about the variation and taxonomy that can show the level and relationship between genotypes used for plant breeding (Nandariyah, 2010).

2.4.2 Genetic divergence in rice germplasm

Genetic diversity generally refers to the amount of genotypic (at the DNA level) variability present in a group of individuals. Every individual species has genes which are responsible for its own distinctive features like days to flowering, number of panicles per plant, plant height, to name but a few (Davi *et al*., 2019).

The scope of distinctiveness in separate generation dangles on the inherent mixture of the parents (Archana *et al*., 2018). Parents that are hereditarily different are most likely to result into heterotic effects and appropriate population (Archana *et al*., 2018). Knowledge on the type of genetic divergence would guide the plant breeder in selecting the ideal genotypes for breeding programmes (Parikh *et al*., 2011). Genetic diversity is one of the main factors that can modify an effective breeding programme for any crop (Archana *at al*., 2018). Hybridization and selection success depend largely on the selection of lines with high genetic dissimilarity (Devi *et al*., 2019). With the aid of molecular markers, genetic diversity could be easily verified thereby assisting in the selection of genetically distinguished parents to obtain desirable combination in the segregating generation (Banumathy *et al*., 2010). Therefore, good information about the genetic variation in germplasm accession and genetic associations among germplasm are crucial consideration in designing an effective breeding programme.

The application of the result of genetic diversity base on molecular data gives repetitively significant result compared to phenotypic data because phenotypic information based on morphological attributes is highly influence by environment.

Many studies on morphological characterization of rice have been carried out, and one of such study is by Qualsat and Shands (2005) who reported that characterization is required for the management of varied heritable reserves of plants to advance the nutritional value of foods to meet changing consumers preference, withstand pest and diseases and adapt to climatic changes.

Archana *et al*. (2018), also evaluated 38 diverse rice genotypes from different origin and geographical origin for 15 yield contributing and nutritional traits and group genotypes into 8 major groups. The genotypes exhibited higher genetic distance with most of the other cluster for the trait under study.

In addition, study by Rao *et al*. (2013), uses distinctiveness, uniformity and stability test (DUS) descriptor to characterized 65 rice landraces using 43 agro-morphological traits. Among the 65 genotypes involved in the study, 32 were distinct base on 22 important and 24 extra traits. This implies the relevance of this study to breeders, to identify relevant germplasm to ensure preservation of useful genes for crop improvement.

2.5. Salinity-stress in rice

Salinity refers to the presence of high concentrations of sodium chloride, magnesium and calcium sulphates and bicarbonates in water and soil (Hoang *et al*., 2014). Salinity is among major production constraints in the world affecting over 830 million ha of land worldwide (Munns, 2006) and about two million ha of arable land annually become excessively salt-stress (Umali, 1993). Salinity is developed naturally. However, the process of salinization has been expedited through human disturbance of the natural ecosystems, leading to significant changes to the hydrology of the landscape, waterways and land. The two major human activities that have expedited salinity are irrigation and extensive deforestation. There are about 200 million hectares of irrigated land globally of which, 45 million hectares (20%) has turned saline (Munns, 2006). Crops growing in salt-stress environments compete for water and implore coping strategies that mitigate ion toxicities, nutrient deficiency and marginal soil physical conditions to thrive (Shrivastava *et al*., 2016).

The effect of salinity on rice is highest among cereal crops having a maximum tolerant limit of 3 dsm-^{1} for several widely cultivated genotypes (USDA, 2016), which is less than the normal ECe (electrical conductivity of its saturation extract) threshold of more than 4 dsm-^{1} (Rengasamy, 2006). Ten percent yield loss has been reported in rice for ECe above 3.5 dsm-^{1} and 50% at ECe at 7.2 dsm-^{1} (Umali, 1993).

2.5.1. Causes of salinity-stress in rice

Salt stress in rice arises from different sources. Salinity primarily occurs when insoluble aluminosilicates, and other mineral compounds like sodium and chlorides salts deposited as components of soil. The basic contributors to salt stress in the soil includes weathering of different insoluble minerals producing anions and cations that take part in primary source of salt stress in rice (Riaz *et al*., 2019).

The other causes of salinity in rice are known as secondary sources. Irrigation of farmlands with salty water is among the secondary sources of salinity. The magnitude of salinity depends on the irrigation water (frequency, type, quantity and salt levels), crop rotation, type of soil and climate. Although water having ECe$\leq$1 dsm-^{1} $^{-}$is ideal for irrigation, irrigating a 4-acre land area with water of ECe dsm-^{1} adds approximately 260 to 300 kg of salts to the soil dependent upon the type of soluble salts present (Razzaque *et al*., 2019).

Salinity is also caused by coastal sea water in coastal areas with ECe reaching up to 50 dsm-^{1}. The salt accumulates through elevated tidal flooding, entry from creeks and rivers and influxes of underground water and salt loaded aerosols (Riaz *et al*., 2019). Salt-stress in rice is also caused by fossil salinity that develops through salt precipitation from sea water. These salts are exposed through excavation of canals or pumping of irrigation water from salty boreholes (Riaz *et al*., 2019). Saline stress in rice can also be attain through minor sources such as early rains after a prolonged dry spell, organic (farmyard manure, poultry manure, sewage sludge and industrial waste) and inorganic fertilizers (Razzaq *et al*., 2020).

2.5.2. Effects of salinity-stress in rice

Salt stress is a significant production constraint that adversely affects crop productivity processes such as seed germination, growth and reproductive aspects that culminate into the economic yield worldwide (Munns and Tester, 2008). About seven, twenty, and fifty, percent land area, cultivated and irrigated lands respectively are under different levels of salt-stress (Rahman *et al*., 2017). Crop yield decreases with accumulation of salts and the extent of it impact depends on the salt concentration, ecological factors, species, genotypes, and developmental stages of plant (Torabi, 2014). Salinity can have harmful effects on the plants by causing oxidative stress, water stress, nutritional imbalance reduction in cell division to name but a few (Zhu, 2007).

Most cereal widely differ in their responses to salinity stress. Rice is the least tolerant among cereal crops, compared to wheat and barley that have higher tolerance levels. Genotypes that are tolerant or sensitive to salt vary in their capacity to store excess salts in their organs, whereas salt-sensitive genotypes cannot transport salts to their vacuoles leading to rapid accumulation in the cytoplasm therefore reducing photosynthesis and assimilation rate (Munns and Tester, 2008). Reactions of plants to salt stress are stimulated by the reduction in the osmotic potential and high ion accumulation (Rahman *et al*., 2000).

Osmotic stress and Ion toxicity stress are the two-phase concept that plants undergo during salinity stress. During osmotic stress, plant growth drastically reduces as a consequence of changes in the osmotic potential within the soil and plant cells, thereby reducing the leaf growth and development (Munns, 2005). During ion toxicity, the salt accumulates and translocate inside the plant which may take several days, weeks or sometimes month which rely on the level of salinity within the root zone leading to leaf senescence (Munns, 2005; Lauchli and Grattan, 2007). Effects of salt stress in rice are also evident in reduce growth resulting from changes in biochemical and physiological features leading to reduction in the total biomass produce as well as grain yield loss (Ghosh *et al*., 2016).

Physiological influences of salt stress on rice include decreased photosynthetic rate, variations in in the sodium to Potassium ion ratio, reduces conductance within the stomata which intensify the synthesis of reactive oxygen species (ROS). Moradi *et al*. (2007) investigated how salt stress affects the performance on three rice genotypes having different levels of stress susceptibility at different growth stages under controlled conditions. The authors found a significant reduction in the photosynthetic carbon dioxide fixation and assimilation, as well as stomatal conductance. Salt tolerant genotypes are more adaptable to salt-stress because they close their stomata within short periods of exposure and have the ability to partially recover following a short period of acclimatization (Moradi *et al*., 2007).

2.6. Rice response mechanism against saline stress

Salinity is a complex mechanism that has remarkable effects on physical and biological pathways in plants (Nabati *et al*., 2011), and has been described as a dehydration process induced by salt stress. Knowledge of different morphological and physiological component traits aids in breeding for salt tolerance stress which affirms previous findings that resistance to salt stress relatively depends on many trait (Abbasi *et al*., 2016). Traits like fertility, panicle length, grain filling, number of spikelets per panicle, tiller number, are affected by salt build up in the plant resulting to significant decrease in yields (Kakar *et al*., 2019). Salt shows undesirable effects on numerous morphological and physiological plant traits (Abbasi *et al.*, 2016). Furthermore, accumulation of Na^+ occurs in the leaves and reduces plant growth (Rahman *et al*., 2019). Osmotic effects distress plants by lowering the osmotic potentiality accompanied by Subsequent ionic effect introducing ion toxicity (Ghosh *et al*., 2016). Report by Rahman, *et al*. (2000), stated that mitochondria and chloroplasts (such as Chlorophyll content) are the most salt stress organs compared to others. Existing variability between chlorophyll fluorescence and accessibility of membranes are efficient and possible lead way in how salinity can decrease the effectiveness of photosynthesis (Ghosh *et al*., 2016).

Plants are immobile; and therefore, cannot mitigate salt stress by moving from one location to another, nature has gifted them with different mechanism of salt tolerance. Salinity tolerance in rice is a prerequisite of the Na^+ to K^+ ratio. Plants alleviate the toxic effect of accumulated sodium salt via the following mechanism (a) salt exclusion (Singh *et al.*, 2008), (b) selective ion uptake (Ghosh *et al*., 2016) and (c) regulation of K^+/Na^+ ratio (Abbasi *et al*., 2016). Among all macro-nutrient, potassium (K^+) plays a key role in the survival of plant under salinity conditions (Abbasi *et al*., 2016).

Investigating rice response under salinity gives a better idea of correlations between the physiological and tolerance mechanisms of the plant activated during stress. A good background of salt tolerance indices in testing local varieties at morpho-physiological level has been cited by Ali *et al*. (2014). In their study, they identified landraces as important source for discovery of new genes for tolerance.

Morpho-physiological evaluation of different cultivars gave a clear understanding of the level of variability to salt stress among cultivars by estimating parameters like panicle length, leaf area, biomass, root length, relative water content (RWC) and Relative Growth Rate (RGR) (Zafar *et al*., 2015). Yancey *et al*. (1982) detected a constant rise in content of leaf RWC in paddy under salt stress and proposed that osmo-protectants prevent cell injury.

Many studies have reported on the existence of genetic variability in salinity tolerance among species in plant response to stress (Akbar *et al*., 1972; Ikehashi and Ponnamperuma, 1978; Bonilla *et al*., 2002). Molecular marker techniques have been used in various studies using several genotypes in salinity tolerance screening. Akbar *et al*. (1972) exploited the RFLP and SSLP markers and genotyped rice landraces for morpho-molecular traits. Separate studies by and Rikke and Johnson (1998) and Mansuri *et al*. (2011) used SSR and RAPD to analyzed diverse rice germplasm.

The nature of inheritance of salinity tolerance in rice is a very complicated paradox, hence several investigations including conventional techniques have been conducted to explain this complexity. Identification of several genes responsible for tolerance such as saltol in rice have been found (Bimpong *et al*., 2013). The ability of plant to fight salt stress is introduced by plant's ability to recognize the osmotic stress. Modification of response in the tissue or cellular level was made explicit by physiological mechanism of the plant measured phenotypically and quantifying the Na^+/K^+ ratio (Ghosh *et al.*, 2016). Thus, control at the genetic level and the regulatory pathway exclusively formed the mainstay behind the understanding of the induced signaling pathway in response to this stress.

2.7. Breeding for salinity tolerance in rice

Salinity could reduce the quantity and quality of plant yield (Bahmani *et al*., 2015). Enhancing yield under salt stress represent a core objective of plant breeding (Hoang *et al.,* 2015), and the procedure employed in the screening is equally very vital. Two major approaches have been prescribed in dealing with salt related problems in the soil which includes 1) altering the growing environment to a suitable one for the normal growth of plants or 2) changing the genetic architecture of the plant to suit the growing environment. The first approach requires major alteration in soil amendment process which could be very expensive for poor resource farmers (IRRI, 2006). The latter approach which seem more visible and promising involves the introgression of tolerance genes into crop varieties. This method is less expensive and a socially acceptable approach. Yield enhancement in plants experiencing salt stress is a vital objective of plant breeding. A successful breeding program requires a reliable salinity screening method (Zeng *et al*., 2002). Both agronomical and physiological parameters have been used as relevant selection benchmarks for salinity tolerance screening in rice (Ashraf, 2004; Ismail, 2007; Hoang *et al*., 2015)

2.7.1. Breeding for salinity tolerance using convention methods.

Application of conventional breeding techniques has been utilized in the improvement of rice populations for salt tolerance through two steps (Breseghello and Coelho, 2013). The first step involves generation of useful variability among breeding population for salt tolerance. The second step involves selection of elite progenies with desired salt tolerance and other useful traits recombined from their parents from the segregating progenies. Two critical factors are often considered for determination of tolerance to salinity which includes threshold of salinity tolerance, and its slope. The acceptable threshold for rice has been pegged at 3.0 dSm-^{1}; at this level, no yield reduction occurs. The slope explains the percent reduction in yield per unit increment in salinity level above the threshold and is measured as 12% per dSm-^{1} (Reddy *et al.*, 2014). The response of rice to salinity depends on several factors which include developmental stage, type and content of salt (Ismail *et al.*, 2007).

Introduction, hybridization and mutagenesis have been utilized to generate highly variable salt tolerant genotypes of rice and identify those with improved salt tolerance (Wang *et al.*, 2013). Despite these successes, the conventional breeding technique is fraught with several constraints. These include the difficulty of establishing the physiological traits that can distinctively describe appropriate salt tolerant genotypes considering such traits to be influenced by many QTLs with many genes that display continuous variation (Fita *et al.*, 2015). Moreover, the limited sources of parents for crossing also contribute to limited successes in rice improvement for salt stress tolerance. The suggestion of utilizing physical criteria to screen towards salinity tolerance in rice was accepted by many rice breeders (Yeo, 1994). This was aimed at increasing salt tolerance above the phenotypic magnitude, hence choosing genotypes based on better physiological traits which enhance in conferring salinity tolerance (Lee *et al.*, 2003). However, the physiological technique has not led to the development and release of any salt tolerant genotypes. Yield loss under salt-stress in rice is considered a more reliable and efficient technique of measuring salt tolerance in test environments than using the physiological traits (Li and Xu, 2007).

Susceptibility levels for salts stress varies from one genotype to another, hence a major restraint in using conventional technique in breeding tolerant genotypes for different growth stages. Seed germination and maximum tillering have been identified as periods when rice tolerates salts; on the other hand, rice exhibits high sensitivity to increase level of salt in it growing environment.

A weak correlation exists between early seedling and reproductive stage salinity tolerance and the two are said to be independent of each other (Jena and Mackill. 2008). Therefore, it is paramount in salinity screening to evaluate genotypes at (i) the seedling stage which implies rapid screening of genotypes in controlled conditions, and (ii) reproductive stage involving the testing for salinity tolerance of promising lines under filed environments (Li and Xu, 2007). Rice gene pool with good salinity tolerance often exhibit low agronomic traits potentials like low yield, tall plant stature, and poor grain quality (Reddy *et al*., 2014) and are used as potential salinity tolerant donor parents in salinity tolerance population improvement studies (Waziri *et al*., 2016). Comparing the relative tolerance of seven wild rice and the popular cultivated species, *O. sativa* and *O. glaberrima* (Akbar *et al*., 1987) observed that the rice variety Nona Bokra (*O. sativa)* are more salt tolerant than the wild types.

Indica rice displays salinity tolerance compared to its Japonica counterpart. Indica rice genotypes that are tolerant to salinity are suitable in eliminating Na+, absorber of high amounts of K+, and maintainer of lesser Na+/K+ ratio in the shoot (Lee et al., 2003). An exceptional genotype FL478 derived from the pedigree Pokkali x IR 29, possesses good seedling stage salinity tolerance, photoperiod insensitive, matures earlier, early flowering, lower Na+/K+ ratio, tillers well and maintains maximum K+, than its parents (Suriya-Arunroj et al., 2004).

2.8. Stability tolerance indices in salt-stress rice

Salinity stress studies involve estimation of different indices of traits in the genotypes studied at different stress conditions (Girma *et al*., 2017). These salinity indices include tolerance indices (Tol) (Senuttuvel *et al*., 2016), mean productivity index (MPI) geometric mean productivity index (GMP) stress tolerance index (STI) (Fernadez, 1992), stress susceptibility index (SSI) yield index (YI) (Lin *et al*., 1986) and yield stability index (YSI). Girma *et al*. (2017) reported stability tolerance indices for final growth stage parameters of different salt-stress genotypes of rice (*Oryza sativa* L.). About 21-60% reduction in plant height was noted in salt tolerant genotypes grown at 12 dSm^{-1}, whereas some susceptible genotypes incurred up to 100% reduction in plant height (Girma *et al*., 2017). Salt susceptible genotypes IR 29, IR 59418, IR 72593, IR 73055 and NERICA 4 were noted to exhibit 100% reduction in number of tillers per plant at 12 dSm^{-1}. Salinity stress reduced panicle length by 23% in tolerant genotypes IR 70023 and IR 71810 (Girma *et al*., 2017). Several other researchers has attributed the reduction in panicle length to stunted growth and the reduction in the seedling survival rates (Mahmood *et al*., 2009; Rad *et al*., 2012; Hakim *et al*., 2014).

The stress tolerance indices (STI) measure the stability of genotypes across different environments. Genotypes exhibiting higher STI possess higher grain yield stability across different environments. According to Fernadez (1992), genotypes with higher GMP are considered tolerant and high yielding. Khan and Kabir (2014), also reported that YSI and YI values are useful for the discrimination of tolerant genotypes in stress conditions, where high value in a genotype indicates its tolerance. Dawit (2010) reported high and significantly positive correlations among MP, GMP and STI for grain yield of rice in stress and non-stress environments. Khan and Kabir (2014) found significant and positive correlations among MPI, GMP and STI in grain yield of heat-stress bread wheat. Girma *et al*. (2017) noted high, significant and positive correlations among YI, YSI, YSI and SSI in grain yield of rice. Strong and significantly positive correlations were also reported for MPI, GMP and STI (Girma *et al*., 2017). These information depict that the indices serve as good indicators for identification of high yielding genotypes in saline and non-saline environments.

2.9 QTL identification and mapping for rice traits

QTLs refers to genes or genomic region responsible for the variation of quantitatively inherited trait (Martinez and Bouza, 2016). "Any chromosomal region associated with a quantitative trait (QT) and a marker is refer to as a QTL (Pervez and Rather, 2007)". QTL mapping includes the combination of linkage mapping and conventional statistical and quantitative genetics approaches using parameters such as variance to elaborate quantitative traits at individual gene level in order to draw conclusion at population level. Two basic approaches are important in mapping QTL, firstly is detecting the QTL and secondly, locating the detected QTL (Pervez and Rather, 2007). Sax (1961) was the first to revealed the concept of detecting QTLs, and Thoday proposed the first statistical method used in QTL studies. Molecular makers may be a promising tool used to distinguish genes and genomic region underlying traits under polygenic control. Therefore, when mapping QTL is important to take into consideration the following, detection and locating a QTL, analyzing certain fundamental aspect such as number, nature of gene action and its effect, epistasis, pleiotropy and Environmental interaction.

The QTL mapping plays a crucial role in marker-assisted breeding (Bimpong *et al*., 2013). Marker-assisted breeding is used in upgrading plant traits, which include; taste improvement, insect resistance, disease resistance, water use efficiency as well as mineral content efficiency (Mehta *et al*., 2019). There is also an increase in the available resources for developing novel marker from DNA sequenced data which result from the sequenced rice genome as well as improved research in functional genomics (Akhtar *et al*., 2010). Many researchers have used SSR in linkage map construction and QTL mapping (Bimpong *et al*., 2013). Nevertheless, SNPs are substantially close and provide a perfect system that can aide in linkage mapping and QTL identification (Shabir *et al*., 2017).

McCouch *et al.* (2010) developed a SNP assay for rice and are commonly being used. The use of SNP markers is becoming a popular and powerful tool for many genetic application. This is because of its low assay cost, high genomic abundance, locus-specificity, co-dominant inheritance, simple documentation, potential for high throughput analysis and relatively low genotyping error rates (McCouch *et al*., 2013). SNP markers developed from a DNA sequence of known gene are abundantly available for use in rice. (McCouch *et al*., 2010).

Advances in QTL identification for biotic and abiotic stresses accompany with yield components of rice in different ecologies has been carried out by Li *et al* 1(995)

2.10 Genetic evaluation for features under salinization at sprouting and maturity stage

Both dominance and additive effects influence the inheritance of the character (Parade and Jha, 2010). Characters such as seed grain yield per plant and plant height exhibit increase adaptive values. These values explain the relevance of the additive gene in acquiring salt-tolerance accessions.

The average of the dominance is used to make suggestions on partial domination of some of the genes which have significant impacts on the plant height, over control of yield per plant, seed set percentage, tillers, as well as the sodium content (Parida & Jha, 2010). It also gives a suggestion of the slight over dominance for calcium and partial domination of genes that influences the plant height.

Two factors must be considered in developing salt-resistant rice varieties, these includes determination of the donors selected based on leaf injury symptoms as well as the seedling survival ability (Ghosh and Gantait, 2016). However, the criteria used may either hold or fail during subsequent developmental stages of the plant. In such situations, diallel cross analysis helps to understand the genetic basis that influences salt tolerance (Ghosh and Gantait, 2016). Research on genetic components both at maturity and seedling stage shows that dominance and additive effects play a significant responsibility in the inheritance of the characters studied (Ogunbayo *et al.*, 2014). For instance, at the shooting stage of development, specific aspects such as roots and shoots revealed a large scale of improvement effects.

It has been revealed through research that the heritability characteristics during seed sprouting and plant maturity are usually high (Mohammadi *et al.*, 2014). This is because several environmental factors highly modify salt tolerance. These characteristics thus appear to have a high value of predictability and may, therefore, be used as a marker in selecting lines that are tolerant at the sprouting stage. Furthermore, the selection of such trait can also be made in the early generation due to the high additive effects that influence inheritance (Ogunbayo *et al*., 2014). However, the selection based on specific aspects, for example, sodium and calcium content, as well as the root length, have little proof of effectiveness. This is because these characters show low heritability values and the prevalence of the leading gene action. Research has revealed that at least three genes play a part in the inheritance of sodium and calcium contents in the shoot at the seedling stage (Saade and Maurer, 2018). Resistance to the dominant character are usually caused by panicle sterility, the resistance can, however, be maintained by at least four sets of genes (Sankar *et al*., 2012).

Also, traits such as plant height and total grain yield per plant exhibit significant indication of additive effects at maturity. Moreover, the heritability values for the characters also increased (Mohammadi *et al*., 2014). These values are ranging from 65% to 74%. Low heritability and high dominance effects for productive tillers and seed set suggest that selection of elite rice genotypes should be postponed to the later generations that exhibit dissolved dominance effect. On the other hand, the high additive effects for the yields show that productive genotypes can be grown in saline environments and conditions (Saade & Maurer, 2018).

CHAPTER THREE

3.0 FARMERS' VARIETAL PREFERENCES AND PRODUCTION CONSTRAINTS IN THE MANGROVE SWAMPS OF SIERRA LEONE

3.1 Introduction

Rice stands as the main staple crop in Sierra Leone and provides 80% of its caloric intake. A steady increase in rice cultivation has occurred over the years, but not sufficient to meet basic requirement of demand, hence a deficit in the country's rice supply chain. Consumption of rice, however, exceeds production in the country. Annual rice production and consumption is estimated at 712,092 metric tonnes and 1,094 million metric tonnes respectively (USDA).

Mangrove swamp also known as tidal swamp, is among ecologies supporting rice cultivation in the country, accounting for 12% of the total annual rice production with an average of 2 t/h as compared to other ecologies (Gborie *et al*., 2016). There is a tremendous increase in demand mangrove rice in the rice market due to shifting consumer taste and preference.

Mangrove swamps in Sierra Leone are laid out along the Atlantic belt approximately 2 to 8 km wide from Mattru Jong in the South to Rokupr in the Northwest. Soils are mostly developed within salt or saline water ecosystem from fresh marine and estuarine mud. These swamps are usually poorly drained and most of them are under water-logged conditions throughout the year and are generally highly acidic (Agyen-Sampong, 1994). It can serve as a major potential ecology to increase rice production, contributing to the national rice self-sufficiency agenda of the country. Despite its high fertility, the mangrove swamp is enshrined with biotic and abiotic factors affecting all round rice cultivation (Baggie *et al*., 2018). Salinity is one of the major abiotic stresses affecting rice cultivation in the area. Enhancing rice cultivation in that ecology can only be effective by breeding varieties that can withstand the salt, while taking into account farmers' preferences and constraints.

Previously, the focus of plant breeders was on developing high-yielding and improved crop varieties at their own conducive and controlled situations at their experimental sites (Banziger and Cooper, 2001). Farmers preference and specific traits attribute where mostly neglected by breeders, and the prevailing environment of small-scale farmers in developing crop verities adaptable to their ecologies (Ceccarelli *et al*., 2000). This lack of incorporating farmers constraint have been identified as the primary reason for the very low adoption of newly developed and improved crop cultivars and their production packages released by scientists, governments and NGOs (Adesina and Baidu-Forson, 1995 Placide *et al*., 2015).

Therefore, for any successful breeding programmes, it is of great importance to take into account the information on existing production system and farmers' preferences. This might help researchers to identify the true problems of the farmers and thus allow their involvement in the development process. Inclusion of farmers and other stakeholders in the improvement of existing cultivars and selection of new varieties is a very crucial factor to be considered in any plant breeding system (Balde *et al.*, 2014).

Use of participatory approaches into conventional breeding programmes has the ability to reduce the timeframe for cultivar development from nine years to six years (Ashby and Lilja; 2004). Rice varieties developed through farmers' involvement have a greater chance of adoption and diffusion by farmers because they are developed to address farmer's constraints and preferences

(Balde *et al., 2014*). Research by Ceccarelli *et al*. (2009), have reported that local farmers have the same ability as breeders in terms of selecting high yielding cultivars. Therefore, a good knowledge of farmer's preferences, production constraints and farming system will enhance in developing appropriate interventions in helping smallholder rice farmers and selecting their target priorities (Mburu *et al.*, 2007).

There is limited information in Sierra Leone on the mangrove rice production constraints and farmers' preferred rice varietal traits. This makes a study in this area very necessary because every successful breeding should be based on distinct identification of farmers' constraints and preferences of end users. Farmers are mostly reluctant to accept technologies which are not in line with their preference and consumer expectation (Placide *et al.,* 2015). Participatory Rural Appraisal (PRA) allows the inclusion of farmers in research decision making, in planning the generation of new technologies, and also serve as a non-formal approach to collect detailed data as well as other relevant information of a system (Uddin, 2013). In this study, PRA is used as a platform that enhances interaction between farmers and researchers as a more effective way to get a detailed insight into the farming system in that ecology.

Hence, the objective of study is to:

1. identify production constraints, farmers varietal preferences and selection criteria for future varietal development;
2. assess the current status of salinity problems in the mangrove ecology; and
3. identify predominant salt mitigation strategies used by farmers.

3.2 Methodology

Focus group discussions (FGD) and semi structured questionnaire were used in this study.

3.2.1. Study area

Kambia, and Port Loko district from the Northern Province and Moyamba district in the Southern province were the study areas (Fig. 1).

These districts were purposively selected based on the availability and importance of mangrove rice production in Sierra Leone. Further, one chiefdom in each of the three districts was purposively selected to avoid bias against Moyamba district, which has only one chiefdom (Ribbi) involved in mangrove rice production. Hence, the three chiefdoms selected were Ribbi (Moyamba), Lokomasama (Port Loko), and Mambolo (Kambia). This study was conducted during the raining season in 2017.

3.2.2 Sampling procedures for FGD participants

For the focus group discussions, farmers were selected by their local leaders and agronomist in each village. Ten to fifteen farmers constituted the FGD, and the groups were classified into, adult men, adult women and youths with a total of forty-five FGDs. Participants for group discussions were selected to represent the spectrum of individual farmers and farmers from various cooperative associations of the villages, taking into account gender balance.

3.2.3 Sampling procedure for Semi-structured Questionnaire Respondent

A multistage sampling technique was used for the study. One chiefdom in each district was sampled. In each chiefdom, five villages were randomly sub-sampled and 16 farmers were randomly selected in each village for the individual questionnaire survey from the list of farmers provided by community leaders. This provided a total of 240 farmers for semi-structured interviews. Pretesting of the questionnaire was done with twenty farmers who were randomly selected in the Mawirr and Royanka communities in the Rokupr environs.

Table 3. 1 List of study area for Individual Questionnaire and Focus Group Discussion

Region	District	Chiefdom	Major River	District Population
Northern	Kambia	Mambolo	Great Scarcies	345,474
Northern	Port Loko	Lokomasama	Rokel	615,376
Southern	Moyamba	Ribbi	Taia	318,588

STAT SL, 2015

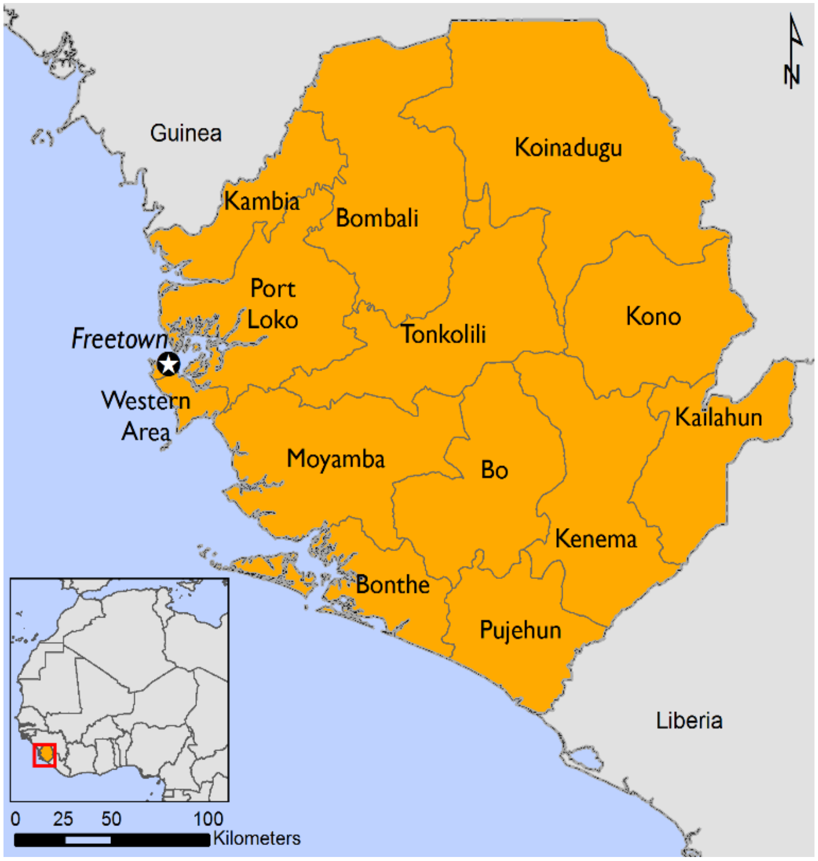

Figure 3. 1 A Map of Sierra Leone showing the district were survey was conducted

3.2.4 Data Collection

3.2.4.1 Semi-Structured Questionnaire

Six researchers including three socio-economists, two breeders and one agronomist from the Rokupr Agricultural Research Centre (RARC), Rokupr, formed the survey research team. A semi-structured questionnaire was used in the survey to collect information related to the importance of individual data, household data, rice production and constraint, types of varieties grown plus farmers preferred varieties, salt mitigation strategies by farmers, constraints affecting rice production in the mangrove, characteristics of good seed, etc.

3.2.5. Focus Group Discussion

Participatory rural appraisal (PRA) approaches were applied to identify periods of rice production, income generation, farmers' preferences, the most serious constraints affecting rice production, and the role of gender in mangrove rice production. A matrix scoring method was used following the approach by Ceccarelli (2012) to rank farmers' and consumers' preferences, constraints of rice production, farmer's rice preferred varieties, and the role of gender in rice production in Sierra Leone. Participants listed the criteria and data were organized on flipcharts followed by ranking using a fixed number of votes. After votes were given by participants, percentage values of each parameter were calculated.

3.2.5 Statistical Analysis

Data collected were subjected to analysis using SPSS (PASW statistics 18.0) computer package (SPSS, 2006), GenStat 15th edition (Payne *et al*., 2011), and Microsoft Excel (Windows Office 2012; Microsoft Inc., Redmond, WA). Variables were subjected to descriptive statistics to explain the socioeconomic characteristics of the farmer's household and farm characteristics. The Kendall's Coefficient of Concordance was used to test the level of Agreement among the ranked constraints and preferences.

3.3 Results

3.3.1 Socio-economic Characteristics of Mangrove Swamp Rice Producers

Table 3.2 and Figure 3.2 show the Socio-economic characteristics identified for the mangrove swamp rice producers within the selected communities in the three districts. Sampled were age, gender, marital status, educational level, main source of income and the average household size. In general, 87.3% of farmers fell within the working age group (21-50 years) with huge farming experience, with Port Loko showing a more aging population with 35.1% grouping within the age category of 41-50 years. The sex representative sample had an average of about 62.7% male mangrove rice owners, with the rest being female. However, there were more (72.7%) representation of male mangrove rice farmers in Port Loko followed by Moyamba (67.1%) than Kambia with the highest (51.9%) female representation.

The findings also show that majority of the mangrove rice farmers were married (81.5%). The situation was the same for all three districts surveyed except for Port Loko District which recorded (92.2%) more married respondents than the other two. About sixty-one percent (61%) of the farmers were illiterate, meaning they may not attend any form of formal education. The level of illiteracy flows right across the three districts, Moyamba recording the highest (73.6%).

Table 3. 2 Socioeconomic characteristics of mangrove swamp rice producers

Socio-economic Characteristics	Districts						Totals	
	Kambia		Port Loko		Moyamba			
	Freq	%	Freq	%	Freq	%	Freq	%
Age (Yrs.)								
Less than 20	0	0	1	1.3	1	1.3	2	0.9
21 - 30	26	33.8	11	14.3	11	14.3	48	20.6
31 - 40	21	27.3	21	27.3	29	36.7	71	30.5
41 - 50	18	23.4	27	35.1	16	20.3	61	26.2
Above 50	12	15.6	17	22.1	22	27.8	51	21.9
Sex								
Female	40	51.9	21	27.3	26	32.9	87	37.3
Male	37	48.1	56	72.7	53	67.1	146	62.7
Marital Status								
Married	48	62.3	71	92.2	71	89.9	190	81.5
Single	19	24.7	5	6.5	4	5.1	28	12
Widow/Widower	9	11.7	1	1.3	2	2.5	12	5.2
Divorced/Separate	1	1.3	0	0	2	2.5	3	1.3
Educational Level								
None	31	40.3	27	35.1	37	46.8	95	40.8
Koranic	8	10.4	18	23.4	21	26.8	47	20.2
Primary	18	23.4	18	23.4	6	7.6	42	18
JSS	4	5.2	7	9.1	7	8.9	18	7.7
SSS	15	19.4	6	7.8	5	6.3	26	11.2
Tertiary	1	1.3	1	1.3	3	3.8	5	2.1

3.3.2 General Information on mangrove swamp rice production

The general information on the main ecology for farmers across the three district is listed in Table 3.3. Overall, the study showed that 94.0% of the respondents rely on mangrove swamp ecology as their main ecology for rice cultivation, with Port Loko (98.7%) recording the highest, and Moyamba (88.6%) district the lowest. The results also show that about thirty-nine percent of farmers have about 6-16 years' experience in mangrove swamp rice cultivation.

Table 3. 3 Showing general information on mangrove swamp rice production

Variable	Name of District			Total
	Kambia	Moyamba	Port Loko	
	%	%	%	%
Mangrove swamp main ecology?				
Yes	94.8	88.6	98.7	94.0
No	5.2	11.4	1.3	6.0
Years of experience				
Less than 6 years	14.5	5.1	6.5	8.6
6-16 yrs.	50.0	46.8	20.8	39.2
17-27 yrs.	19.7	31.6	42.9	31.5
28-38 yrs.	9.2	12.7	20.8	14.2
39-49 yrs.	6.6	3.8	9.1	6.5
Cropping System Practice				
Sole cropping	97.4	98.7	92.2	96.1
Mixed cropping	2.6	1.3	7.8	3.9
Number of times farmers cultivate in a season				
Single cropping	84.4	88.6	54.5	76.0
Double cropping	15.6	11.4	45.5	24.0
Land preparation methods				
Brush, dig and puddle	81.6	86.1	54.5	74.1
Brush, and puddle	17.1	11.4	23.4	17.2
Puddle	1.3	0	0	0.4
Brush	0	0	3.9	3.9
Others	0	2.5	10.4	4.3

3.3.3 Main Sources of Income and Primary activities of farmers

Figure 3.2 shows that 78.1% of the respondents' main source of income is from farming and 14.6% from business. Same trend is recorded for all three districts, with Moyamba (91.1%)

recording the highest on farming and Kambia (22.1%) recording the highest on business, as opposed to the other two districts.

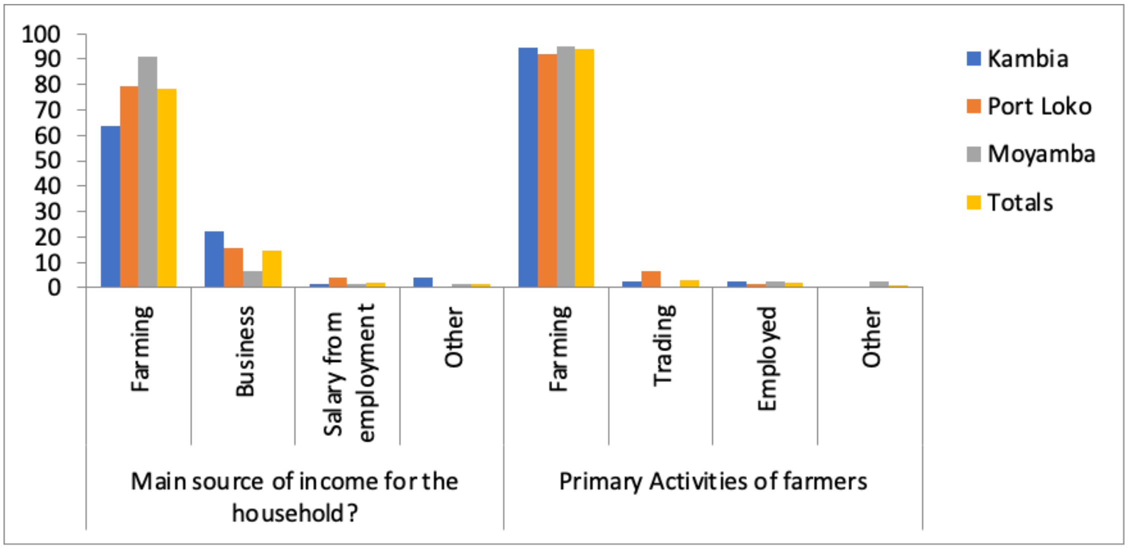

Figure 3. 2 Main source of income and primary activity of the farmers

3.3.4 Rice Varieties Grown Across the Study Areas

Local and improved rice varieties are both grown by Mangrove swamp rice farmers to meet their multiple objectives in rice farming (Table 3.3). Some farmers did not know the true name and origin of the varieties they cultivate. Therefore, they mostly name varieties in ways that reflect their source. A total of 91 different types of varieties were mentioned, with Kambia district recording the highest number of varieties (46).

Table 3. 4 Names of rice varieties grown in the mangrove swamps across the three districts

		Districts			
S/N	Kambia (N=46)	S/N	Moyamba (N=30)	S/N	Port Loko (N=35)
1	Amie	1	Abijan	1	Amaya
2	Bamp Kabie	2	After War	2	Bamp Kabie
3	Ben Kanu	3	Black	3	Chief Coker
4	Butter Cup	4	Foster	4	Falma
5	Compound	5	Guinea	5	Fatiyando
6	Conlaka	6	Jegay	6	Indian Rice
7	Cowuka	7	Kamara Morlia	7	Kalma Mayei
8	CP4	8	Kobo	8	Kamara Morlia
9	CPK	9	Lanikuru	9	Kankosu
10	Frama Malie	10	Mismis	10	Kulma

Districts					
S/N	**Kambia (N=46)**	**S/N**	**Moyamba (N=30)**	**S/N**	**Port Loko (N=35)**
11	Iye Thorley	11	NERICA	11	Maya
12	Jegay	12	Pa Bunch	12	Merican
13	Kabebdy	13	Pa Fant	13	Mobi
14	Kali Safie	14	Pa Fondu	14	Momoyegbeh
15	Kalisedu	15	Pa Gbessay	15	Morlai Kanu
16	Kamalaka	16	Pa Gbinti	16	Munafa
17	Kamara Kadie	17	Pa Kiamp	17	NERICA
18	Kamara Morlia	18	Pa Laak	18	Or Matei
19	Kinther	19	Pa Laoth	19	Pa Gbessay
20	Kulma	20	Pa Rant	20	Pa Kiamp
21	Nerica L19	21	Pa saidu	21	ROK 1
22	Mamie Yilla	22	Pafed	22	ROK 10
23	Marasam	23	Palark	23	ROK 20
24	Munafa	24	Palenakoro	24	ROK 22
25	Nerica L9	25	ROK 24	25	ROK 3
26	NGO	26	ROK 5	26	ROK 5
27	Oda Farm	27	ROK 6	27	Samphany
28	Pa Fondu	28	Safinatu	28	Sanfera
29	Pa Gbessay	29	Wuldeh	29	Super Sit
30	Pa Kiamp	30	Wuteteh	30	Sweet Mother
31	Pa Lahai			31	Unumsil
32	RARC Improve			32	Wuteteh
33	ROCK 3			33	Ya Kanu
34	ROK			34	Yaabu
35	ROK 10			35	Yabashe
36	ROK 20				
37	ROK 22				
38	ROK 5				
39	ROK10				
40	Safinatu				
41	Salia				
42	Salim				
43	Samphany				
44	Sanamise				
45	Ya gbessa				
46	Yabashe				

The five main varieties grown in the three districts in order of ranking are presented in Table 3.5. In Kambia, ROK 10, ROK 5, Kamara Morlia, Kalisedu, NERICA and Mamie Yilla were recorded as the top five varieties grown by mangrove farmers. In Moyamba district, Pa Kiamp, Guinea, Mismis, Pa Fondu, Wuteteh and Pa Gbessay were recorded as the top six varieties; while ROK 10, Kamara Morlia, Munafa, ROK 5, Unumsil and Sweet Mother were the top five varieties grown in Port Loko. From these findings, ROK (also known as Pa Kiamp by many farmers) is the most widely grown rice variety across the mangrove swamps in Sierra Leone.

Table 3. 5 Main rice varieties grown across the districts

Districts					
Kambia		Moyamba		Port Loko	
Variety Name	Rank	Variety Name	Rank	Variety Name	Rank
Kalisedu	4	Guinea	2	Kamara Morlia	2
Kamara Morlia	3	Mismis	3	Munafa	3
Mamie Yilla	5	Pa Fondu	4	ROK 10	1
NERICA	5	Pa Gbessay	6	ROK 5	4
ROK 10	1	Pa Kiamp	1	Sweet Mother	6
ROK 5	2	Wuteteh	5	Unumsil	5

Results of different rice varieties grown by farmers in the mangrove swamps of Sierra Leone are indicated in Table 3.6. Majority (69.1%) of farmers cultivate local rice varieties. The trend was the same across the three districts, with Moyamba (82.4%) recording the highest number of farmers growing local varieties. The main source of seed grain for cultivation by the mangrove swamp rice farmers was from other farmers or their own seed bank (68.9%), followed by research institution (28.2%). Farmers sourced less of their seed grains for cultivation from seed companies (1.4%), Ministry of Agriculture and Forestry (1.2%) and Non-Governmental Organization (0.3%). Same trend was recorded across all the three districts, except for Kambia which recorded the least source of seeds from farmers but the highest source of seeds from research (43.2%) and seed companies (2.2%).

Table 3. 6 Type and sources of varieties grown in the mangrove swamps in three districts of Sierra Leone

Variables	Districts			Total
	Kambia	**Moyamba**	**Port Loko**	
	Percent	Percent	Percent	Percent
Type of Variety				
Local	**52.7**	**82.4**	**70.8**	**69.1**
RARC Improved	46.3	16.7	27.7	29.7
Other Improved	0.5	0.5	1.5	0.8
Don't Know	0.5	0.5	0.0	0.3
Source of Variety				
Farmers	**51.9**	**81.5**	**71.2**	**68.9**
Research	43.2	17.1	26.2	28.2
Seed Companies	2.2	1.4	0.5	1.4
MAFS	2.2	0.0	1.6	1.2
NGOs	0.5	0.0	0.5	0.3

3.3.5 Farmers' choice of varieties

Mangrove rice farmers used many parallel criteria for selecting the rice varieties they grew. The results from Figure 3.3 revealed that, majority said they considered all the traits presented to them but the five most important traits were high tillering ability (93.9%), late maturing (89.7%), tall plant height (87.3%), tolerance to abiotic stress (88.5%) and panicle type (85.3%). The other traits that majority of the respondents also considered before selecting rice variety for cultivation were leaf angle (84.4%), grain size (83.5%), leaf senescence (80.8%), taste (78.9%), tolerance to biotic stress (75.35) and yield (68.7%).

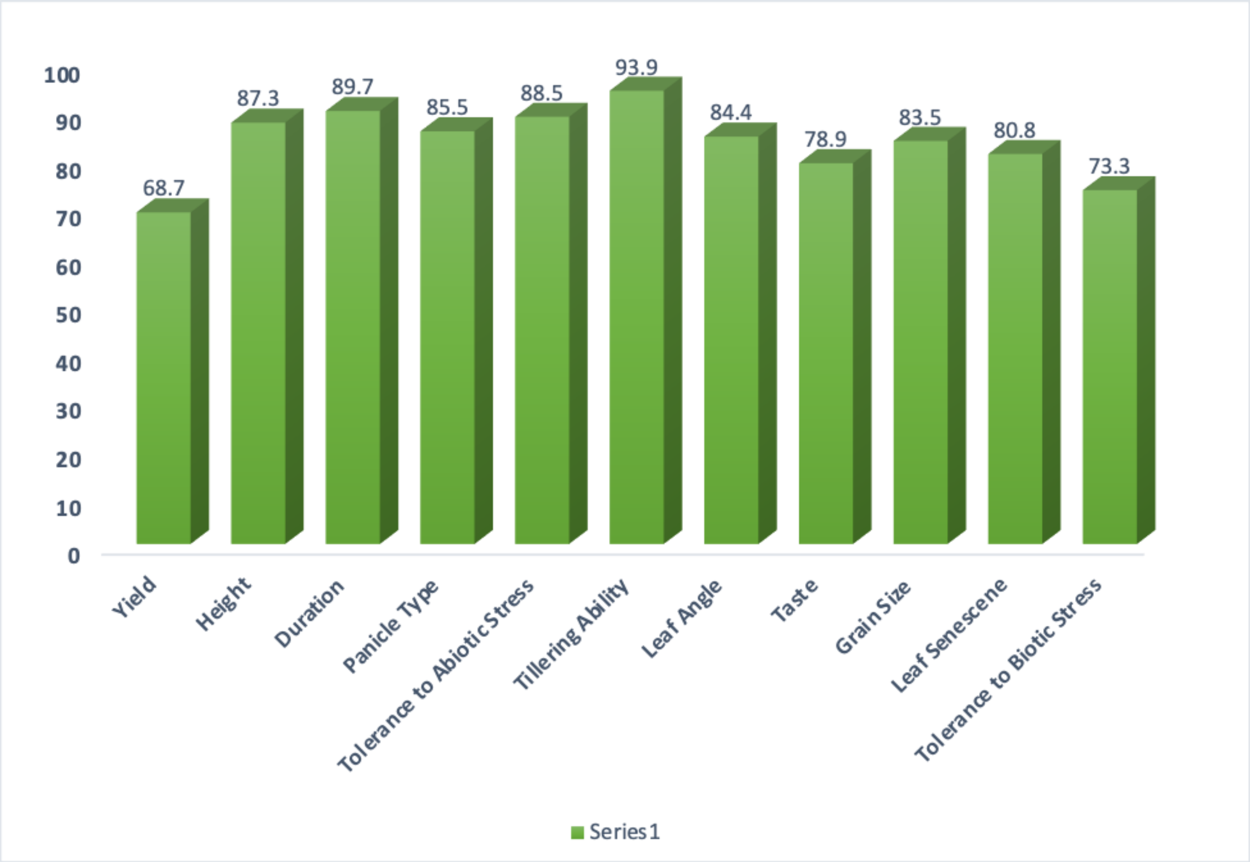

Figure 3. 3 Criteria or Traits guiding farmers' selection of varieties

Table 3.7 presents results of the mean rank of farmers' preference in choice of varietal traits for mangrove swamp rice cultivation. The result shows that most farmers preferred high yielding varieties, tall plants, late maturing, tolerance to abiotic stress and high tillering ability.

The Kendall's coefficient of concordance (W) is used to highlight the level of association of ordinal valuations made by different appraisers when assessing the same sample. From the results, the model indicates a moderate agreement among the mangrove swamp rice farmers because the Kendall's W=0.53 as to the ranking of the rice varietal traits. The P-value (<0.000) in the test statistics was highly significant (Table 3.7).

Table 3. 7 Mean rank of farmers' preference in choice of varietal traits for mangrove swamp rice cultivation

Traits	Mean	[a]Rank
Yield	1.27	1
Height	3.75	2
Days to Maturity	4.03	3
Taste	6.21	6
Tillering ability	5.51	5
Leaf angle	7.33	8
Tolerance to Abiotic Stress	5.09	4
Grain Size	6.66	7
Leaf senescence	8.36	10
Panicle Type	8.14	9
Tolerance to Biotic Stress	9.65	11
Kendall's W[a]	0.529	
P Value	**<0.000**	
Chi-Square	**534.150**	

[a]Rank: Kendall's ranking*;* ***Highly significant (*P*< 0.0001)

3.3.6 Farmers' Perception on the Salinity Status of Mangrove Swamps in Sierra Leone

Response of mangrove swamp farmers to the salinity status of their farms is shown in (Table 3.8) Different soil samples where displayed with descriptors and farmers were asked to select which sample matches the soil in their mangrove swamps. From the analysis, majority of the farmers (over 90%) across all three districts believe their soils are not sandy or silty in texture, but clayey (90.1%). Majority of the farmers (at least 86.1% in Moyamba district) also agreed that the soils in their swamps have high water retention capacity. All respondents assessed across the three districts agreed that the swamp water tastes salty and also answered in the affirmative when asked if the salt content affects crop growth and yields. Also, farmers did not agree that their soils had temperature, with the highest percentage of disagreement recorded in Kambia district (95.0%) and the least in Port Loko district (93.2%). This disagreement implies lack of knowledge among the local farmers with regards to soil temperature conditions in their mangrove swamps.

Table 3. 8 Status of soil salinity in mangrove swamps

Soil Property	District						Total	
	Kambia		Moyamba		Port Loko			
	Freq	%	Freq	%	Freq	%	Freq	%
Sandy soil texture								
Yes	2	3.3	1	1.3	5	6.8	8	3.8
No	58	96.7	78	98.7	69	93.2	205	**96.2**
Silty soil texture								
Yes	3	5.0	3	3.8	10	13.5	16	7.5
No	57	95.0	76	92.2	64	86.5	197	**92.5**
Clayey soil texture								
Yes	57	95.0	71	89.9	64	86.5	192	**90.1**
No	3	5.0	8	10.1	10	13.5	21	9.9
Organic matter accumulation								
Yes	57	95.0	22	27.8	47	63.5	126	**59.2**
No	3	5.0	57	72.2	27	36.5	87	40.8
Soil water retention capacity								
Yes	57	95.0	68	86.1	70	94.6	195	**91.5**
No	3	5.0	11	13.9	4	5.4	18	8.5
Soil porosity								
Yes	3	5.0	2	2.5	1	1.4	6	2.8
No	57	95.0	77	97.5	73	98.6	207	**97.2**
Soil temperature								
Yes	3	5.0	5	6.3	5	6.8	13	6.1
No	57	95.0	74	93.7	69	93.2	200	**93.9**
Other soil qualities								
Yes	2	3.3	3	3.8	2	2.7	7	3.3
No	58	96.7	76	96.2	72	97.3	206	**96.7**

3.3.7 Mitigation measure employed by mangrove swamp farmers in addressing soil salinity

Soil salinity mitigation measures by farmers in the three districts is shown in Table 3.9. The results showed that majority of the farmers across the districts did not practice semi-direct production system. For example, only 16.5% had practiced semi-direct production system in Moyamba, which was the highest compared to the other districts. Small percentage (5.0 to

39.2%) of the respondents used conventional (farmers' traditional method of land cultivation using a long wooden handled hoe or mattock) production system across the districts while only 2.7 to 5.0% adopted pre-germination production system across the three districts. In the case of the use of Other production systems as mitigation measures, only respondents in Kambia (5%) indicated the use of these.

Table 3. 9 Soil salinity mitigation measures employed by mangrove swamp farmers

Salinity Mitigation Measures	**District**						**Total**	
	Kambia		**Moyamba**		**Port Loko**			
	Freq	**%**	**Freq**	**%**	**Freq**	**%**	**Freq**	**%**
Semi direct production system								
Yes	4	6.6	13	16.5	1	1.4	18	8.4
No	57	93.4	66	83.5	73	98.6	196	**91.6**
Conventional production system								
Yes	3	5.0	24	30.4	29	39.2	56	26.3
No	57	95.0	55	69.6	45	60.8	157	**73.7**
Pre-germination production system								
Yes	3	5.0	2	2.5	2	2.7	7	3.3
No	57	95.0	77	97.5	72	97.3	206	**96.7**
Other productions systems								
Yes	3	5.0	0	0.0	0	0.0	3	1.4
No	57	95.0	79	100.0	74	100.0	210	**98.6**

3.3.8 Access to services by mangrove swamp farmers

Results on farmers access to services is presented in Table 3.10. Overall, about fifty-six percent (56.2%) of farmers had access to milling machines (Table 3.10). However, 97.5% of farmers in Moyamba district did not have access to such services. Seventy-seven percent (77.7%) of farmers in all three districts did not have access to community stores for rice storage, with this situation being more pronounced in Port Loko (92.2%) and Moyamba (100%) districts. The results for access to concrete drying floors was very similar to that of the storage facilities, as majority (77.3%) of the farmers did not have access to concrete drying floors for their seed grains.

Table 3. 10 Mangrove farmers' access to services

Variables	**Name of district**			**Total**
	Kambia	**Moyamba**	**Port Loko**	
	Percent	**Percent**	**Percent**	**Percent**
Do you have access to Milling machine?				
Yes	94.8	2.5	72.7	56.2
No	5.2	97.5	27.3	43.8
Do you have access to community store?				
Yes	59.7	0.0	7.8	22.3
No	40.3	100.0	92.2	77.7
Do you have access to concrete drying floor?				
Yes	62.3	0.0	6.5	22.7
No	37.7	100.0	93.5	77.3
Is access to improved mangrove rice variety/ies a constraint?				
Yes	60.3	65.8	64.0	63.5
No	39.7	34.2	36.0	36.5
Is accessing labor for your mangrove rice cultivation a major constraint?				
Yes	46.8	59.5	57.1	54.5
No	53.2	40.5	42.9	45.5
Do you have access to credit for mangrove rice farming?				
Yes	81.8	22.8	63.6	55.8
No	18.2	77.2	36.4	44.2

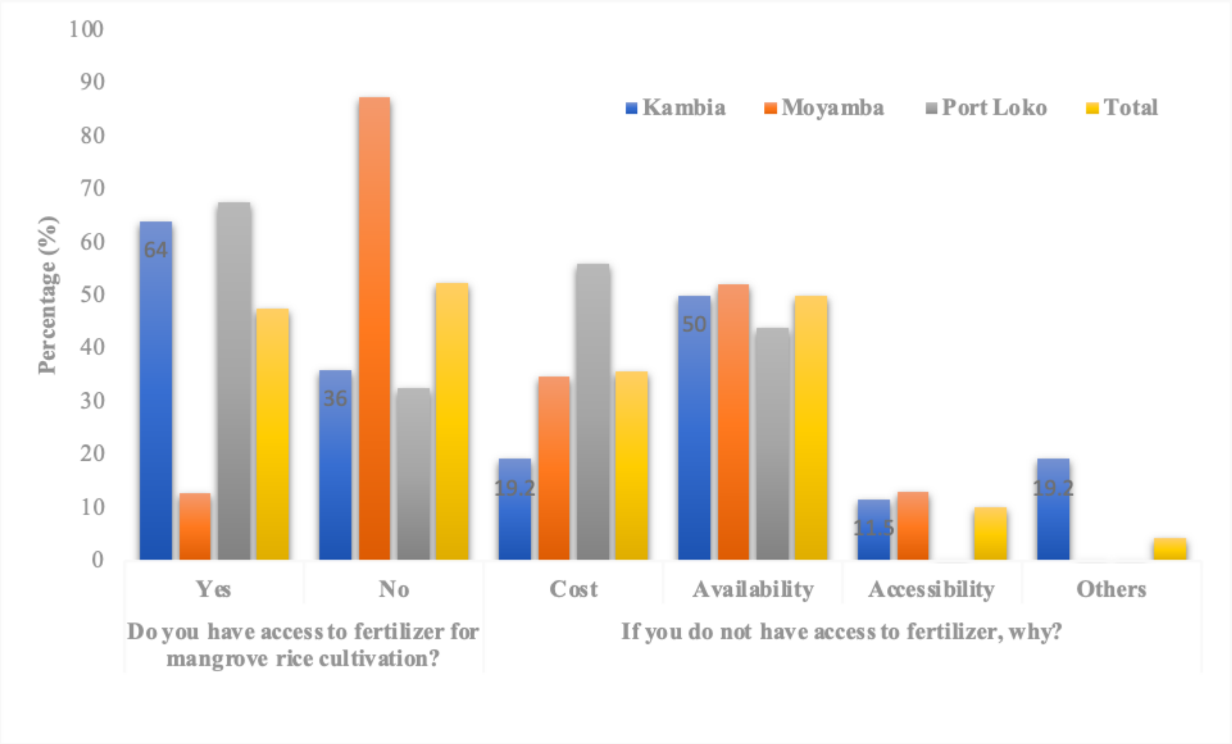

Figure 3.4 Access to fertilizer by mangrove rice farmers

As shown in Figure 3.4, most farmers (52.4%) have no access to fertilizers. Comparing across districts, a large proportion of farmers in Moyamba (87.3%) district have no access to fertilizer, hence non fertilizer usage, as compared to Kambia (36.0%) and Port Loko (32.5%) districts. The limited access to fertilizer is as a result of non-availability (50.0%) in the market and the high cost (35.8%) of the fertilizer.

Access to land is a key constraint limiting mangrove rice production. Therefore, assessing land constraint in relation to access by men, women and the youth is important. In the case of men, a slight majority said “Yes” (51.9%) to having access to land. Comparing across districts, men in Port Loko had more access to land (64.9%) than in Moyamba (45.6%) and Kambia (45.4%) districts. This result indicated that sixty percent (60.1%) of women do not have access to mangrove swamp for rice cultivation. Comparing across districts, women in Port Loko had more access to land (48.1%) than those in Moyamba (36.7%) and Kambia (34.7%) districts. For the youth, the majority also responded that they did not have access to land (56.2%), youth in Port Loko reported more access to land (54.5%) than their counterparts in Moyamba (39.2%) and Kambia (37.1%) districts. Therefore, high percentage of the respondents did not have much access to land, and the major source of land access was from family (59.3%), and land is mostly controlled by men (84.0%).

Table 3. 11 Linkage between mangrove swamp rice farmers and other stakeholders in agriculture

Activities	District							
	Kambia		Moyamba		Port Loko		Total	
	Freq	%	Freq	%	Freq	%	Freq	%
Attend agricultural field day								
Yes	6.0	8.0	3.0	3.8	8.0	10.4	17.0	7.4
No	69.0	92.0	78.0	76.0	69.0	89.6	14.0	92.6
Field day organized by								
Research	0.0	0.0	0.0	0.0	1.0	12.5	1.0	5.9
MAFS	5.0	83.3	2.0	66.7	3.0	37.5	10.0	58.8
NGO	1.0	16.7	1.0	33.3	3.0	37.5	5.0	29.4
Others	0.0	0.0	0.0	0.0	1.0	12.5	1.0	5.9
Contact extension officers								
Yes	12.0	16.0	0.0	0.0	13.0	16.9	25.0	206.0
No	63.0	84.0	79.0	100.0	64.0	83.1	10.8	89.2
Visit by Extension technician								
Yes	4.0	5.5	0.0	0.0	9.0	11.8	13.0	57.3
No	69.0	94.5	79.0	100.0	67.0	88.2	15.0	94.3
Visited by								
Research	1.0	20.0	0.0	0.0	1.0	11.1	2.0	14.3
MAFS	3.0	60.0	0.0	0.0	8.0	88.9	11.0	78.6
NGO	1.0	20.0	0.0	0.0	0.0	0.0	1.0	7.1
Membership into organization								
Yes	12.0	16.7	28.0	35.4	21.0	27.3	61.0	26.8
No	60.0	83.3	51.0	64.6	56.0	72.7	16.0	73.2
Type of organization								
FBO	6.0	50.0	7.0	25.0	11.0	50.0	24.0	38.7
FSA	1.0	8.3	3.0	10.7	8.0	36.4	12.0	19.4
Social Clubs	5.0	41.7	13.0	46.4	3.0	13.6	21.0	33.9
Others	0.0	0.0	5.0	17.9	0.0	0.0	5.0	8.1
Benefit agricultural input from organization								
Yes	2.0	20.0	4.0	14.3	6.0	30.0	12.0	20.7
No	8.0	80.0	24.0	85.7	14.0	70.0	46.0	79.3

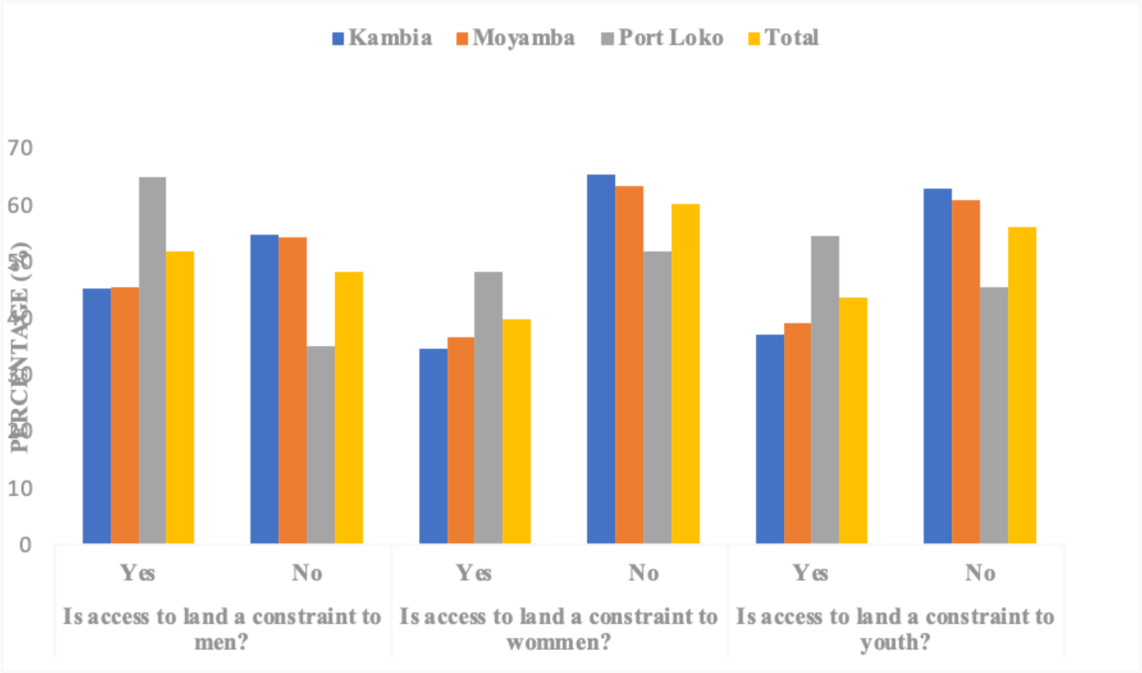

Figure 3. 4 Access to land by Mangrove rice farmers

3.3.9. Gender roles in mangrove swamp rice production

Different labour allotment roles by male, female and youths in rice production is shown in Table 3.12. Division of labour within the household implies an equally high degree of knowledge by gender on labour duties in the study area. Most of the activities such as nursery preparation (48.7%), brushing (63.3%), bird scaring (56%), harvesting (43.6%), transportation (36.3), and storage (57.0) were predominated by adult men while threshing (54.1%), ploughing, (48.9%), puddling (62.1%), winnowing (49.9%), drying (44.8%), and parboiling (43.2%) are mainly carried out by youths. From the result, it is shown that involvement of adult women in mangrove rice farming is very minimal.

Table 3. 12 Roles of Gender and labour contribution in various activities in mangrove swamp rice production in the survey district.

	District											
Variables	**Kambia**			**Moyamba**			**Port Loko**			**Total Mean**		
	Male	Female	Youth	Male	Female	Youth	Male	Female	Youth	Male	Female	Youth
Nursery Preparation	**80.6**	4.2	**15.3**	**16.4**	0	83.5	52	1.3	46.7	49.5	1.8	48.7
Brushing	83.8	0	16.2	11.8	0	86.8	33.3	1.7	63.3	42.2	0.5	55.4
Ploughing	62.9	4.2	15.3	8.9	0	80	1.9	1.3	42.7	33.5	1.8	48.9
Swamp Puddling	70.6	15.5	13.8	7.7	0	92.3	23.7	3.4	72.9	31.3	5.6	62.1
Transplanting	67.7	12.9	17.7	2.6	1.3	96.2	14.9	9.5	75.7	25.7	7.5	66.9
Weeding	78.5	7.7	13.9	13.6	11.4	75	31.7	14.6	53.7	46.7	10.7	42.7
Fertilizer Application	85.3	5.9	8.8	41.5	9.8	48.8	58.5	7.5	34	65.4	7.4	27.2
Bird Scaring	88.9	3.2	7.9	6.8	1.7	91.5	13.6	9.1	77.2	39.8	4.2	56
Harvesting	84	8	8	13.5	0	86.5	31.9	0	64.2	43.6	2.8	8.7
Transportation	81.1	2.7	16.2	5.2	7.2	90.6	23	2.3	74.3	36.3	3.1	5.4
Threshing	80	1.3	18.7	9.1	7.8	83.1	32.9	6.8	60.3	40.4	5.3	54.1
Winnowing	9.3	76	14.7	24.7	5.2	70.1	17.6	20.3	62.2	17.3	33.6	49.9
Drying	77	9.5	12.5	5.5	31.5	63	13.5	28.4	58.1	32.1	23.1	44.8
Parboiling	78.8	8	13.4	8.5	23.7	67.8	14.9	31.1	54.1	36.1	20.7	43.2
Storage	86.5	5.4	8.1	27.7	9.2	63.1	53.3	10.7	36	57	8.4	34.5

3.3.10 Identifying major production constraints, and recommendation of mangrove rice farmers

Results for major production constraints of mangrove swamp rice production are indicated in Table 3.13. Constraints were classified into four categories including strengths, weaknesses, opportunities and threats (SWOT). High productivity (2 t/ha), fertile soils, and experience in mangrove swamp rice production were the most mentioned and ranked strengths. In a similar vein, the most mentioned and ranked opportunities to mangrove farmers include availability of improved seeds, vast area of mangrove swamps, availability of agro-chemicals, availability of financial institutions and availability of agricultural equipment (Table 3.11). These are the major drivers of mangrove swamp rice production in Sierra Leone. Major weaknesses outlined are high cost or lack of access to improve seeds and fertilizer, high labour cost, lack of capital, poor infrastructure and transportation facilities. On the other hand, crab damage, flooding, salt stress and climate change were major threats and barriers to rice cultivation in mangroves.

Table 3. 13 SWOT analysis for rice farmers across three districts under study

SWOT ANALYSIS	
STRENGTH	**WEAKNESS**
• High productivity • Access to fertile soil • Experience in mangrove • Organisation of farmers • Government support	• High cost or lack of access to improved seed and fertilizer • High labour costs • Lack of capital • Scarcity of good quality water • Poor infrastructure and transportation
OPPORTUNITY	**THREATS**
• Availability of improved seed • Vast area of mangrove swamp • Availability of agro-chemicals • Availability of financial institutions • Availability of agricultural equipment	• Crab damage • Pests and diseases • Flooding • Salt stress • Climate change

The results of recommendations from mangrove swamp rice farmers are summarized in Table 3.14. Provision of improved seed adaptable for mangrove swamp rice production ranked the first by 29.1% of the respondents followed by need of government support and provision of credit facilities ranking second and third by 23.6% and 15.0% of the respondents respectively. The results showed that need for support from NGOs was the least recommended among farmers in the three districts with only 7.9% of the respondents.

Table 3. 14 General Recommendations and ranking of priorities by the mangrove swamp rice farmers

General Recommendations	Percent (%)	Rank
Provision of improved rice seeds	29.1	1
Need of government support	23.6	2
Provision of credit facilities	15.0	3
Infrastructural development	13.4	4
Provision of farming equipment and tools	11.0	5
Support from NGO	7.9	6

3.4 Discussion

This study was set out to elicit information on the existing production systems, production constraints, and farmers' preferences as end users of new or improved rice varieties or cultivars. The distribution of the farmers by age showed that the mean age for farmers was 40 years, which is comparatively lower than the mean age (50) of farmers for other important crop commodities like cocoa and coffee (PEMSD, 2010). This implies that the rice farming population are within the active age and can efficiently engage in rice production. Rice cultivation in the mangrove swamps is a tedious exercise which is labour intensive and requires good energy for land preparation, nursery, transplanting, weeding and harvesting. Similar findings have been reported

(Chidiebere-Mark *et al*. 2018), indicating mean age of rice farmers in different rice production systems to be 49 years. In this studied, it was discovered that majority of the farming population in the mangrove swamps range from 21-30 years of age. This is encouraging, indicating that the farming population belongs to the productive and active age group, implying realistic sustainability and productivity for the rice sector if given the right support. Younger farmers were found in the Kambia district compared to Port Loko and Moyamba. Rahman, (2008) reported that farmers in the age bracket of 40 years were still energetic and have a lot of positive implications for the production of rice as a crop.

The socio-economic distribution by sex showed that male farmers are more involved (62.7%) in rice farming than women (37.3%). This shows that rice farming activities in the mangrove are dominated by men and suggest that more effort is required to mainstream gender in rice production. It is also clear in the result that involvement of adult women in mangrove rice production is very minimal. This result is consistent with the findings of Sow *et al.* (2015), who had earlier reported low involvement of women in rice production in Niger. This, however, differs with the findings of Zossou *et al* (2010), who reported that women in Benin were principal actors in rice production such as processing, parboiling and marketing. The involvement of women in the rice value chain is very important because women is crucial in rice sector development, especially in the processing and marketing aspects. Therefore, it is important to encourage more women into rice cultivation. Marriage is held in very high esteem in the farming communities under study, which showed that 81% of the farmers were espoused. This indicates that majority of the respondents were married indicating that majority of the respondents are stable and could command societal respect. Being married could mean that the respondents are responsible. In a farming household, all members of the household assist each other with farming activities and other household chores. This is more the reason why marriage is paramount among the farming communities, because most farmers depend on their families as primary source of labour. Most farmers (46.8%) in Moyamba, (40.3%) in Kambia, and (35.1%) in Port Loko Moyamba, had no formal education. Few farmers (2.5%) had tertiary education. This implies that most of the mangrove rice farmers were illiterate, which pose a significant challenge in adopting new technologies. Education is paramount and can play a key role in the adoption of agricultural technologies and equally empowers individuals to make informed decision. Bala *et al*. (2010) have also isolated illiteracy as a key hindrance to institutional support towards agriculture while Amos (2007) and Nyagaka *et al*. (2010) revealed that education had positive relationship with agricultural efficiency and production.

The choice of mangrove swamp rice cultivation may be largely due to the availability of mangrove in the study area and the suitability of the swamp for rice cultivation. This is consistent with findings of Agyen-Sampong *et al* (1984), who described the mangrove swamps as most fertile compared to other rice ecologies. Farming represents the main income source of the farm families coupled by other businesses. In the Moyamba district more farmers (91.1%) solely depend on farming for their daily income and livelihood. Few farmers form the Kambia district were engaged in trading as another source of income.

Majority of the respondents has vast knowledge of experience in mangrove swamp rice farming due to long (6-16years) years of continuous operation, and according to Nwoye (2007) this has a positive implication for increased rice production.

The most prevalent cropping system practice by most of the farmers (76.0%) in the swamp is the single cropping system. Single cropping starts from July to January. Few farmers (24%) engage in doubling cropping. The type of cropping system adopted by farmers is determining by the water regime (salt free period) during the cropping season. Similar findings have been reported by Sow *et al*. (2015) in Niger.

The single cropping system is mostly found along the Great Scarcies and Taia rivers which have long periods of fresh water flooding the swamps. In these areas, mostly long duration (4 to 7 months) varieties are appropriate for cultivation (Baggie *et al*., 2018). Double cropping is found only in the Port Loko district where 45.5 % of the mangrove farmers cultivate twice in the cropping season. Another finding from this studies shows that most farmers planted only rice (96.10%) in the mangrove swamp, with Moyamba recording the highest (98.7%) across the districts.

Most farmers across the 3 districts use old rice varieties or landraces which have been recycled over the years. This may possibly be due to lack of access to new and modern cultivars or the absence of the acceptable alternatives to their landraces (Maurice *et al.*, 2018). Another possible reason is lack of linkage between the farmers and the extension agents who are obliged to disseminate new research technology to farmers. Farmers mostly used their own seed saved from the previous harvest. This agrees with findings from Jenkins *et al*. (2019) when they assessed the indigenous knowledge systems of farmers, and reported that 53.8 % of the farmers interviewed across ten districts use traditional varieties for rice cultivation. In this survey, names of 91 different varieties were mentioned across the 3 districts with few common across district and few specific to a particular district. These varieties might be improved or local or recycled seed from improved varieties. Local varieties were mostly named after the particular individual who introduced the variety to that locality. This implies the possibility for the same varieties to carry different names across communities. Some cultivars were mostly grown by some farmers than others, and the main varieties mostly grown across the mangrove swamps in Sierra Leone include ROK 10, ROK 5, Kamara Morlai and Pa Kiamp. This result agrees with the findings of Gborie *et al.* (2016), who also reported ROK 10 and ROK 5 as the most adopted RARC improved mangrove rice in their study. However, the present study showed that 55.8% of farmers had access to improved seeds, which was a bit encouraging. This implies the availability of the technology for the mangrove swamp rice cultivation, though limited by high cost. Labour was also noted to be available, but constrained by cost.

Salinity mitigation in the mangrove swamps is purely controlled by natural mechanisms. Fresh water from the rains overflow the major rivers during the raining season, which washes the excess salt from the swamps, making soil suitable for rice cultivation. Nutrients are also supplied to the swamps through the inflow of sea water during the dry season. Soils are also enriched through the microbial activities within the root zones. Most farmers transplant overgrown (8-12 weeks) seedlings to avoid crab damage. The responses with regards to soil texture reflect actual soil characteristics of mangrove swamps.

Mangrove soils generally have low sand content (10-15%), with clay and silt constituting the bulk of the soils (Odell *et al*., 1974). This is a result of the soils being formed from recent marine and estuarine mud (Wiel and Brady, 2017). However, most of the farmers disagree to the presence of silt in the soils, which could be attributed to the fact that distinguishing it from clay is an extremely difficult task on-farm and can pose serious challenges even in laboratory analysis as well (Jones, 2001). Moreover, a previous study by Odell *et al*. (1974) classified these soils as silty clay as a result of the high percentage of the two textural classes in those soils. The high agreement recorded for soil water retention is a reflection of the fact that most of the mangrove swamps are water-logged for prolonged periods and in most cases throughout the year. The high amount of clay (which has high water holding capacity) and the daily inflow of water from the sea at high tides, are the major factors responsible for the soil's water retention. The high amount of clay coupled with low sand content, as responded by farmers, are responsible for the low soil porosity in the mangrove swamps. The salt content of mangrove swamps is usually high due to the salt or brackish water environment.

Most farmers have access to credit, thereby allowing farmers to increase the size of their farms and the use of inputs. However, the high interest rate serves as a barrier to some farmers who see farming as a non-profit venture. There is no formal financial institution for credit facility for farmers in the Moyamba district (Table 3.9). Only few farmers (25%) across the three districts have contact with extension officers; hence, visitations by extension officers to mangrove swamp rice farmers was extremely low (5.7). This low far-extension interaction is an indication of poor linkage among major players in the agricultural sector. Few farmers belong to farmer Based Organization (FBOs) and social clubs.

The revelation that only 35.4 % of the respondents belonged to an association was not encouraging. Among farmers that belonged to organizations, majority belonged to Farmer Based Organizations (FBOs), with Social Clubs being a close second with 33.9%. Membership in farmer-based organizations enables the farmers to work together to achieve common goals. Additionally, most of the farmers (79.3%) did not derive any benefits in the form of agricultural inputs from the organizations they belonged to. This indicated low involvement of agricultural organizations in mangrove rice cultivation (Table 3.10). In other to match the increasing demand of the farmers to satisfy the growing demand of mangrove swamp rice producers in the future, the limitations faced by the farmers must be addressed. The result of the SWOT analysis thus revealed the need for an integrated approach to addressing these constraints.

The Kendall's coefficient of concordance (W) explains the level of relationship and assessments made by multiple appraisers when assessing the same sample. From the results of labour access constraints, the Kendall's coefficient of concordance indicates a fair understanding among the mangrove swamp rice farmers because the Kendall's W=0.414 as to the ranking of the rice production constraints in relation to labour access. The P-value (<0.000) in the test statistics was highly significant. Ranking was done by farmers for all the three labour constraints that affect them more during mangrove swamp rice production. The labour constraint with the smallest mean rank is the major problem faced. Therefore, results indicated that farmers are faced with mostly cost constraint followed by availability and accessibility constraint (Table 3.13). From table 3.13, the results of access to land constraints show that the Kendall's coefficient of concordance indicates a fair agreement among the mangrove swamp rice farmers because the Kendall's W=0.223 as to the ranking of the rice production constraints in relation to land access by the different categories of farmers. The P-value (<0.000) in the test statistics was highly significant. Farmers were asked to select and rank all the categories involved in land access constraints that were more evident during mangrove swamp rice production. The land constraint with the smallest mean rank is the “farmer with limited access to land”. The result indicated that, women were more constrained in accessing land as compared to men and youths.

The results of access to improved rice constraints showed that, the Kendall’s coefficient of concordance indicated a poor agreement among the mangrove swamp rice farmers because the Kendall’s W=0.141 as to the ranking of the rice production constraints in relation to accessing improved rice varieties by farmers. The P-value (<0.000) in the test statistics was highly significant. Farmers were asked to select and rank all the constraints that affected them in the process of accessing improved rice seeds towards mangrove swamp rice production. The improved rice constraints with the smallest mean rank is the key constraint faced by farmers in accessing improved rice seeds. Therefore, results indicated that, farmers were more faced with cost constraints, then followed by availability and accessibility constraints (Table 3.13). From Table 3.13, the results of access to fertilizer constraints showed the highest percentage meaning that it was the major problem faced by mangrove swamp rice producers.

3.5 Conclusion

This study identified different production constraints such as high cost of labour, salinity, lack of credit, lack of access to improved seeds and fertilizer, crabs, pests and diseases as the most important. High yield, tall plant height, late maturity, good taste, and high tillering ability were the top five traits farmers considered in selecting a variety. Farmer’s knowledge on salinity tolerant varieties was very low. It was found that the availability of rainfall/ fresh water to leach the salts from the swamp’s soils and transplanting of overgrown seedlings were the major mitigation mechanisms for avoiding salinity effects and crab damage on rice production in the mangrove ecology. Farmer’s knowledge on salinity tolerant varieties was very low but were willing to adopt salinity tolerant varieties. There was also willingness by farmers to adopt fertilizer usage, but the farmers complained about the increasing high cost of inputs and the risk of the flood water washing away the nutrients. The agricultural extension support to farmers should be intensified to improve agronomic practices of farmers.

CHAPTER FOUR

4.0 AGRO-MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF MANGROVE RICE CULTIVARS IN SIERRA LEONE

4.1 Introduction

Rice (*Oryza sativa* L.) belongs to the family *Poaceae* and subfamily *Oryzoide.* It feeds over 50 percent of the global population (Rasel *et al*., 2018). Rice plays a major role in the Sierra Leonean diet (Baggie *et al*., 2018), with the annual rice consumption estimated at 530,000 tonnes (Baggie *et al*., 2018). Rice germplasm is a rich reservoir of useful genes which researchers can rely on for expanding rice production (Singh *et al*., 2015). Rice contributes a minimum number of the large *ex situ* germplasm accessions in the world (Vanniarajan *et al*., 2012).

Previous research affirm that local varieties continually cultivated by indigenous farmers are possible source of high genetic diversity that can serve as potential genetic banks for improving yield and developing new rice varieties (Vanniarajan *et al*., 2012; Bidhan, 2013; Singh *et al*., 2015). These diverse traits are mostly prone to different range of abiotic and biotic stresses and agronomic performance (Rabara *et al*., 2014). Banumathy *et al.* (2010) have defined genetic diversity as the amount of genotypic (at the DNA level) present in a group of individuals. Genetic diversity studies occupy an important position in breeding and crop improvement programmes as they ensure efficient utilization of germplasm resources and related crop species.

Huge amount of dissimilarity exists among landraces there is a huge amount of dissimilarity existing in rice among and within landraces, this shows that there is a wide gap and scope for future improvement (Singh *et al*., 2015). According to Vanniaraja *et al*. (2012), landraces have shown to thrive perfectly in their local environment and are known to harbour valuable genetic traits for rice improvement, especially in breeding for stress tolerance.

Understanding the presence of genomic difference with regards to salt tolerance within species is a key component in crop breeding (Kumari *et al*., 2018). Hence, success of every plant breeding program is based on the selection decision of the germplasm. Considerable collection of germplasm offers a valuable platform of genetic diversity for the studied trait (Amaranatha *et al., 2014).* Genetic divergence is a useful tool which gives accurate information about the choice of parents used for hybridization programme (Vanniaraja et al., 2012). Most landrace have the potential of carry a new gene, hence its utilization is important for the discovery of new gene and further genetic improvement of germplasm (Ahmed *et al., 2015*).

In Sierra Leone traditional landraces are still under cultivation by resource poor farmers practicing subsistence farming (National Rice Development Strategy, 2009). It is important to note that new germplasms have evolved over the years from cross boundary introductions and other means, and most of these are found in the hands of farmers. Due to this there is a lot of duplication of varieties in the hands of farmers, the same varieties may have different names across locations. The diversity of the wild species is gradually being eroded for series of reason which may include destruction of natural wild rice habitat to pave the way for expanding agricultural activities resulting from increasing human population pressure, change in land use and deforestation (Kiambi *et al*., 2005).

Chromosomal markers hold a vital component in plant breeding (Nadeem *et al*., 2017). Molecular level screening for genetic diversity is more resourceful and meaningful than at the phenotypic level. Phenotypic screening depends on morphological trait data which can be manipulated by environmental influences, labour intensive and time consuming. Molecular markers on the other hand offer a rapid, efficient and cost-effective measure of genetic dissimilarity. These markers have been reported to be an important tool in the characterizing and evaluating genetic diversity within and between species and populations (Sharma *et al*., 2018). Preservation of crop diversity requires collection, characterization and conservation of traditional landraces and wild relatives. The diverse traits can be used to generate new varieties and to make crosses so as to transfer important traits between varieties. Consequently, farmers' owned cultivars can be improved to meet their preferences.

Hence the objectives of the study were to:

(i) Assess the nature of genetic diversity present among the mangrove swamps rice; and

(ii) Select desirable diverse genotypes as parent for further utilization in crop improvement programs.

4.2 Materials and Methods

4.2.1 Experimental Site

The present study was conducted in the screen house in 2018 during the dry cropping season in Mbe, AfricaRice Centre, Cote d'lvoire located between 7.5^o N and 8.5^o N and between 4.5 ^{o} W and 5.5^o W, with an average annual rainfall of 985 mm. The station is characterized by 3 main seasons: a long dry season starting in early November with an end in mid-March; a long rainy season from mid-March to mid-July; a short rainy season from mid-July to mid-August, and an inter-season rainy period which starts in mid-August and ends in October.

4.2.2 Plant Materials

Ninety (90) rice accessions including both landraces and improved varieties from three districts (Kambia, Port Loko, and Moyamba) in Sierra Leone were used for this study. Kambia and Port Loko are located in the Northern region and Moyamba in the Southern region in the country. For convenience, the assembled genotypes were coded as ARB-ROK-2017-1 to ARB-ROK-2017- 90 (Table 1).

Table 4. 1 List of rice accessions collected from the Mangrove agro-ecologies of northern and southern Sierra and were genotyped for diversity using DArTseqTM in IGSS, Kenya.

S/N	Accession	S/N	Accession	S/N	Accession
1	ARB-ROK-2017-1	31	ARB-ROK-2017-31	61	ARB-ROK-2017-61
2	ARB-ROK-2017-2	32	ARB-ROK-2017-32	62	ARB-ROK-2017-62
3	ARB-ROK-2017-3	33	ARB-ROK-2017-33	63	ARB-ROK-2017-63
4	ARB-ROK-2017-4	34	ARB-ROK-2017-34	64	ARB-ROK-2017-64
5	ARB-ROK-2017-5	35	ARB-ROK-2017-35	65	ARB-ROK-2017-65
6	ARB-ROK-2017-6	36	ARB-ROK-2017-36	66	ARB-ROK-2017-66
7	ARB-ROK-2017-7	37	ARB-ROK-2017-37	67	ARB-ROK-2017-67
8	ARB-ROK-2017-8	38	ARB-ROK-2017-38	68	ARB-ROK-2017-68
9	ARB-ROK-2017-9	39	ARB-ROK-2017-39	69	ARB-ROK-2017-69
10	ARB-ROK-2017-10	40	ARB-ROK-2017-40	70	ARB-ROK-2017-70
11	ARB-ROK-2017-11	41	ARB-ROK-2017-41	71	ARB-ROK-2017-71
12	ARB-ROK-2017-12	42	ARB-ROK-2017-42	72	ARB-ROK-2017-72
13	ARB-ROK-2017-13	43	ARB-ROK-2017-43	73	ARB-ROK-2017-73
14	ARB-ROK-2017-14	44	ARB-ROK-2017-44	74	ARB-ROK-2017-74
15	ARB-ROK-2017-15	45	ARB-ROK-2017-45	75	ARB-ROK-2017-75
16	ARB-ROK-2017-16	46	ARB-ROK-2017-46	76	ARB-ROK-2017-76
17	ARB-ROK-2017-17	47	ARB-ROK-2017-47	77	ARB-ROK-2017-77
18	ARB-ROK-2017-18	48	ARB-ROK-2017-48	78	ARB-ROK-2017-78
19	ARB-ROK-2017-19	49	ARB-ROK-2017-49	79	ARB-ROK-2017-79
20	ARB-ROK-2017-20	50	ARB-ROK-2017-50	80	ARB-ROK-2017-80
21	ARB-ROK-2017-21	51	ARB-ROK-2017-51	81	ARB-ROK-2017-81
22	ARB-ROK-2017-22	52	ARB-ROK-2017-52	82	ARB-ROK-2017-82
23	ARB-ROK-2017-23	53	ARB-ROK-2017-53	83	ARB-ROK-2017-83
24	ARB-ROK-2017-24	54	ARB-ROK-2017-54	84	ARB-ROK-2017-84
25	ARB-ROK-2017-25	55	ARB-ROK-2017-55	85	ARB-ROK-2017-85
26	ARB-ROK-2017-26	56	ARB-ROK-2017-56	86	ARB-ROK-2017-86
27	ARB-ROK-2017-27	57	ARB-ROK-2017-57	87	ARB-ROK-2017-87
28	ARB-ROK-2017-28	58	ARB-ROK-2017-58	88	ARB-ROK-2017-88
29	ARB-ROK-2017-29	59	ARB-ROK-2017-59	89	ARB-ROK-2017-89
30	ARB-ROK-2017-30	60	ARB-ROK-2017-60	90	ARB-ROK-2017-90

4.2.3 Experimental Design and Data Collection

Ten seeds per accession were pregerminated in petri dish lined with moist tissue paper at the Biotechnology lab of AfricaRice in Bouake. Some of the seeds had very low germination rates and could not produce the required amounts of seedlings. This narrowed the collections to ninety which were then transplanted ten days after germination. The experiment was laid out in screen house in a randomized complete block design (RCBD) designs with two replications and eight plants per row. One seedling was transplanted per hill, spaced at 20 cm x 20 cm between and within rows.

Agronomic practices were maintained and chemical fertilizer (N.P.K) dosage of 60-50-40 kg/hm^2 was applied. The field was irrigated twice a day. Other recommended cultural practices were adopted and weeding was done by hand whenever weeds appeared. Four middle plants from each accession were used in the characterization of the collection following 12 morphological descriptors outlined by the fifth edition of the standard evaluation system (SES) (IRRI, 2014). In summary, plant height was measured as length from the base of the plant to the tip of panicle in cm, tiller number was recorded as the number of tillers per plant.

4.2.4 DNA Extraction and genotyping of rice accessions

Two leaf discs of 6mm diameter were sampled from each of the four middle plants into 96 deep well polymerase chain reaction (PCR) plates 15 days after transplanting. 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 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. The samples were genotyped using 34 single nucleotide polymorphism markers for stress tolerance developed by IGSS.

4.2.5 Statistical Analysis

4.2.5.1 Phenotypic analyses

The statistical package XLSTAT software was used to calculate the mean value of all the twelve morphological characters and for sample matching. Analysis of variance was run to test the variation among different genotypes under study. Also, Principal component analysis (PCA) was used to depict non-hierarchical relationships among the genotypes. Eigen values and eigenvectors were calculated by the Eigen program using a correlation matrix as inputted (calculated using standardized morphological data). Genetic distance between and within plants were calculated using the Mahalanobi's D2 (1936).

Intra- and inter-cluster distance and mean performance of the clusters for the character were computed. The percent contribution of each trait to total variability was manually calculated from table of cluster means generated be the software as follow:

$$TC\% = \frac{\sum T_i}{\sum C_i} * 100$$

Where, TC% = Percent contribution of specific trait to total divergence, T_i = Total contribution of a trait i^{th} obtained by adding all contributions of the trait across clusters, Ci = Total sum of clusters contribution obtained by adding contribution of each cluster across the traits.

4.2.5.2 Molecular analysis

Power Marker version 3.25 was use in estimating genetic parameters such as number of alleles (Na) major allele frequency, number of observed genotypes per marker locus, genetic distance, and polymorphic information content on the SNP (Liu and Muse, 2005).

A model-based software known as STRUCTURE (Pritchard *et al*. 2000) was used to deduce the population structure of the tested accessions; with an assumption that, frequencies of the alleles are correlated with unlinked loci. Among the parameters used included a population size (K) of 10, with 10 repetitions each based on a burning time of 100,000 iterations and 200,000 iterations of Markov-Chain Monte Carlo (MCMC) method. Two separate analyses using same parameters were performed. The actual value for K was estimated using an online post STRUCTURE analysis called Structure Harvester (Earl et al., 2012) base on the second-order rate of change of likelihood of data between consecutive K values as illustrated by Evanno *et al*. (2005). In this method, accessions having ≥90% resemblance were grouped in the same cluster while those with lower resemblance (<90%) to any particular group were assigned to an ‘‘admixed’’ group. Further, a matrix of genetic dissimilarity was computed using simple matching index and used in cluster analysis to generate a dendrogram based on ‘‘unweighted neighbor joining’’ method (Saitou and Nei 1987) in DARwin (Perrier and Jacquemoud-Collet 2006) and to show a graphical representation of the genetic relationships among the accessions, a principal coordinate analysis (PCoA) was performed using molecular data, and a scatter plot of the first two principal coordinates constructed.

4. 3 Results

4.3.1 Phenotypic characterization of 90 rice accessions for diversity

Mean performances of the genotypes varied for the ten traits recorded in the current study (Table 4.2). The means ranged from 2.02 for flag leaf area to 63.54 for leaf length. Highest coefficient of variation (23.56 %) was obtained for number of tillers per plant (NTP) with the lowest being 0.52 % for leaf width. The least significant difference of means among the traits from 0.16 for LBP to 16.57 for plant height. The results showed standard deviations ranging from 0.22 for seed vigor to 44.30 for flag leaf respectively.

Table 4. 2 Mean agronomic performances of 90 rice genotypes

Trait	Minimum	Maximum	Mean	Standard deviation	CV%	LSD (5%)
FLA (cm^2)	1.00	5.00	2.03	1.54	17.55	0.20
LA	1.00	5.00	2.42	176	0.52	5.20
LBP	1.00	3.00	2.33	3.69	3.90	0.16
LL (cm)	44.30	82.70	63.53	7.90	3.38	7.08
LW (cm)	0.90	2.40	1.43	0.24	1.50	0.20
NTP	3.00	49.00	21.41	9.73	11.48	9.82
TBi	1.00	8.50	3.59	1.85	23.56	2.07
PT	1.00	3.00	1.63	0.68	11.84	2.10
PHT (cm)	52.08	129.60	97.48	14.37	14.76	16.57
Seed-Vig	1.00	7.00	1.66	1.44	18.46	0.15

LSD= Least Significant difference at 5%, CV= % Coefficient of variation, SE= Standard error of difference, FLA= Flag leaf area, LA= Leaf area, LBP= Leaf blade Pubescence, LL= Leaf length, LW= Leaf width, NTP=No. tillers/plant, TBi= Tiller ability, PT=Panicle type, PHT= Plant height, and Seed-Vig= Seedling vigor.

The results for analysis of variance are presented in Table 4.3. The results showed significant variations among the accessions ($p \leq 0.01$) however, the blocking effect was not significant. The mean squares due to genotypes varied from 0.08 for leaf width to 416.06 for plant height.

Table 4. 3 Mean squares for twelve traits of ninety rice genotypes evaluated under salt stress and non-salt stress (control) conditions in 2017.

Source of		**Mean Squares**									
variation	**DF**	**FLA**	**LA**	**LL**	**LW**	**PT**	**LBP**	**NTP**	**PHT**	**Seed-Vig**	**TBi**
Block	1	0.00	0.03	4.10	0.02	0.00	0.01	102.76	91.87	0.01	40.14
Genotype	89	4.73**	6.30	80.86**	0.08**	0.98	0.90**	192.04**	416.06**	4.09**	4.86**
Residual	89	0.01	1.00	20.87	0.01	0.00	0.01	24.43	69.69	0.01	1.09
Total	179										

Significant at $p< 0.01$; Flag leaf area (FLA), leaf area (LA), leaf length (LL), leaf width (LW), panicle type (PT), leaf blade pubescence (LBP), No. of tillers per plant (NTP), plant height (PHT), seedling vigor (Seed-Vig), tiller ability (TBi).

4.3.2 Phenotypic correlation among traits of 90 rice genotypes

The number of tillers per plant (NTP) and Leaf blade pubescence (LBP) were high positively, significantly correlated ($p\leq 0.01$) (Table 4.4). Similar trends of correlation ($p\leq 0.01$) were observed between Leaf area (LA) and flag leaf area (FLA), Plant height (PHT) and leaf blade pubescence (LBP), plant height (PH) and number of panicle per plant (NPP), seedling vigor (SeedVig) and leaf blade pubescence (LBP), and seedling vigor (SeedVig) and plant height (PH) respectively. Additionally, the study found significant correlations ($p\leq 0.05$) between leaf blade pubescence (LBP) and leaf area (LA), number of panicles per plant (NPP) and leaf area (LA), plant height (PHT) and flag leaf area (FLA), plant height (PHT) and leaf area (LA), and seedling vigor (SeedVig) and leaf area (LA). Generally, the trait Leaf area (LA) had significant correlations with majority of the traits investigated in this study. No significant correlations were observed between seedling vigor and leaf blade pubescence (LBP), panicle type (PT) and all other leaf architecture parameters.

Table 4. 4 Pearson correlations among 10 traits of rice accession evaluated

Variables	TBi	NTP	SeedVig	LBP	PT	PHT	LL	LW	LA	FLA
TBi	1									
NTP	**-0.93****	1								
SeedVig	0.58**	**-0.53****	1							
LBP	-0.25**	0.25**	-0.24**	1						
PT	0.05	-0.09	0.09	-0.26**	1					
PHT	-0.54**	0.51**	**-0.73****	0.30**	-0.17	1				
LL	-0.15**	0.14**	-0.02	0.02	-0.07	0.25**	1			
LW	-0.02	0.01	0.11*	-0.26**	-0.07	0.08	0.13	1		
LA	0.20**	-0.14*	0.10*	0.05	-0.01	-0.10*	-0.04	-0.01	1	
FLA	0.17**	-0.11*	0.04	-0.04	-0.12*	-0.04	-0.04	-0.06	0.53**	1

TBi = Tiller Ability, FLA= Flag leaf Area, LA= Leaf area, LBP= Leaf blade Pubscence, LL= Leaf Length, LW= Leaf width, NTP= No. of Tiller per plant, PHT= Plant height, SeedVig= Seedling vigor (**=at 1% level of significance).

4.3.3 Principal component analyses (PCA)

The PCA was performed for all traits among the 90 rice genotypes as indicated in Figure 4.1. Out of ten, four principal components showed major contribution with each component exhibiting eigen value greater than 1. Collectively, the four vectors contributed 71.53% variability among the traits studied. The PC1 had 31.59%, PC2 showed 15.54%, PC3 exhibited 12.52% and PC4 contributed 10.98 variability among the genotypes for the traits under study. The eigenvalues ranged from 4 to 1 while the percent variance explained was between 0.4 to 1.0 respectively. The remaining components had variance explained values below 1%.

4.3.4 Eigenvalues and eigenvectors for major agronomic traits

Principal component one (PC1), principal component two (PC2), principal component three (PC3) and principal component four (PC4) had eigenvalues of 3.16, 1.55, 1.25 and 1.09 respectively. The first PC was highly and positively correlated to number of tillers per plant,

plant height. The PC2 was positively correlated with leaf area and lag leaf area. The results showed positive correlations between PC3 and leaf width. The PC4 was positively correlated with panicle type. However, it was negatively correlated to leaf area, tiller ability, and seedling vigor. In the second PC, leaf area, flag leaf area, where highly positively correlated trait. On the other hand, it was highly and negatively correlated to number of tillers per plant, seedling vigor, leaf length, panicle type and leaf width. The third PC exhibited high positive correlation between leaf width and leaf length. However, it was highly and negatively correlated to panicle type and leaf blade pubescence. In PC4 only panicle trait was highly correlated, the rest of the other traits were negatively correlated.

Table 4.5 Eigenvalues and Eigenvectors for major agronomic traits in rice accessions collected from the mangrove swamps in northern and southern Sierra Leone in 2018.

Trait / Cluster	**PCI**	**PCII**	**PCIII**	**PCIV**
Trait	**Eigenvectors**			
TBi	-0.500	0.069	-0.028	-0.202
NTP	**0.485**	-0.017	0.037	0.204
SeedVig	-0.445	-0.110	0.083	-0.166
LBP	0.234	0.291	-0.381	-0.430
PT	-0.105	-0.289	-0.128	**0.714**
PHT	**0.453**	0.102	0.160	-0.012
LL	0.131	-0.056	0.484	-0.256
LW	-0.013	-0.205	**0.702**	-0.061
LA	-0.124	**0.602**	0.186	0.284
FLA	-0.114	**0.630**	0.205	0.212
Eigenvalue	3.160	1.55	1.25	1.098
Total variability (%)	31.598	15.54	12.52	10.98
Cumulative (%)	31.598	47.14	59.65	70.63

At least four variables demonstrated high eigenvalues and explained variance for tolerance to abiotic stresses. The eigenvalues ranged from 4 to 1 while the percent variance explained was between 0.4 to 1.0 respectively. The remaining components had variance explained values below 1% (Figure 4.1). The results showed assortment of traits into different clusters-based o the four major actors as presented below (Figure 4.2).

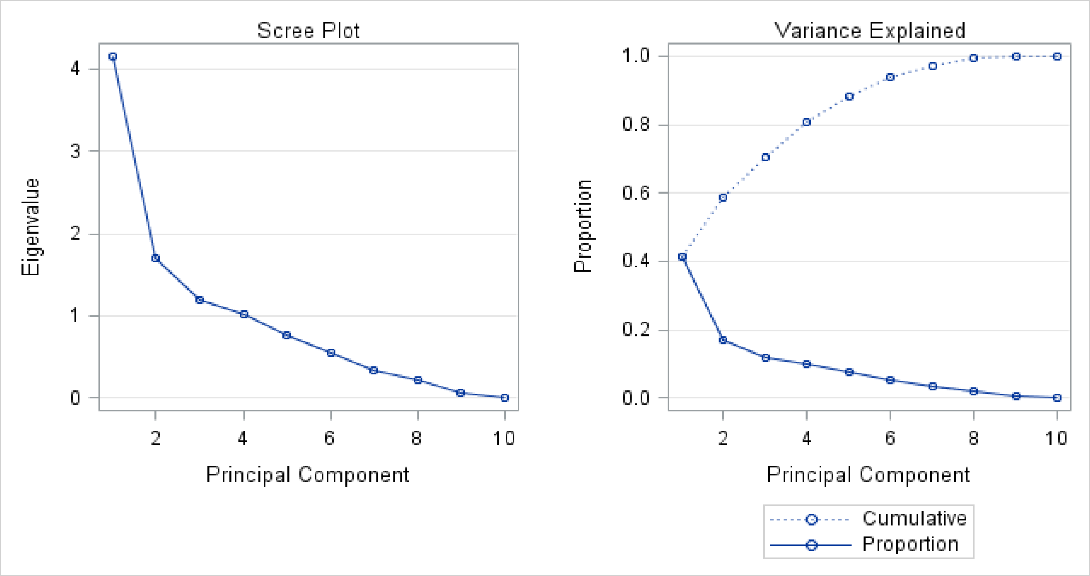

Figure 4. 1The eigenvalues and variance explained by four major principal components for diversity.

Table 4. 6 Inter-class distances and their P-probabilities for major agronomic traits evaluated in 90 accessions of rice.

Class	Inter-class distance				*P*-probability			
	I	II	III	IV	I	II	III	IV
I	0	24.96	20.72	**29.09**	1.0000	**< 0.0001**	< 0.0001	< 0.0001
II	24.96	0	15.02	**46.45**	**< 0.0001**	1.0000	< 0.0001	< 0.0001
III	20.72	15.02	0	**39.69**	**< 0.0001**	**< 0.0001**	1.0000	< 0.0001
IV	29.09	**46.45**	**39.692**	0	**< 0.0001**	**< 0.0001**	**< 0.0001**	1.0000

4.3.5 Cluster analyses

The Dendrogram results of the rice accessions showed various patterns of four distinct major groups of clusters (Figure 4.3). Each main group was further divided into four clusters and sub-clusters with each sub-cluster having a number of fewer accessions contained in smaller clusters. The correlation among the four Principal Components and accessions within each cluster is shown in biplot diagram below (Figure 4.4). Accessions in cluster 1 are positively correlated with plant height, number of tillers per plant. Cluster 2 consist of genotypes positively correlated with flag leaf length and leaf area. Accessions 9, 25, 27 and 77 were grouped in cluster number 2. Cluster number 3 included accessions number 18, 31, and 80 while cluster 3 had accessions 3, 15 and 35 respectively.

Table 4. 7 Distribution of 90 genotypes of rice among 4 clusters on the basis of D^2 analysis

Cluster	Cluster size	Cluster composition	Intra cluster D2	Cluster mean (No. Till)
I	15	1,5,16,18,26,30,31,33,35,38,41,61, 80,82	134.06	14.400
II	46	2,3,4,6,7,9,11,12,14,14,17,19,30,33,37,32,34,36,37,39,40,43,44,46,47,48,49,50,51,54,57,58,59,62,6664,65,67,68,69,70,73,74,76,79,82,83,	127.94	28.967
III	21	8,10,15,21,22,24,25,29,42,45,52,53,55,62,63,66,75,77,78,84,87	180.05	15.131
IV	08	56,71,72,81,85,88,89,90	131.36	8.563

4.3.6 Trait contribution to genetic diversity

Mean values of 4 clusters for different quantitative and qualitative characters in mangrove rice and their contribution to total divergence is shown below (Table 4.8). The highest contribution to genetic divergence was from panicle type with 15.52 %, followed by leaf width with 13.47% and 12.34 for Flag leaf area.

Table 4. 8 Mean values of 4 clusters for different quantitative and qualitative characters in mangrove rice and their contribution to total divergence

Traits	I	II	III	IV	Contribution %
TBi	4.967	2.144	4.667	6.406	7.40
NTP	14.4	28.967	15.131	8.563	6.95
SeedVig	1.533	1.056	1.667	5.25	6.12
LBP	2.267	2.467	2.333	1.625	11.75
PT	1.667	1.614	1.476	2	15.52
PHT	89.125	104.248	101.957	63.338	6.04
LL	52.273	64.927	68.51	63.675	8.01
LW	1.313	1.427	1.486	1.55	13.47
LA	3.133	1.8	3.19	2.5	12.31
FLA	2.2	1.578	2.81	2.25	12.34

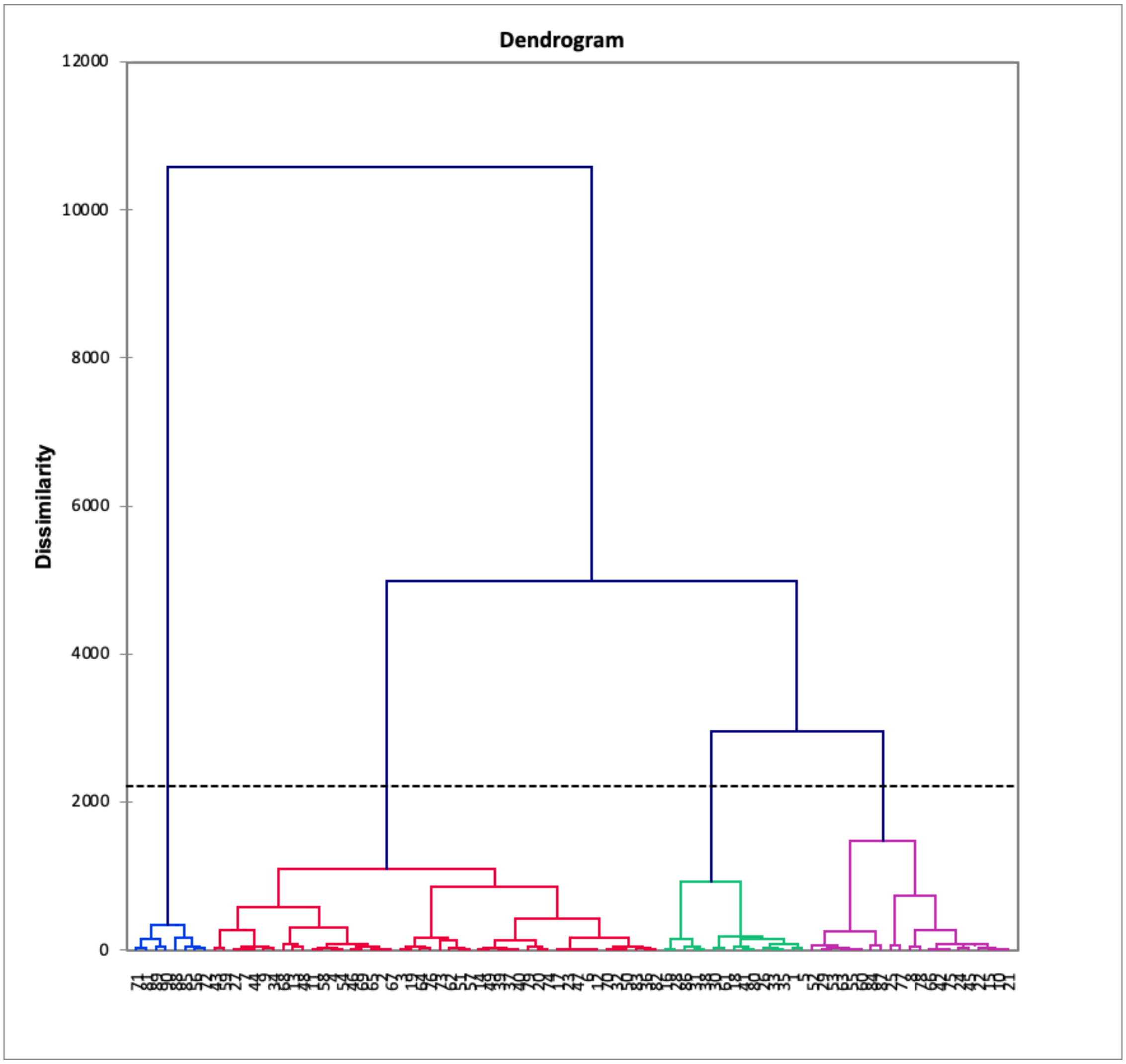

Figure 4. 2. Morphological base dendrogram of 90 rice accession using 10 discriminate phenotypic characters

Results of correlation among the four principle components are shown in Figure 4.4. Cluster 2 and Cluster 3 showed large overlapped and shared most of genotypes between them. Cluster 4 had clear separation from rest of the clusters in terms of genotypes while Cluster 1shared some genotypes with clusters 3 and 2.

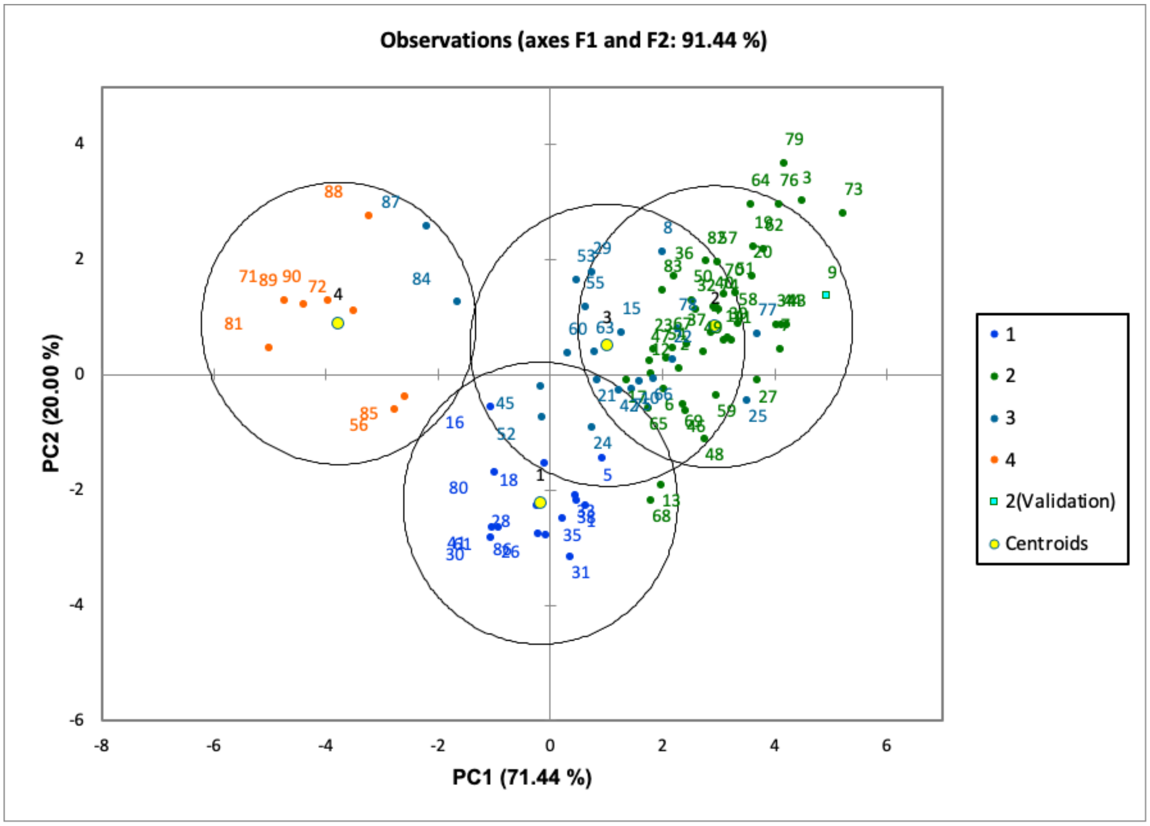

Figure 4. 3 Biplot showing correlation among the four major Principal Components for 90 rice accessions

4.3.7. Genetic diversity analysis among the 90 rice accessions

A total of 34 SNPs loci were evaluated for their polymorphism across 90 accessions of rice assembled. Among the 34 SNPs, 29 were polymorphic and retained for further analysis (Table 4.9). 30 alleles with a mean of 1.85 alleles per locus were obtained from these twenty-nine SNPs. Each SNP contained a locus with two alleles (tolerance and susceptible). Major allele frequency (MAF) ranged from 0.51 to 0.99 with an average of 0.71. Gene diversity (D) fluctuated between 1% to 50% with an average of 36%. PIC range from 0.01(xa5-S1_SKEP) to 0.37(snpOS0301) with a mean of 0.28. The SNPs snpOS0305, snpOS0305, snpOS0314, snpOS0317, snpOS0318 were the most informative with polymorphic information content (PIC) values ranging from 0.34

to 0.37 and gene diversity values of 0.44 to 0.50. These SNPs also had the lowest levels of heterozygosity ranging from 0.00 to 0.01. The SNPs snpOS0295, snpOS0304, xa5-S1_SKEP, snpOS0009 and snpOS0040 had the lowest PIC values that ranged from 0.01 to 0.18. Generally, low level of heterozygosity was observed among the SNPs ranging from 0.00 to 0.01 respectively.

Table 4. 9 Summary statistics for the SNPs used in the study for genotyping of 90 rice accessions collected from mangrove swamps in Sierra Leone

Marker	Major allele frequency	Genotype Number	Number of observations	Number of allele	Availability	Gene Diversity	Heterozygosity	PIC
snpOS0295	0.89	2	88	2	0.98	0.20	0.00	0.18
snpOS0296	0.57	2	87	2	0.97	0.49	0.00	0.37
snpOS0297	0.74	3	90	2	1.00	0.38	0.02	0.31
snpOS0298	0.69	2	88	2	0.98	0.43	0.00	0.33
snpOS0299	0.77	3	88	2	0.98	0.36	0.01	0.29
snpOS0300	0.70	3	86	2	0.96	0.42	0.01	0.33
snpOS0301	0.56	2	85	2	0.94	0.49	0.00	0.37
snpOS0302	0.70	3	85	2	0.94	0.42	0.01	0.33
snpOS0303	0.72	2	86	2	0.96	0.40	0.00	0.32
snpOS0304	0.98	2	89	2	0.99	0.04	0.00	0.04
snpOS0305	0.56	3	88	2	0.98	0.49	0.01	0.37
snpOS0306	0.77	3	89	2	0.99	0.35	0.01	0.29
snpOS0307	0.51	3	67	2	0.74	0.50	0.01	0.37
snpOS0308	0.57	3	86	2	0.96	0.49	0.02	0.37
snpOS0309	0.76	3	87	2	0.97	0.36	0.01	0.30
snpOS0310	0.87	3	88	2	0.98	0.23	0.01	0.20
snpOS0311	0.70	3	83	2	0.92	0.42	0.02	0.33
snpOS0312	0.57	2	84	2	0.93	0.49	0.00	0.37
snpOS0313	0.52	2	88	2	0.98	0.50	0.00	0.37
snpOS0314	0.52	2	85	2	0.94	0.50	0.00	0.37
snpOS0315	0.63	3	88	2	0.98	0.47	0.01	0.36
snpOS0316	0.82	2	84	2	0.93	0.29	0.00	0.25
snpOS0317	0.67	2	79	2	0.88	0.44	0.00	0.34
snpOS0318	0.59	2	85	2	0.94	0.48	0.00	0.37
S12_17618268	0.86	3	88	2	0.98	0.24	0.01	0.21
xa5-S1_SKEP	0.99	2	87	2	0.97	0.01	0.01	0.01
snpOS0009	0.98	2	82	2	0.91	0.05	0.05	0.05
snpOS0024	0.51	3	87	2	0.97	0.50	0.02	0.37
snpOS0040	0.99	2	86	2	0.96	0.02	0.00	0.02
Mean	0.71	2	86	2	0.95	0.36	0.01	0.28

PIC = polymorphism information content.

Results showed that best K value for clustering of the genotypes was K=2 as shown in Figure 4.5a. The population structure was generated using Structure Plot version 2.0 software (Figure 4.5 b). Populations with scores >0.80 were considered pure while those with scores <0.80 were admixtures (Figure 4.5b). The results showed two distinct sub-populations and a group of admixtures. Results of factorial analysis of the two first principal axes were shown in the factorial analysis in which (Figure 4.6). The dendrogram results indicted clustering of the populations into two main groups (Figure 4.7)

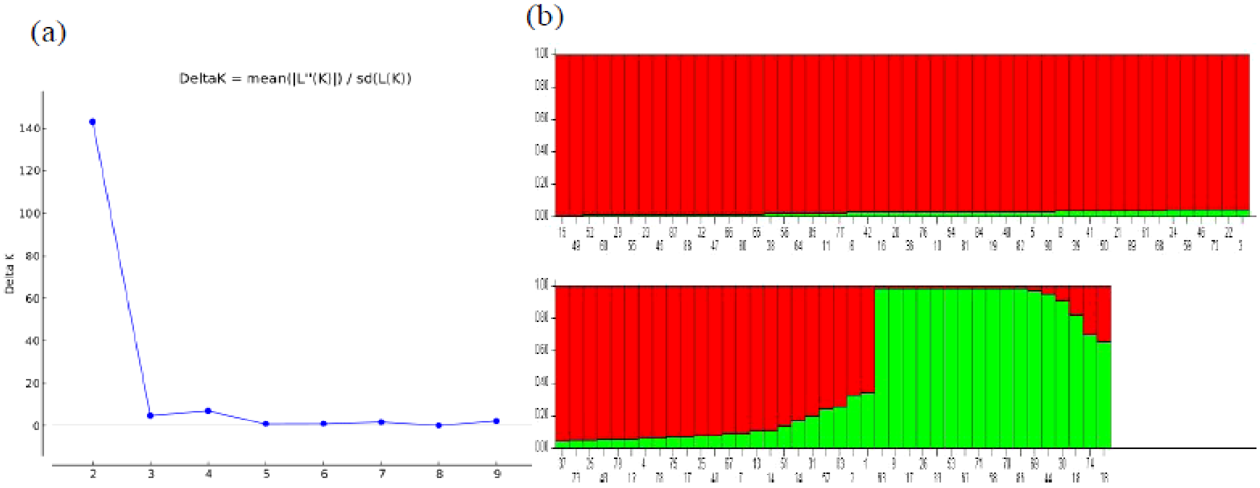

Figure 4. 4 Model-based clustering of the 90 mangrove rice genotypes using SNP markers (k=2): (a) estimation of population using LnP9D) derived Δk for k from 1 to 20 using SNP marker data; estimation by Evanno method markers for K = 2; (b) estimated population structure

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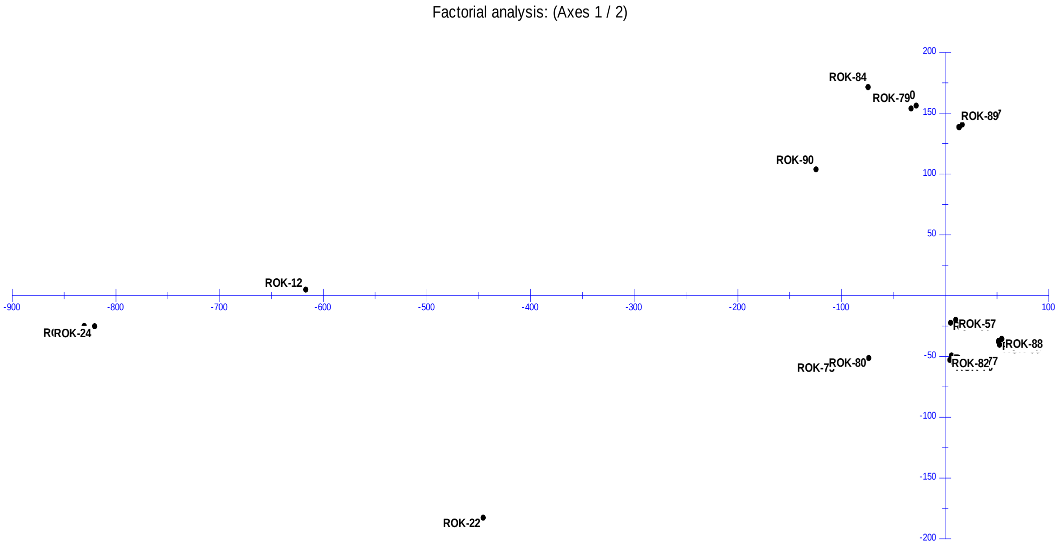

Figure 4. 5 Factorial analysis among the 90 mangrove rice accessions.

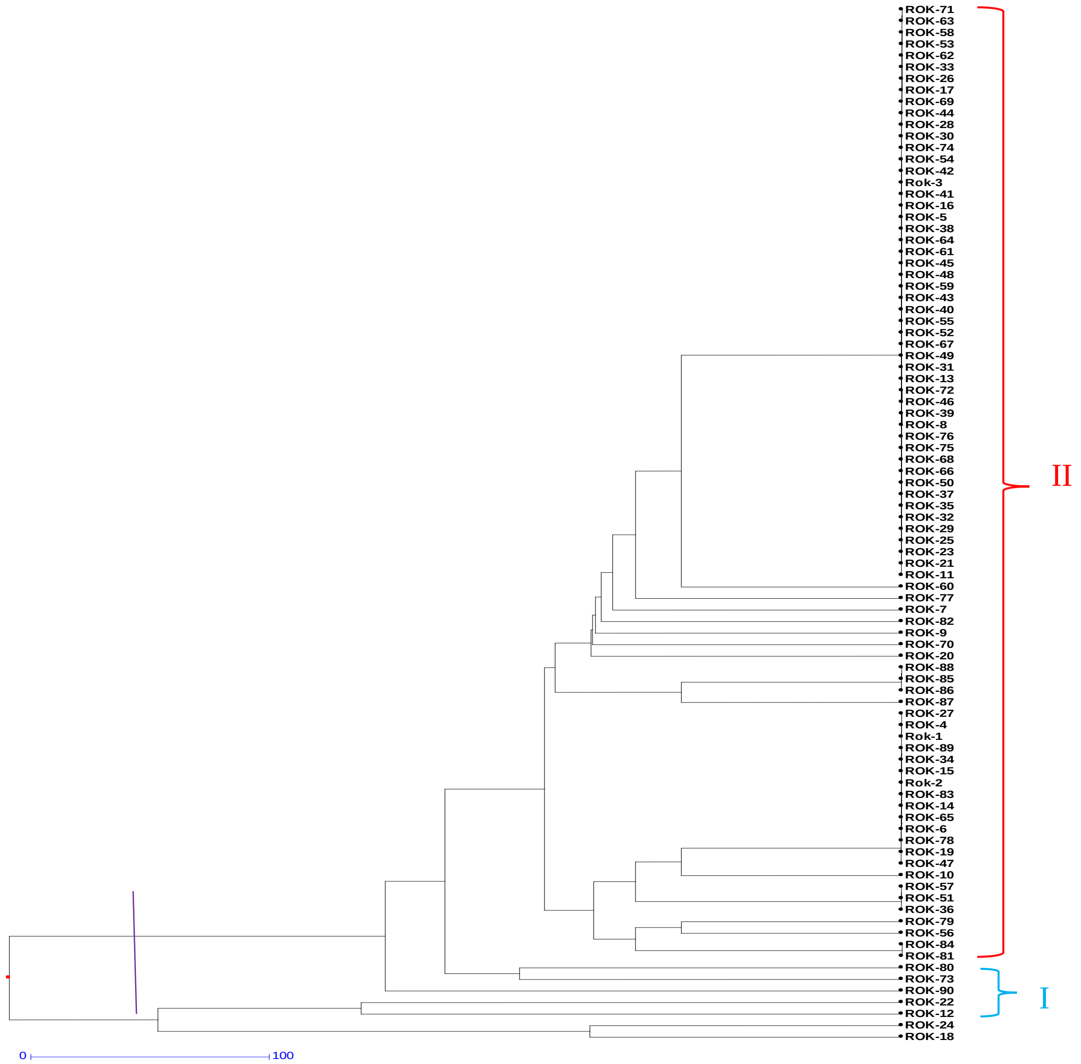

Figure 4. 6 UPGMA dendrogram base on SNP data showing relationship of mangrove rice accession

4.4. Discussion

Agro-morphological characterization of germplasm

The significant mean squares for genotype observed in this study might suggest genotypes were genetically different than environment and that might have resulted into variability in mean performances among the 90 rice accessions. The non-significant means squares detected for the block could imply that blocking was not important and did not cause variability in performances among the accessions. The non-significance of mean squares of blocking could be because the experiment was conducted in screen house whereby uniformity of environment within the screen house had resulted into blocks being environmentally similar. It was evident from the results that the treatment differences were highly significant for all traits except leaf area. This indicates that there exists an inherent genetic difference among the germplasm. Kumari et al. (2018) investigated the genetic diversity among 45 rice germplasm using Mahalanobi's D^2 and found reported significant variations among the genotypes for the studied traits.

The strong correlations observed among traits in the present study imply that the studied traits are genetically related. Therefore, information obtained for one trait can be used to understand or predict the behaviour of another related trait. The importance of correlation among traits has been reported. Zeng *et al.* (2003) in their studies showed that there is a highly significant ($p<0.001$) correlations between Na-Ca selectivity and the rankings among genotypes for grain yield stated that Na-Ca detection could be one salt tolerance component and useful selection criterion in screening for salt tolerance. Rahman *et al*. (2019) argued that for complex traits like salinity, information on the relationship among phenotypic traits can provide useful information for designing breeding programmes. In the current study, for example, positive correlation was observed between seed vigor and tillering ability, meaning high seed vigor could lead to high tillering ability. However, the study found negative correlations among some of the traits for example between plant height and tillering ability were negatively correlated, implying genotype with high tillering ability could indirectly, through interactions with other traits, reduce plant height and *vice versa*. Genetic correlation among traits has implications in designing breeding programmes. This is critically important especially when funding is limited therefore, more traits can be studied with limited resources.

In the present study, panicle type had a maximum contribution followed by leaf width and flag leaf area, suggesting the importance of contributions of these traits towards divergence among the among the rice accessions. These findings are in agreement with Islam *et al*. (2018), who reported high genetic divergence for panicle type in qualitative trait analysis. Kumari *et al*. (2018) found that grain per panicle and days to maturity contribute least towards genetic diversity whereas days to 50 per cent flowering, plant height and panicle length were found to be the most contributing traits towards genetic diversity implying these traits could be useful for selection while improving grain yield. Odjo *et al.*, (2017) reported high genetic divergence for qualitative and quantitative traits in rice. Generally, the present results indicate that panicle type, leaf width, flag leaf area, leaf blade pubescence, leaf length, and tiller ability are potential contributors to genetic divergence.

Molecular characterization of germplasm

The current study observed low heterozygosity values among the SNP markers which might indicate that these markers are highly informative for the rice accessions under study. Suvi *et al.* (2019) reported low heterozygosity among 54 rice genotypes from Tanzania using 14 polymorphic SSR markers. Similarly, Yelome *et al.* (2018) reported low heterozygosity in 42 rice accessions from six West African countries using 20 polymorphic SSR markers. Many other studies have reported minimal amount heterozygosity in rice (Chourhury *et al.* 2014; Nachimuthu *et al.* 2015).

Polymorphism information content (PIC) is an important measure of allele frequency of a marker since markers with higher PIC are usually preferred for germplasm characterization (Boopaathi, 2013; Singh *et al*., 2013). Furthermore, marker informativeness can be correlated to high values of heterozygosity and PIC statistics. Low PIC levels were observed in the present study which could be attributed to low number of SNPs use. Hoque *et al.* (2014) screen 30 rice genotypes using three SSR markers and they found that marker RM167 was the best in screening the thirty rice genotypes due to its highest PIC value followed by marker RM147 and RM215. Contrarily, Mvuyekure *et al.* (2017) genotyped 25 rice accessions using 94 SNP markers and they observed relatively low PIC values. The authors suggested that the low PIC values could be attributed to the usage of released lines or adapted local varieties which may have been subjected to increase selection. In the present study, the high PIC values detected could be due to diverse nature of the mangrove rice accessions and which have not undergone selection before. Anupam *et al.* (2017) reported high PIC > 0.5 for the study of Tripura with trait linked markers. This result is also in agreement with findings by Chen *et al.* (2011), who also reported low PIC values of 0.28 for three hundred inbred lines using SNP. They also observed low gene diversity 0.35 compared to the findings of the present studies with 0.28 and 0.36 for PIC and gene diversity respectively. However, Behera *et al.* (2012) reported higher mean diversity indices (4.69 alleles and PIC of 81%) among 36 Indian landraces with different therapeutic properties using SSR markers.

The various clusters observed within the 90 rice accessions under the present study indicate that these accessions belonged to different genetic groups. This underscores the genetic diversity within the rice germplasm from the mangrove swamps. Also, the large dissimilarity values detected by the SNP markers shows the importance of marker application in cluster analysis of rice germplasm. The clusters observed in this study could be a useful tool for designing breeding programmes targeting hybridization among the accessions. Similarly, the cluster analysis provides useful information for genetic conservation since the different clusters have been revealed. Reig-Valiente *et al*., (2016) observed that grain type contributed to the genetic classification of rice cultivars, which suggest that the trait is important in the morphological stature of the plant. The authors also found in their study that use of genetic distance provides an explicit variability pattern in rice genotypes. The inter-cluster distances observed in this study were higher than the intra-cluster distances suggesting presence of wider genetic diversity among accessions in different groups. This could provide good genetic resources for hybrid development. Study by Maurya (2005) suggested parental selection for hybridization that should be based on two clusters having wider inter-cluster dissimilarity.

PCA value shows the how individual characters influence the classification of accession. The higher values observed for PC1 in the present study indicates the importance of the principle component one in influencing the mean phenotypic performance of the traits. Study by Ahmed *et al.* (2016) reported the contribution of 82.90% and 75% of the first five and four component to the total variation in rice respectively. In the present study, only two main groups were detected in the population structure, suggesting: (i) either the SNP primers used were too small and not discriminative enough to distinguish among these pairs of accessions (ii) The accessions have close genetic relations but different names across locations.

4.5 Conclusion

Cluster analysis of the agro-morphological traits grouped all the 90 accessions into four groups. Significant differences ($p \leq 0.05$ and $p \leq 0.01$) were found for most of the traits studied, which indicated the existence of diversity in the ninety-rice germplasm. On the other hand, results from the molecular analysis using SNPs markers revealed two cluster groups, which were confirmed by population structure analysis to show all the 90-rice accession assembled into two populations. This study concluded one of two things; that the SNP markers used in the study were not discriminatory enough to differentiate between the bulk of the genotypes clubbed into major group II, or a lot of duplicate accessions were identified. The low diversity index also reported could be due to low number of SNP markers used.

CHAPTER FIVE

5.0 MOLECULAR ANALYSIS AND IDENTIFICATION OF NOVEL QTL ASSOCIATED WITH SALT STRESS TOLERANCE IN RICE USING DArT MARKERS

5.1 Introduction

Anticipated increase in climate change will influence a number of abiotic stresses affecting rice such as salinity, flooding, drought, heat and submergence which pose serious constraint to rice production affecting the overall future food security (Rena *et al.,* 2019). Salinity is one of the major abiotic stress affecting rice production in soil affected areas and especially in coastal areas, causing more than 50% yield reduction in crop (Rahman *et al.*, 2017) and is projected to deteriorate more due to unprecedented change in climate. Hence the need to breed climate-smart crop to match up with abiotic stresses and to maintain food production cannot be over emphasize (Khan *et al.,* 2016; Rahman *et al.*, 2017).

Rice is a staple food that feeds half of the world's population but is reported to be highly sensitive to salinity during different developmental stages (Zhu *et al.,* 2001, Singh *et al.* 2010; Hossain *et al.* 2015). Seedling stage salinity have been reported to be very crucial in the overall yield of a rice crop (Bimpong *et al.,* 2013). Rice cultivation in costal mangrove areas is highly dependent on rainfall which is crucial for salinity tolerance at the seedling stage. According to Baggie *et al.* (2018), soil salinity is high at the beginning of the raining season which is very critical to seedling establishment. Salt affected soils are associated with the occurrence of high amount of sodium ions (Na+) which is detrimental to the normal growth and development of rice plants. High (Na+) in the soils can have negative effect on photosynthesis which correlate with reduction in plant growth, chlorophyll content and metabolic processes (Munns and Gilliham 2015; Rahman *et al.* 2019). It has been established by researches that rice is tolerant to salinity stress during germination and active tillering, whereas it displays more sensitivity during early seedling and reproductive stages (Lutts *et al.*, 1995; Bimpong *et al.* 2013, Rahman *et al.* 2016; Zhu *et al.*, 2018,) A detailed understanding of the genetic nature of salinity tolerance would aid in breeding for salt stress.

With the introduction of molecular marker technology, much significant contribution has been made in the discovery of quantitative trait loci (QTL) influencing important genetic variation in crops (Ismail and Horie, 2017; Rahman *et al.* 2017). QTL identification aides in the quick discovery of genes related to trait variation under polygenic control and in developing salinity tolerant rice varieties is of great importance (Yao *et al.*, 2005; Sabouri and Sabouri, 2009; Ahmadi and Fotokian, 2011; Islam *et al.*, 2011; Thomson 2014; Hossain *et al.*, 2015; Rahman *et al.*, 2019. Rena *et al.* 2019). The use of molecular marker technology in breeding appear to be the best resourceful technique for evaluation and rapid selection of planting materials (Chen *et al.*, 2017). In contrast, relying on conventional breeding methods for screening for salinity tolerance is time consuming and quite enormous due to large environmental effect and low narrow sense heritability of salt tolerance (Gregorio, 1997). Several QTLs have been identified for salinity tolerance in rice using different mapping populations such as RIL, DH or $F_{2:3}$ (Zhu *et al.*, 2018; Bimpong *et al.*, 2013),

The recent development of the diversity arrays technology-based sequencing (DArTseq) platform (Sansaloni *et al*., 2011) have enhance the efficiency and use of SNP markers, it generates high density SNP information with significantly reduced genotyping cost, allowing detailed molecular characterization of germplasm preserved in gene banks. Compared to genotyping by sequencing (GBS), DArTseq can produce more result and lesser occurrence of missing data (Chen *et al.*, 2016). DArTseq assay has gain popularity in the studies of several species including Oryza sativa spp and japonica (Courtois *et al*., 2013).

Therefore, the objectives of this study were to:

(1) Identify and map QTL controlling salt tolerance at vegetative and reproductive stage in Sahel 317×Nerica L9 F4 RIL populations using 13,432 SNP markers under field conditions; and

(2) Select best performing RILs for their further use as breeding materials or for cultivar development.

5.2 Material and Methods

5.2.1 Experimental Site

The study was conducted under field conditions in 2017/2018 dry cropping season in Mbe, AfricaRice Centre, Cote d'lvoire. The study site is located between 7.5^{o} N and 8.5^{o} N and between 4.5 ^{o} W and 5.5^{o} W, with an average annual rainfall of 985 mm. The station is characterized by 3 main seasons: a long dry season starting in early November with an end in mid-March; a long rainy season from mid-March to mid-July; a short rainy season from mid-July to mid-August, and an inter-season rainy period which starts in mid-August and ends in October.

5.2.2 Plant Materials

Plant material used for QTL mapping included 462 $F_{3:4}$ progenies evaluated for tolerance at the vegetative stage and their 2 parents Sahel 317 and NERICA-L9, were planted in 2018 in Mbe, AfricaRice, Bouake. Nerica L 9, is said to have some tolerance to salinity and was used as the donor parent and Sahel 317 was use as recipient parent. The objective of the cross was to transfer tolerance from Nerica L9 into Sahel 317. These two parent lines were developed by Africarice for tolerance to salinity and have good yield attributes.

5.2.3 Phenotypic evaluation of mapping population

This experiment was carried out during the dry season at the Africarice Center, Bouake, Cote D'dvoire. Seedlings were phenotyped under field conditions. Seeds were heat treated for 5 days in a convection oven set at 50 °C to break their dormancy, surface-sterilized with a fungicide (Vitavax-200, Syngenta) and rinsed with distilled water, then placed in petri dishes lined with moistened filter paper and incubated at 30 °C for 48 h to germinate. The germinated seedlings were transfer to the nursery beds, and transplanted to the main field after 21 days.

Phenotyping of the mapping population was carried out for sterile spikelets, filled spikelets, Plant height, days to 50% flowering, 100 grain weight, number of tillers per plant, and number of panicles per plant at Mbe, AfricaRice in February to May in 2018. Data was collected on the following parameters:

Number of panicles (NPP): Total number of panicles per individual plant

Plant Height (PHT): Height of Individual plants from the base to longest panicle

Number of unfilled Spikelet: Manual counting was done by pressing individual spikelet to determine empty spikelets.

Number of Filled Spikelets: manual counting was done by pressing individual spikelets to determine those that were filled

Days to 50% flowering: Number of days from sowing to 50% of flowering of plants.

Number of Tillers Per Plant: Manual counting of total number of tillers per individual plants

100 Grain Weight: 100 grains were selected and weighed

5.2.4 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 50ºC 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.

5.2.5 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 2677 DArTseq informative SNPs (out of 10219) and 462 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.

5.2.6 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 NERICA-L9 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 Correlation studies of salinity related parameters

Correlation analysis was performed on 8 quantitative plant traits including days to 50% flowering and plant height (Table 5.1). All the yield related rates under study were found to have a strong correlation (at $p< 0.0001$) except for plant height which shows week correlation with the other traits. Days to 50% flowering was highly and significantly correlated with tiller number ($r=0.948$, $p<0.0001$), panicle number ($r=0.728$, $p<=0.0001$), and weight of field grain ($r= 0.899$, $p<0.0001$). Plant height shows significant but negative correlation with days to 50% flowering, tiller number, panicle number, weight of field and empty grain. For a complex trait like salinity an understanding of the relationship among the yield parameters is important.

5.3.2 Genetic Linkage map for the F3 derived between Sahel 317 and Nerica L9

Summary of the genetic linkage groups is presented in Table 5.1. Linkage analysis was performed on 462 F_3 families genotyped by sequencing with 2677 SNP markers. Twelve (12) linkage groups (LG) were constructed which corresponded to the 12 gametic rice chromosomes, spanning a total length of 1600.05 cM at an average marker interval of 0.70 cM (Table 5.2). Linkage group 1 was the longest (198.16 cM) and had the most markers (363 markers) with an average marker interval of 0.55 cM, while 4 LGs (9, 10, 11 and 12) were the least with lengths less than 100 cM. Linkage group 12 with a length of 99.50 cM and 291 markers had the best coverage with a marker at every 0.34 cM. Linkage group 9 had the widest marker interval of 1.45 cM. No QTLs were identified on LGs 3,5,6-10 and 12, thus only 4LGs (chromosomes 1, 2, 4 and 11) contained QTLs for the 4 traits studied.

5.3.3 QTLs for Days to 50% Flowering.

A total of 5 QTLs were mapped for days to 50% flowering (Table 5.2); two on chromosome 1 (*qDTF1.1* between 73.4-73.5 cM and peaked at 73.51 cM; *qDTF1.2* which peaked at 93.21 and flanked between 92.9-96.1 cM), one each on chromosome 2 (*qDTF2* flanked between 104.3-105 cM at a peak of 104.91 cM), chromosome 4 (*qDTF4* at 61.2-67.5 cM and peak 64.53 cM), and on chromosome 11 (*qDTF11* at 32.91 flanked within 32.8 - 33 cM). All 5 QTLs had minor effects and explained 4.5 - 11.2% of the phenotypic variance. The *qDTF4* contributed most to the phenotypic variance (11.2%) with LOD score of 4.19 and additive effect of 0.01. This was followed by *qDTF11* with R^2 of 6.5%, LOD of 4.14 and an additive effect of -0.02. The least contribution to the phenotypic variation came from *qDTF1.2* (R^2 = 4.5%) with 3.64 LOD and additive effect of -0.01.

5.3.4 QTLs for Plant Height.

Five QTLs were identified for plant height (Table 5.2). Among these five, two were mapped to chromosomes 1 *qPH1.1* (between 24.7-27.5 cM and at a peak of 25.71 cM) and *qPH1.1* (at a peak of 132.11 cM within 132.0-133.1 cM). The remaining three were mapped on chromosome 2 (*qPH2*), chromosome 4 (*qPH4*) and chromosome 11 (*qPH11*). The *qPH1.1* was a major effect QTL explaining almost 28% of the variation in plant height under salt stress. This QTL had an LOD of 3.0 and additive effect of 0.03 with the increasing alleles from the donor parent

NERICA-L9. All other four QTLs were of minor effects with R^2 ranging from 1.4 – 5.9% with additive effect range between -0.86 – 0.88.

5.3.5 QTLs for Panicle Number

Of the 4 QTLs identified for panicle number, one each was identified on chromosome 1 (*qPN1;* within 134.4-134.5 cM and peak at 134.51 cM) and chromosome 2 (*qPN2;* peak at 45.71 cM flanked between 44.8-46.1 cM). The remaining two QTLs were mapped to 47.6-49.3 cM (*qPN11.1*) and 59.5-62 cM (*qPN11.2*) on chromosome 11. The qPN2 made the highest contribution to the variation in panicle number with R^2 of 8.4%, alleles coming from the donor

5.3.6 QTLs for Weight of filled grains.

Two QTLs were identified for weight of filled grains on chromosomes 1 and 11. The qWFG1 contributed 3% to the total phenotypic variation and was mapped between 175.6-176.9 cM at a peak of 176.81 cM on chromosome 1. The other QTL was mapped on chromosome 11 between 32.8-33.0 cM with an R^2 of 6.5%.

5.3.7 Colocalization of some QTLs within the same region.

Some loci on chromosomes 4 and 11 harbored more than one QTL for different traits (Figures 3 and 4). On chromosome 4 between 61.2-67.5, *qDTF4* for days to 50% flowering and *qPH4* for plant height colocalized. A similar observation was made on chromosome 11 for days to 50% flowering *qDTF11* and weight of filled grains *qWFG11* at 32.91 cM.

Table 5.1 Pearson correlation coefficient among salinity related rice trait

Variables	Days to 50% Flowering	Plant Height	Tiller Number.	Panicle Number.	Weight of filled grains	Weight of empty grains	Sterility (%)	100 Grain weight
Days to 50% Flowering	1							
Plant Height	0.262*	1						
Tiller Number	0.948***	-0.003ns	1					
Panicle Number	0.728***	-0.002ns	0.821***	1				
Weight of filled grains	0.899***	-0.005ns	0.854***	0.813***	1			
Weight of empty grains	-0.044ns	-0.008ns	0.760***	0.763***	0.820***	1		
Sterility (%)	0.208*	0.758***	0.976***	0.691***	0.752***	0.654***	1	
100 Grain weight	0.41**	0.314*	0.888***	0.931***	0.921***	0.174	0.625***	1

Significant at: $p<0.01$** and $p<0.001$*** respectively.

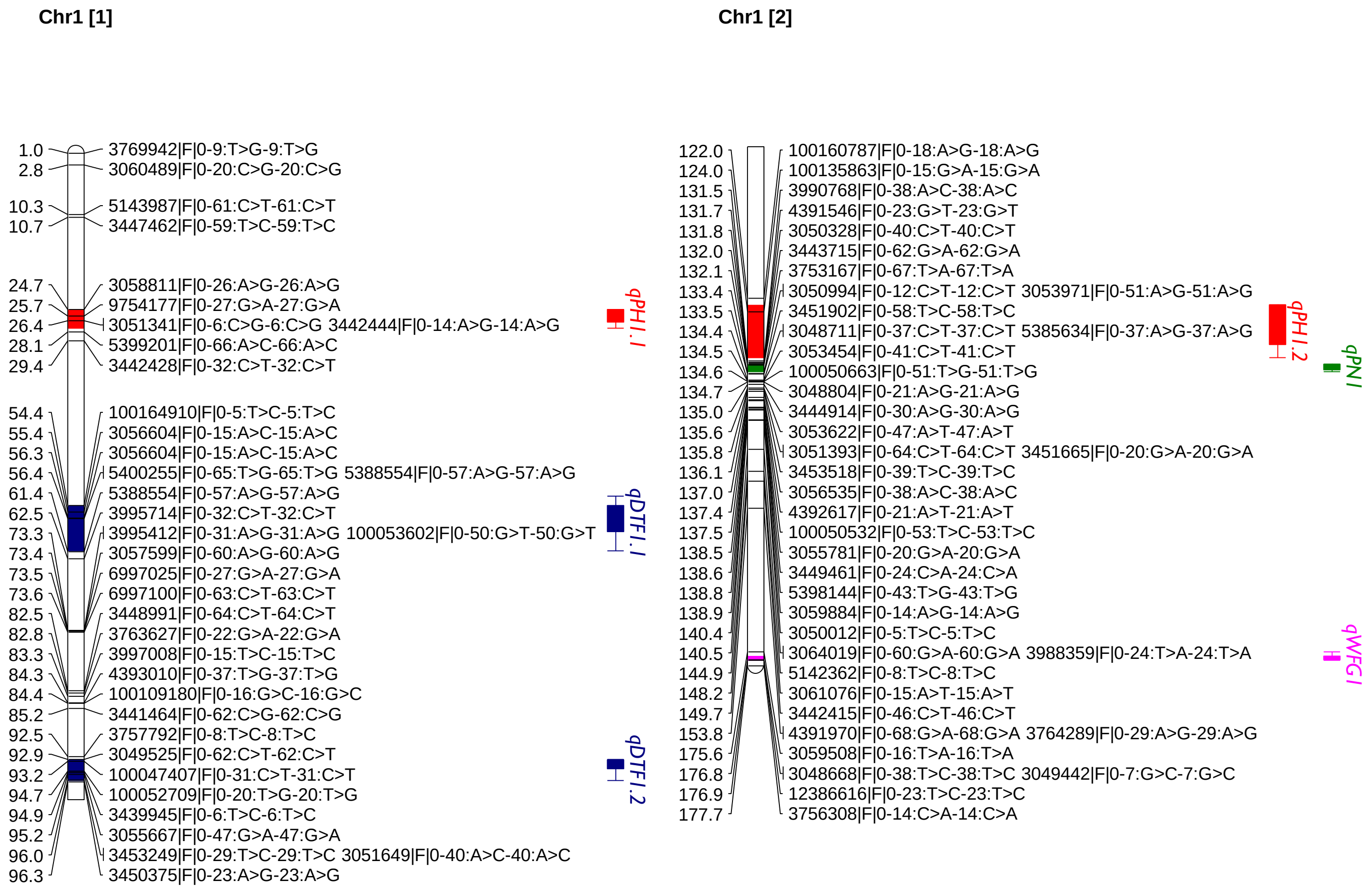

Figure 5. 1 Molecular linkage map showing positions of two major QTLs identified for plant height on chromosome 1.

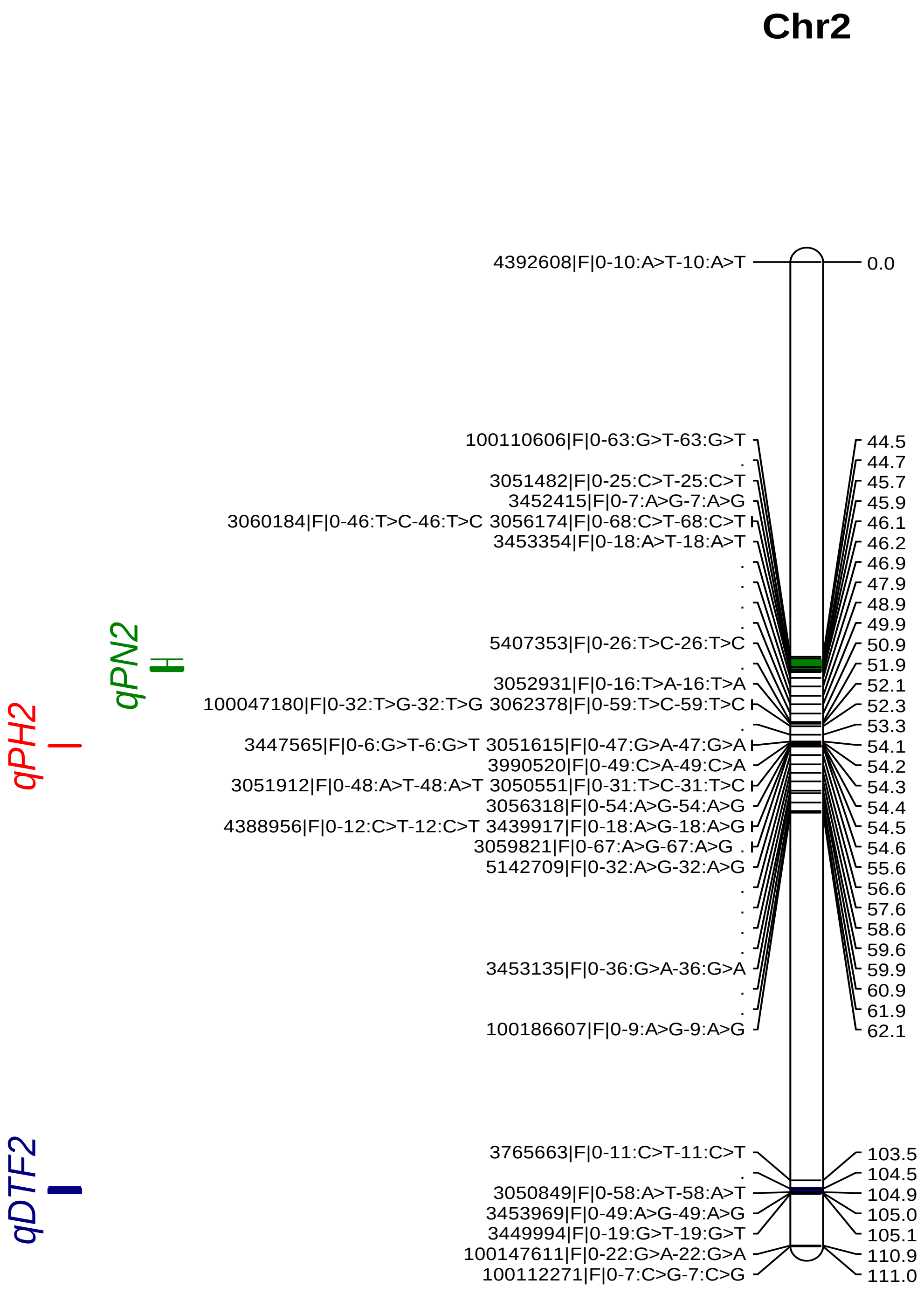

Figure 5. 2 Molecular linkage map showing positions of QTLs identified for plant height and days to 50% flowering on chromosome 2.

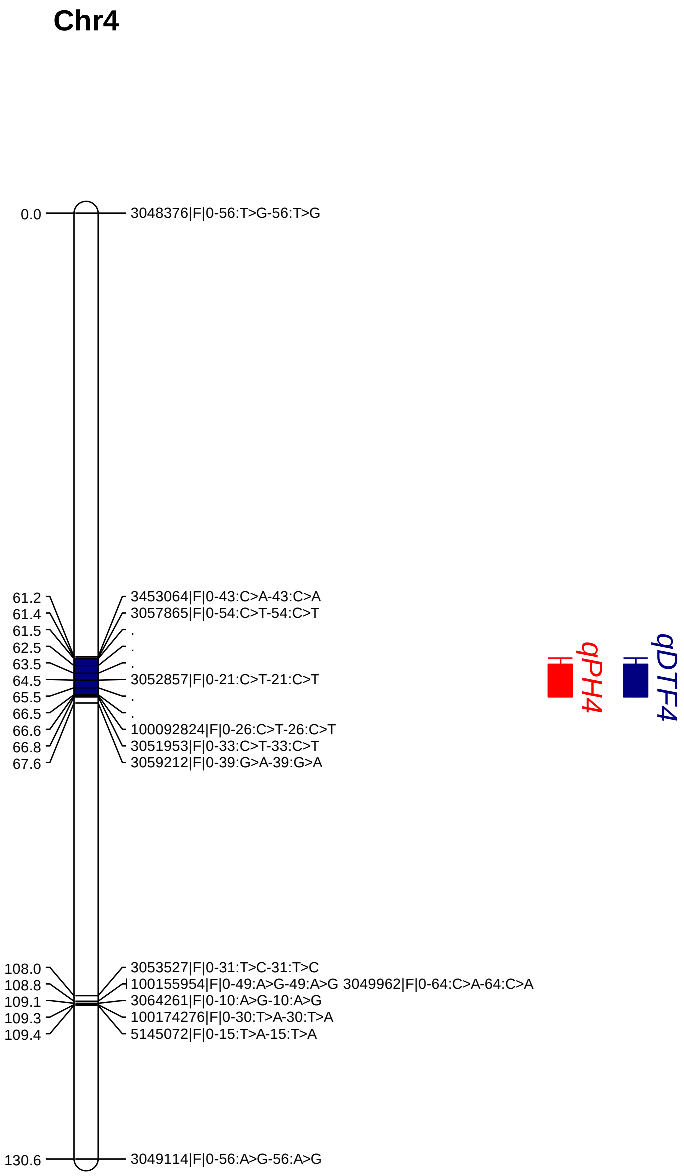

Figure 5. 3 Molecular linkage map showing positions of QTL identified for plant height and days to 50% flowering located on chromosome 4.

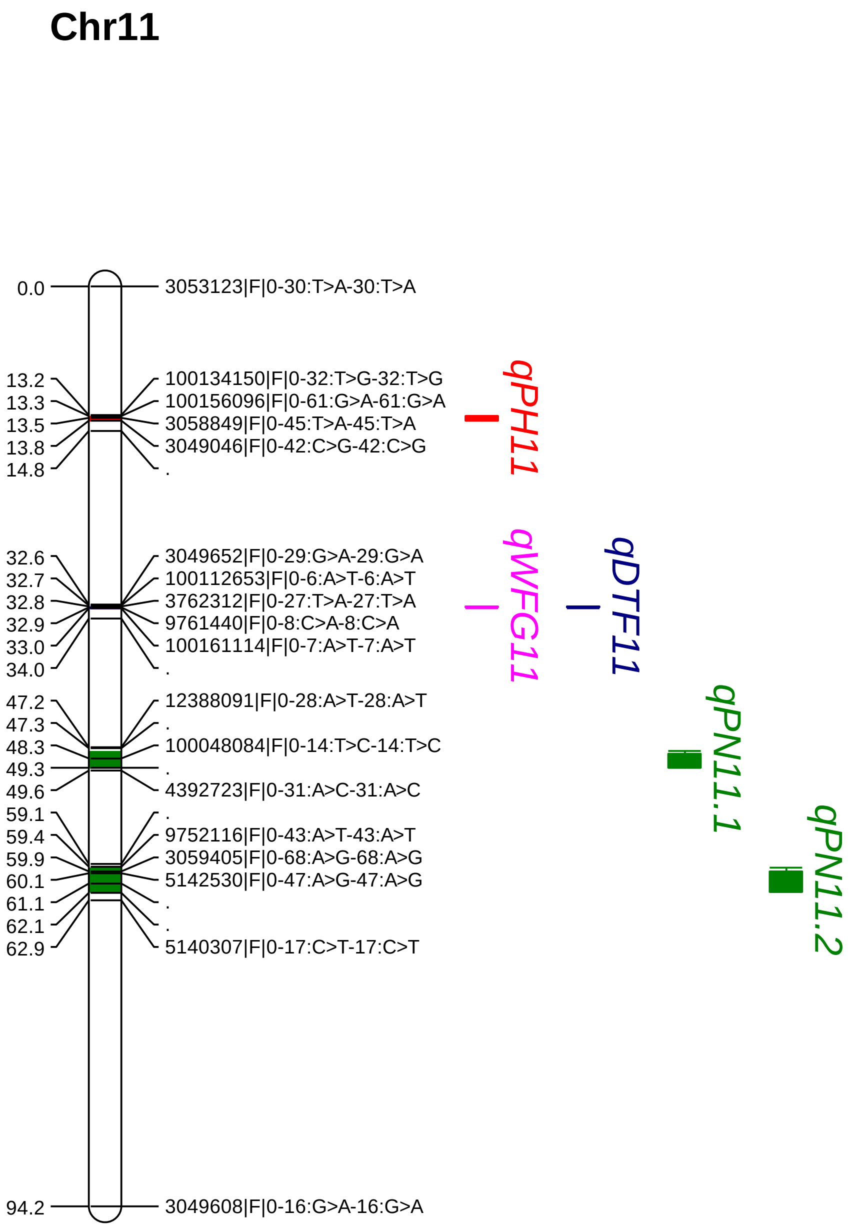

Figure 5. 4 Molecular linkage maps showing positions of QTL for plant height, grain weight, days to 50% flowering, number of panicles localised on chromosome 11.

Table 5. 2 Genetic linkage groups for the 2677 SNP markers

Linkage Group/ Chromosome No.	Genetic Length (cM)	Number of SNPs	Average Linkage Distance (cM)
1	198.16	363	0.55
2	170.77	342	0.50
3	194.39	255	0.76
4	127.26	201	0.63
5	132.96	129	1.03
6	131.41	300	0.44
7	151.02	305	0.50
8	117.33	177	0.66
9	91.45	63	1.45
10	92.63	145	0.64
11	93.17	106	0.88
12	99.50	291	0.34
Total/Average	1600.05	2677	0.70

Table 5. 3 QTLs identified for days to 50% flowering, plant height, panicle number and weight of filled grains under salt stress

Trait	QTL Name	Chr.	Position (cM)	LOD	Additive Effect	Parental Source of Increasing Allele	R^2 (%)	Total PVE (%)	Interval (cM)
Days to 50% Flowering	*qDTF1.1*	1	73.51	6.59	4.37	Sahel 317	6.1	10.6	73.4-73.5
	qDTF1.2	1	93.21	3.64	-0.01	NERICA-L9	4.5		92.9-96.1
	qDTF2	2	104.91	5.14	**1.12**	Sahel 317	**11.2**	**11.2**	104.3-105
	qDTF4	4	64.53	4.19	0.01	Sahel 317	5.2	5.2	61.2-67.5
	qDTF11	11	32.91	4.14	-0.02	NERICA-L9	6.5	6.5	32.8-33
Plant Height	*qPH1.1*	1	25.71	3.00	**0.03**	NERICA-L9	**27.9**	31.8	24.7-27.5
	qPH1.2	1	132.11	5.48	0.88	NERICA-L9	3.9		132-133.1
	qPH2	2	54.51	2.86	-0.16	NERICA-L9	5.7	5.7	54.4-54.6
	qPH4	4	64.51	4.13	-0.86	NERICA-L9	5.9	5.9	61.2-67.5
	qPH11	11	13.51	4.54	0.45	Sahel 317	1.4	1.4	13.3-13.8
Panicle Number	*qPN1*	1	134.51	7.65	**1.01**	Sahel 317	**8.3**	**8.3**	134.4-134.5
	qPN2	2	45.71	4.39	-0.55	NERICA-L9	**8.4**	**8.4**	44.8-46.1
	qPN11.1	11	48.31	5.05	0.69	Sahel 317	6.3	9.7	47.6-49.3
	qPN11.2	11	60.01	3.16	0.36	Sahel 317	3.4		59.5-62
Weight of filled grains	*qWFG1*	1	176.81	2.74	-0.78	NERICA-L9	3.0	3.0	175.6-176.9
	qWFG11	11	32.91	4.14	-0.02	NERICA-L9	6.5	6.5	32.8-33

5.4 Discussion

Several breeding programs around the world have used QTL mapping as a major gene discovery approach targeting those involved with quantitatively inherited traits. These efforts have led to the identification of stable QTLs with major phenotypic effect for most of the important stresses that reduces the productivity of crop plants. For instance, in rice, the mapping of a major effect

QTL *Saltol* which controls the Na/K ration on chromosome 1 (Gregorio, 1997; Bonilla *et al*., 2002) has been useful in MAS and other breeding purposes. These studies were conducted in an era where marker density was not scanty yet costly. With advances in marker technology, cost of DNA sequencing has considerably reduced, leading to the detection of single nucleotide polymorphism (SNPs), a marker technology with these highest resolution in recent times. 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 0.70 cM on the average. Molecular marker technology has enabled scientists to identify and map on rice chromosomes hundreds of genes using QTL analysis. QTL analysis is essentially a statistical creation that identifies a particular region of the genome as containing one or more genes and that region is associated with the trait being measured or phenotyped (Toure et al., 2000). A QTL is shown as an interval across a chromosome where the probability of association is plotted for each marker used in the mapping experiment (Collard and Mackikill, 2008). QTL analysis forms an early step in discovery and sequencing the actual genes is responsible for the trait variation or employing a marker in MAS (Young, 2000).

A total of 16 QTLs were detected for 4 (plant height, days to 50% flowering, panicle number, weight of filled grains) out of the 8 studied traits. For each trait, more than one QTL was detected with varying contributions from both parents, suggesting possible epistatic interactions across both parental genomes with all loci acting together to express the trait. This observation supports 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).

Salt stress limits plant growth, biomass production and productivity (Zhu, 2001) in rice. The ability for plants to maintain vigorous growth under salt stress 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, five QTLs were identified for plant height on chromosomes 1, 2, 4 and 11. A major effect QTL (*qPH1.1*) that increased plant height, hence vigorous growth under salt stress by 27.9% was identified on chromosome 1 at 25.7 cM. The increase in this QTL was influenced by alleles from the donor parent NERICA-L9. Different studies have mapped QTLs for plant height under salt stress at different loci on chromosome 1 (Mohammadi *et al*., 2013; Hossain *et al*., 2015; Mondal *et al*., 2019). Three out of the remaining four plant height QTLs on chromosomes 1, 2 and 4 were influenced by alleles from NERICA-L9 while the fourth on chromosome 11 were contributed by the recipient parent Sahel 317. Given the high effect of *qPH1.1* locus had increasing growth effect from the donor parent NERICA-L9, great progress could be made in breeding salt tolerant lines by introducing alleles of NERICA-L9 into elite germplasm in an attempt to exploit the dilution effect associated with vigorous growth.

In this study, five QTLs for days to 50% flowering were identified on chromosomes 1, 2, 4 and 11. All five QTLs were of minor effect with contributions from both parents. In a related study, Mohammadi *et al*. (2013) identified three QTLs for days to flowering on chromosomes 4, 6 and 10 in rice. Similarly, Bimpong *et al*. (2014) identified 4 QTLs for days to flowering on chromosomes 1, 5, 7, 8, 10 and 11. Days to flowering under salt stress in rice has been suggested to be an adaptive mechanism (especially when delayed) where plants conserve energy to maintain ion homeostasis rather than transition from vegetative to reproductive stage (Li *et al*., 2007).

It was observed that, the same region on some chromosomes controlled more than one QTL in this study. For instance, 2 QTLs *qPH4* (for plant height) and *qDTF4* (for days to 50% flowering) were found in the 61.2 - 67.5 cM region on chromosome 4. Similarly, on chromosome 11, two QTLs (*qWFG11* for weight of filled grains and *qDTF11* for days to 50% flowering) were mapped around the same region between 32.8 – 33.0 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 different QTLs that are tightly linked and 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 *qSESF12.1* and shoot dry weight QTL *qSDW12.1* in the same region on chromosome 12.

5.5 Conclusion

The present study identified two major QTL for time to 50% flowering (*qDTF1.1* and *qDTF1.2*) on chromosome one with total PVE% of 10.6. In addition, a major QTL (*qDTF2*) explaining 11.6% PVE was detected for the same trait on chromosome two. The study also found two major QTL (*qPH1.1* and *qPH1.2*) for plant height on chromosome one with a total phenotypic variance of 31.8%. Two QTL were found on chromosome 1 (*qPN1)* and chromosome two *(qPN2*) explaining 8.3% and 8.4% of the phenotypic variance respectively.

CHAPTER SIX

6.0 ANALYSIS OF SELECTION INDEX AND MORPHOLOGICAL CHARACTERS FOR PHENOTYPIC SELECTION OF BC2F3-DERIVED RICE POPULATIONS UNDER SALT STRESS

6.1 Introduction

Rice is a diverse and is adapted to various agro-ecologies but is susceptible to erratic weather variations which leads to low crop productivity (Kakar *et al., 2019*). Weather variations is causing series of abiotic stresses, sea water intrusion, eras of drought, recurrent floods etc. (Jagadish *et al.,* 2012) which inhibit tnormal crop growth and hence decreasing the total yield harvest of current mangrove rice varieties. Salt stress is the most destructive abiotic stress diminishing rice production after drought (Kakar *et al.,* 2019). Salinity is a condition when ECe (Electrical Conductivity of Saturated extract), exceed it threshold of 3 dsm-^{1}, sufficient to cause yield reduction in most crops (*Rana et al., 2019*). The crop is sensitive to salinity at various growth stages, especially at early vegetative and reproductive stages (*Lutts et al., 1995*; *Zhu et al., 2001*).

Recent reports show that over 12% of rice produced in Sierra Leone is from the mangrove belt along the coast which is prone to salinity (Baggie *et al., 2018*). Rice is moderately tolerant to salt stress during germination, active tillering and grain filling and is sensitive during the early seedling and reproductive stages including panicle initiation (PI), anthesis and fertilization (Zeng *et al.,* 2001; Fukuda *et al*., 2007; Singh and Flowers 2010; Batayeva *et al.,* 2018; Wu *et al.,* 2019).

Soil salinity severely decreases rice performances as the salt competes with plant solutes resulting into osmotic pressure hence plant dehydration. Similarly, soil toxicities, due to excess sodium ions in the soil, leads to poor metabolism and plant death (*Abbasi et al., 2016*). Also, the increase of excess sodium chloride (NaCl) in the leaf laminae decreases net photosynthesis and growth (Dubey, 1994). Soil salinity also induces ionic stress which causes homeostatic imbalances of ions in plant cells, especially the essential elements like potassium (K+) (Munns, 2002). Reproductive stage salinity can cause appreciable reduction in number of spikelets per panicle, tiller number, fertility, panicle length (PL) and the number of primary branches per panicle (Singh *et al.,* 2010, Mohammadi *et al.,* 2014).

Development of salt tolerant variety can enhance rice production (kg/ha) in farmer fields. Various selection criteria have been adopted to select for stress tolerance in rice (Gregorio *et al., 1997*). Successful development of salt tolerant rice variety is dependent on viable selection criteria and the environments. Various methods have been used for selection of salt tolerance in rice, however, 'saline soil method' is the most robust because it allows selection of genotype at different growth stages (Ismail and Horie, 2017; Anshori *et al., 2018*). Under saline conditions genotypes can be selected based on phenotypic characteristics resulting from their gene expressions for salinity tolerance (De Costa *et al*., 2012). The agronomic traits are considered as selection criteria and should manifest high heritability and strong correlation with yield under saline conditions *(*Acquaah*, 2012).* In situation where heritability is low, selection criteria are considered as selection indices and are used to select for yield. Selection indices provide useful information about which trait to be combined (Asghar and Mehdi, 2010). Therefore, selection index becomes an integral part of an approach for improvement of rice under saline environments.

The objective of this study was to:

(1) Determine the efficiency of selection criteria; and
(2) Identify agronomic characters as selection criteria that can be used to screen for salinity tolerance in rice under saline soil conditions.

6.2 Materials and methods

6.2.1 Experimental sites

The present study was conducted under field conditions in 2018 dry cropping season in Mbe, AfricaRice Centre, Cote d'lvoire located between 7.5^o N and 8.5^o N and between 4.5 ^{o} W and 5.5^o W, with an average annual rainfall of 985 mm. The station is characterized by 3 main seasons: a long dry season starting in early November with an end in mid-March; a long rainy season from mid-March to mid-July; a short rainy season from mid-July to mid-August, and an inter-season rainy period which starts in mid-August and ends in October.

6.2.2 Plant materials

Sahel 317, Nerica L9, and it BC_1F_3 seeds with three popular checks from Africarice where used in this study (Table 6.1). Sahel 317 has good yield attribute and Nerica L 9 is said to have some tolerance to salinity.

Table 6. 1 List of F3-derived rice populations, with two parent and two check evaluated for agronomic performances under salt stress conditions in the field during 2018.

S/N	Population	Number of lines
1	Progeny of Sahel x Nerica L 9	476
2	Sahel 317	20
3	Nerica L9	20
4	Giza 178_1	30
5	ARS 1071SMK87-15WB-2-41	20
6	ARS1071SMK-03-15WB-1-1	34

6.2.3 Experimental design and data collection

6.2.3.1 Screening for salinity tolerance at the reproductive stage

The experiment was conducted at Mbe in the lowland breeding plots. The 21-day-old seedlings of BC_1F_3 and parents, plus three checks from Africarice were transplanted in the controlled and saline conditions using 50 x12 alpha lattice design in two replications with 3 m long rows spaced at 0.2 m between plants and 0.4 m between rows respectively. Rice seeds were germinated in containers and were transferred to the field 25-30 days after seedling emergence. One plant was transplanted per hill. Salinity stress in the saline blocks was imposed initially by applying NaCl to the field and adjusting the saline water (using the electrical conductivity meter), ECe 3–4 dS m^{-1} during transplantation at the seedling stage, which increased to ECe 5– 6 dS m^{-1} after the active tillering stage and was maintained until maturity. This level of salinity stress at the reproductive stage is optimum to differentiate between sensitive and tolerant genotypes.

Low salinity levels can pose difficulty in differentiating between sensitive and tolerant genotypes, also two much high stress levels result in a lot of sterility among the tolerant genotypes as well. Agronomic practices were maintained and fertilizer (NPK 15:15:15) was applied at a dose of 6g/plot one week after planting. Urea was applied at three and nine days after emergence at a dose of 1.5g/plot. Seven different qualitative traits associated with reproductive and ripening growth stages were measured according to the standard evaluation score for rice by Gregorio *et al.* (1977). The IRRI standard evaluation system for rice (IRRI) was use to aid the trait selection process and method of measurement. Days to 50% flowering was determined as the number of days from sowing to when 50% of the rice population had flowered. Plant height was recorded in centimeters from the base of the shoot to the tip of the tallest leaf blade. Numbers of tiller were considered as the number of shoots that grew after the initial parent shoot. The panicle number is a key indicator of rice yield, and was counted as the number of all panicles except panicles shorter than the half of culm length and late emerging heads. Filled grains reading was obtained from the count of well-developed spikelets in proportion to the total number of spikelets. Unfilled grains were counted as number of empty spikelets per panicle. 100-grain weight was determined from a random sample of 100 well developed, whole grains within a population, dried to 13% moisture content and weighed on precision scale to obtain the 100-grain weight. Experimental units were harvested by hand when 89-90 % of the panicle reaches physiological maturity. Details of phenotypic data collected are presented below (Table 6.2).

Table 6. 22 Agronomic traits recorded in F3-derived populations evaluated under saline stress and control conditions in 2018.

Trait	Description
50% flower	Number of days to 50 % flowering
PH	Plant height measured in centimetres
No. TIL	Number of tillers per plant
No. PAN	Number of panicles per plant
Filled-grain	Number of spikelets with developed grains per panicle
Unfilled Grain	Number of spikelets with undeveloped grains per panicle
Grain Weight.	Weight of 100 grains was measured in grams

6.2.4 Statistical analyses

Analysis of variance was performed following the statistical model for single location based on alpha incomplete block design and was fitted as follow (Ott and Longnecker, 2010):

$$Y_{ijk} = \mu + G_i + R_j + B_{jk} + \varepsilon_{ijk}$$

where Y_{ijk} was phenotypic observation of the i^{th} genotype in the j^{th}replication *in the* k^{th} incomplete block; μ was population mean; G_i was genetic effects of the i^{th} genotype; R_j was effect of j^{th} replication; B_{jk} was effect of k^{th} incomplete block in j^{th} replication; and ε_{ijk} was normally distributed error term with mean=0 and variance $=\sigma^2_\varepsilon$. The salinity tolerance indices were computed as below (Kumawat *et al.*, 2017):

Yield Index (YI) = $Ys / \hat{Y}s$

Stress Tolerance Index (STI) = $Yp \times Ys / \hat{Y}p^2$

Yield Stability Index (YSI) = Ys / Yp

Geometric Mean Productivity (GMP) = Sqrt $(Yp \times Ys)$

Stress Susceptibility Index (SSI) = $(1 – Ys/Yp) / SI$ and $SI = (1-\hat{Y}s / \hat{Y}p)$

Mean Productivity (MP) = $(Ys + Yp) / 2$

Tolerance Index (TOL) = $Yp – Ys$

Where *Yp*= mean performance of a genotype under control condition, *Ys*= mean performance of a genotype under stress condition, Ŷs= average performance of all genotypes under stress condition, and Ŷp= mean performance of all genotypes under control conditions. The SSI as an index provides an assessment of the relative performance of a given entry with regard to mean performance of all the genotypes. Data obtained were subjected to analysis of variance using GenStat Statistical package 14 edition. Comparison between treatment means was done using the Tukey Honestly Significant Test (HSD).

6.3 Results

6.3.1 Mean performance of genotypes under saline and control conditions

Analysis of variance for agronomic traits is presented below (Tables 6.3). The results showed variations among the genotypes for the traits measured. High significant difference ($p \leq 0.01$) was detected among the genotype for most traits. Similarly, significant interactions ($p \leq 0.01$) were observed between genotypes and NaCl levels. Based on filled grain data, some genotypes showed superior performances compared to population mean for grain yield (Table 6.4). The genotype means for the selected seven best lines and three checks ranged from 5.45 to 15.97 for filled grain and which were far above the population mean of 1.89 for the same trait under salt stress. However, superior genotypes with high filled grain were not the same genotypes with large number of tillers per plant. Similarly, plant height was not the highest in genotypes with the highest filled grain (Table 6.4).

Table 6. 3 Analysis of variance for agronomic traits in F3-derived rice populations grown under saline and control conditions in 2018

Source of Variation	Df	Mean Squares						
		50%FL	PHT	No. TIL	No. PAN	Filled-grain	Unfilled Grain	Grain weight
Rep	1	0.004655	1941546	493.97	3639.42**	10.73	1.46441**	8.73
Rep. Block	22	1.640E+03**	830.87	82.77*	366.62**	5.421	0.12312**	3.42
Entry	481	11.21.588**	87.61*	12.48**	70.08**	3.005*	0.08689*	6.005*
NaCl level (L)	1	1.188E+01**	37.61*	2.48**	34.08**	1.005*	0.01689*	2.18*
E x L	481	0.888E+01**	21.61*	1.08**	20.08**	1.105*	0.10689*	2.20*
Error	479	0.004076	94.2	13.28	72.96	3.427	0.07405	1.28
Total	1465	44.48	2036.47	14.66	80.6	3.245	0.08315	2.69

*, ** Significant at $p\leq0.05$ and $p\leq0.01$, 50%FL= Days to 50% flowering, PHT= Plant height, No. PAN= Number of panicles, No. Tiller=Number of tillers,

Table 6. 4 Means of some selected rice genotypes that showed superior performance for major agronomic traits under salt stress in the field during 2018.

S/N	Genotype	**Control**				**Salt stress**			
		PHT (cm)	No. TIL	Filled-grain	Grain weight	PltHe (cm)	No. TIL	Filled-grain	Grain weight
1	SAHEL317/NERICA-L-9_38	109.00	15.00	33.58	23.01	63.74	10.4	15.97	9.97
2	SAHEL317/NERICA-L-9_444	108.00	18.00	6.85	3.29	62.52	9.55	11.18	7.01
3	SAHEL317/NERICA-L-9_196	104.00	14.00	22.89	15.07	95.00	9.00	11.10	6.31
4	SAHEL317/NERICA-L-9_119	102.00	16.00	22.79	14.95	60.02	12.05	11.04	5.19
5	SAHEL317/NERICA-L-9_320	89.00	9.00	7.62	4.01	57.24	7.9	9.58	4.58
6	SAHEL317/NERICA-L-9_384	99.00	8.00	37.36	37.36	59.02	6.55	8.94	4.01
7	SAHEL317/NERICA-L-9_452	97.00	14.00	13.11	9.47	94.00	8.00	5.90	3.87
8	ARS 1071SMK 87-15WB_2-31	95.00	17.00	7.62	3.84	69.79	12.57	5.86	3.04
9	ARS 1071SMK 87-15WB_2-21	95.00	10.00	10.37	6.00	101.00	8.00	5.47	3.00
10	ARS 1071SMK-03-15WB_2-21	103.00	11.00	19.99	14.71	105.00	7.00	5.45	2.97
	Mean (μ)	101.99	10.77	20.47	17.3	61.10	6.17	1.89	1.26.
	LSD (5 %)	17.46	10.00	3.55	2.65	19.81	8.03	4.55	1.39
	CV %	20.09	363.40	110.01	36.49	14.72	43.08	109.92	24.81
	SE	6.78	7.15	3.12	2.17	9.00	3.65	2.07	1.521

PHT= Plant height (cm), No. TILL= Number of tillers per plant, Filled-grain=filled spikelet μ= Population mean, LSD= Least significant difference at 5 % level, CV= Coefficient of variability at 10 %, SE= Standard error of difference

6.3.2 Correlation among traits

Results of phenotypic correlations among seven agronomic traits are shown in Table 6.5. Strong and positive and negative correlations were observed between the traits (p = 0.01). The positive correlations were between 100-grain weight and all other traits except with unfilled-grain. Similarly, filled-grain and unfilled-grain were negatively correlated. Another negative correlation was observed between number of tillers and number of panicles.

Table 6. 5 Phenotypic correlation among traits for tolerance to saline stress conditions at the reproductive stage.

Trait	50%FL	PHT	No. TIL	No. PAN	Filled-grain	Unfilled-grain	Grain weight
50%FL	0	24.39**	349.69**	225.19**	414.03**	433.48**	38127**
PHT		0	34.71**	30.65**	38.82**	39.27**	26.73**
No. TIL			0	-16.10**	49.12**	53.46**	38.98**
No. PAN				0	39.54**	40.30**	36.85**
Filled-grain					0	-2.66**	1.54**
Unfilled Grain						0	-3.11**
Grain weight							0

*, ** Significantly different at $P \leq 0.05$ and $P \leq 0.01$, a strong evidence that means of two of the indices have unequal variances.

6.3.3 Estimation of tolerance index

The results of pairwise t-test of difference among selection indices for filled grain trait in F_3-derived rice populations are shown in Table 6.6. The results indicated that the indices were significantly different ($p \leq 0.01$) for 100-grain weight among the genotypes. Indices STI, YSI and SSI were highly significantly different ($p \leq 0.01$) while Ys, GMP, YI and TOL were significant at $p \leq 0.05$. Variation due to MP was not significantly different.

Table 6. 6 Results of Student's pairwise t-test of difference among selection indices for filled grain trait in F3-derived rice populations.

Mean1	Mean2	Df	*t*-value
Ys1	Ys2	7	-2.98*
YI1	YI2	7	-0.89*
STI1	STI2	7	-3.42**
YSI1	YSI2	7	-4.33**
GMP1	GMP2	7	-2.76*
SSI1	SSI2	7	-3.31**
MP1	MP2	7	-1.78
TOL1	TOL2	7	-2.19*

*, ** Significantly different at $p \leq 0.05$ and $p \leq 0.01$, Mean1= Mean under stress conditions, Mean2= Mean under control conditions, Ys= Genotype mean under stress conditions, YI= Yield index, STI= Stress tolerance index, YSI= Yield stability index, GMP=Geometric mean productivity, SSI= Stress susceptibility index, MP= Mean productivity, TOL= Tolerance index,

To assess the linear relationship between filled-grain and the indices, correlation analysis was conducted (Table 6.7). The results indicated that the indices were highly correlated with filled-grain under stress conditions (Ys). Indices TOL and SSI had negative correlations with the filled-grain under saline conditions.

Table 6. 7 Pearson correlation of selection indices for 100-grain weight per plot recorded in F3-derived rice populations evaluated under saline stress conditions in 2018.

	Ys	STI	YSI	YI	GMP	TOL	MP	SSI
Ys	1							
STI	0.99**	1						
YSI	0.99**	0.98**	1					
YI	0.38	0.27	0.34	1				
GMP	0.96**	0.96**	0.96**	0.34	1			
TOL	-0.98**	-0.97	-0.98**	-0.56	-0.95**	1		
MP	0.96**	0.98**	0.96**	0.12	0.94	0.92	1	
SSI	-0.67*	-0.59*	-0.78**	-0.47	-0.49	-0.74	-0.57*	1

Ys= Genotype mean under stress conditions, STI= Stress tolerance index, YSI= Yield stability index, YI= Yield index, GMP=Geometric mean productivity, TOL= Tolerance index, MP= Mean productivity, SSI= Stress susceptibility index.

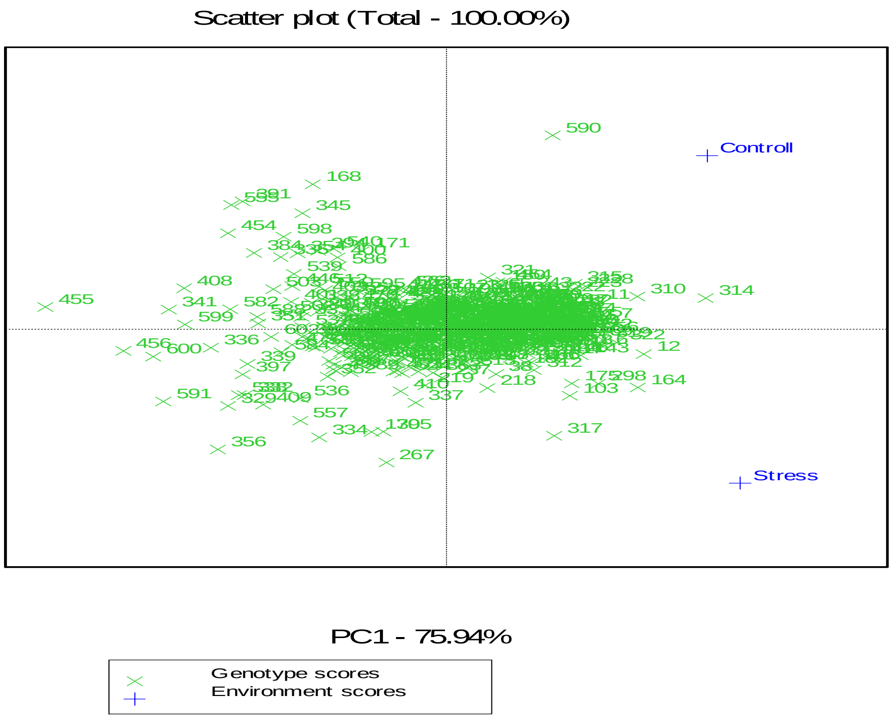

Figure 6. 1 The graphical representation of principal component analysis (PCA) of the first two principal components (PC1 and PC2) and identification of most salt tolerant genotypes conducted using 476 F3 rice populations, with two parent and two popular checks.

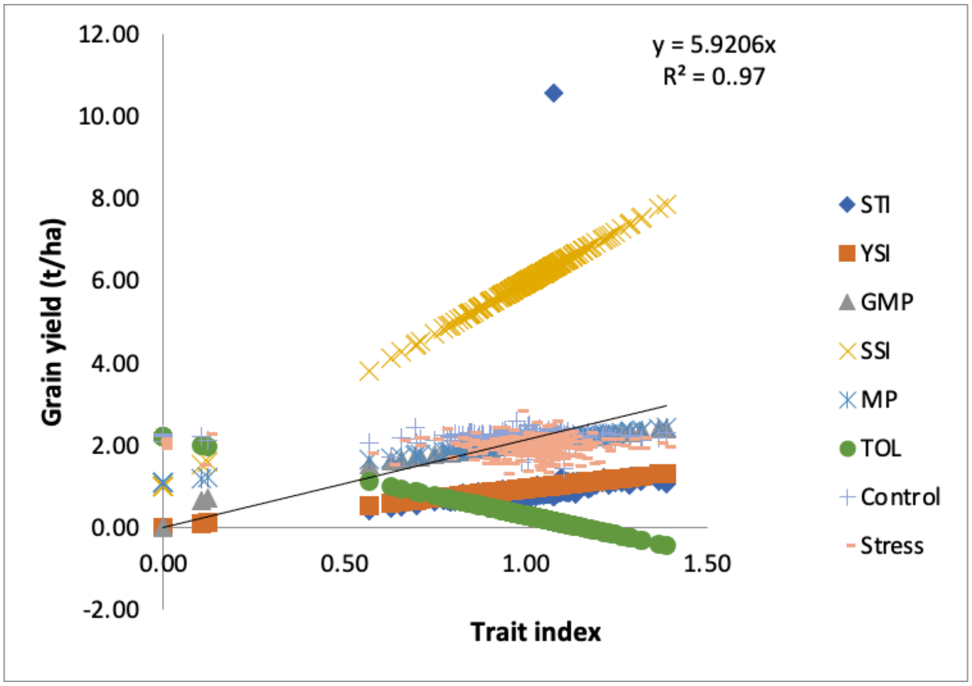

Figure 6. 2 Relationship of seven salinity tolerance indices with grain yield under saline and control conditions in rice.

6.4 Discussions

6.4.1 Performance of genotypes

Variance analysis output of genotypes indicated differences among the genotypes, such differenceS are due to dissimilarity in the genetic makeup of the genotypes used in this study. Also, the difference between stress and control conditions was significant for all characters investigated in the current study. This indicates the genotypes responded differently under both environments. Caser *et al.* (2010) evaluated 30 rice genotypes and found high dissimilarity among the genotypes for salt tolerance at seedling stage. Similarly, significant variability for the traits measured at the reproductive stage was reported. The interactions of genotypes × salinity treatments were significant for pollen viability, number of unfilled grain and grain yield. The authors found that reduction in grain yield due to salinity was more sever for control to 60mM than for 60mM to 100mM. Ma *et al.* (2018) found significant variability in response among rice genotypes when subjected to salt stress.

The present study found that interaction between genotype and stress condition was significant for most traits. This variation could be due to difference in response to salinity between the susceptible and resistant genotypes. This can also indicate that combination of genetic effect and stress level can be a good criterion for selection for tolerance to salinity in rice. Comparatively, average performances of the genotypes were different under control and stress conditions. Significant variations were observed in most of the traits under study. Plant height and number of tillers per plant were significantly reduced under stress conditions. This could be attributed to high salinity in the soil which might have affected plant metabolism and growth. Study by Ma *et al.* (2018) reported that tolerant lines managed to maintain their water and chlorophyll content with lower incidence of sodium ion accumulation. They found that antioxidant activities of these lines: production of ascorbate peroxidase (APX) and catalase (CAT) were significantly higher in the sensitive line while superoxide dismutase (SOD) was higher in the tolerant line. The authors detected an up regulation of non-polar metabolites and production of sucrose and GABA and acetic acid in the tolerant lines, suggesting an important role in salinity adaptation. In contrast, they found that glutamine and putrescine were noticeably high in the susceptible rice. Nayyeripasand *et al*. (2019) evaluated a total of 155 rice accessions, taken from the International Rice Research Institute (IRRI), were phenotypically evaluated under salt stress. The analysis of variance (ANOVA) showed that treatments (normal and saline) had significant or very significant differences for all the studied characteristics except for the shoot length (SL). There was a significant difference between genotypes (G) and G×T interaction for all the studied traits

(Table 1). Results showed that shoot length (SL), turgescence weight (TW), root length to shoot length ratio (RL:SL) and root dry weight to shoot dry weight ratio (RDW: SDW) at saline condition were not significantly changed compared to normal condition.

6.4.2 Estimation of tolerance indices

The *t*-test results revealed tolerance indices that were able show variation in yield (filled-grain) between the susceptible and tolerant genotypes. The results found that indices STI, YSI, GMP and SSI were able to segregate for yield between susceptible and tolerant genotypes under salt stress. This implies the importance of these indices to select for yield in rice under saline environments. Nayyeripasand *et al.* (2019) evaluated 155 rice genotypes at two levels of NaCl namely non-stress (Ec 1.2 dS m^{-1}) and salt-stress (Ec 10 dS m^{-1}) under hydroponic condition. The authors found that the effect of genotypes (G), Salt treatment (T) and G×T interaction were significant for all investigated traits (except for the effect of genotypes on shoot length). Their study revealed that under salt stress most genotypes showed reduced performances for all traits. In a related study, Faiyue *et al.* (2012) recommended the use of bypass flow as a new screening technique for salt tolerance in rice.

6.4.3 Correlation of tolerance indices with grain weight

Evaluating yield potential of a genotype in a harsh environment requires a certain criterion known as a tolerant index. Different approaches has been employed to understand the tolerance index and are usually determined based on grain yield (Singh *et al.*, 2015). The method has been commonly used to identify genotypes and parameters with substantial ability to survive saline conditions. In the current study, tolerance index results showed significant correlations between grain yield and the tolerance indices implying that the significant indices were correlated to grain yield under saline conditions. However, some indices did not correlate to yield under saline condition for example, indices YI and SSI were able to differentiate between susceptible and tolerant genotypes but were not significantly showed correlation with filled grain (Ys). Indices STI, YSI, GMP and TOL showed significant correlation with 100-grain weight and were significant in *t*-test therefore, they could be utilized as selection indices for tolerance to salinity in rice. Many studies have reported selection indices in rice and other crops. Caser *et al.* (2010) reported that pollen viability was found to be a robust criterion to screen the genotypes for salt tolerance at the reproductive stage. The authors also reported that a major quantitative trait locus (QTL) for salt tolerance at seedling stage (Saltol) mapped on chromosome 1 was used as reference for haplotyping. Thirty rice genotypes divided into 16 different haplotypes based on Saltol QTL.

Additionally, they observed RM8094 and RM10745 microsatellite markers to be the most effective markers for discriminating the salinity tolerant genotypes. The result of this studies is in agreement with Nayyeripasand *et al.* (2019) who reported seven stress tolerance indices including STI, SSI, HM, RDI, SSPI, ATI and TOL to identify salt tolerant genotypes. The authors found high positive correlation (r=1.0) between TOL and SSPI and also between SSI and RDI at stress condition. Phenotypic performance was significantly and positively correlated with TOL, SSI, STI, HM, ATI, MP, GMP, STI and SSPI. Also, the performance was significantly and positively correlated with MP, GMP, STI and RDI, indicating that these criteria were more effective in identifying high performance genotypes under salt conditions. However, they found negative correlations between SSI with HM, MP, GMP, STI and RDI. Their results indicated that there were high positive and significant correlations among performance with HM, MP, GMP and STI.

Application of salt tolerance indices as parameter for selection has been reported in other crops. Kumawat *et al*. (2017) investigated the relationship among several salt tolerance/resistance indices in lentils under normal (0.0 mM NaCl) and saline (40mM NaCl) conditions during seedling stage using ten lentil genotypes in laboratory conditions. On the basis of the dry matter yield and stress indices the authors reported that genotypes SAPNA, RLG-258 and RLG-234 were identified as the most salt tolerant genotypes. Their results indicated that indices STI, MP and GMP exhibited good correlation with dry matter yield under both the conditions therefore selection should be based on these indices in salinity tolerance programmes. Asghar *et al.* (2010) employed three selection indices to determine yield and quality traits in sweet corn. The authors found that the three indices were important in improving the aggregate genotype of yield traits for most of the selection strategies.

In the present study, indices STI, YSI and GMP showed the highest correlation values with 100-grain (Ys) indicting that these indices were the most effective for selection of rice genotypes for salt stress. The least correlation value vas observed between YI and grain yield implying that the index was not strong enough for selecting of rice genotypes under salt stress. The negative correlations between some indices and grain yield observed in the present study could suggest that these indices are not efficient for direct selection for yield performance under salinity.

6.4.4 Phenotypic correlation among traits for tolerance to saline stress conditions at the reproductive stage

Correlation coefficients estimated for yield and its components showed that 100-grain weight had positive and highly significant correlation ($p \leq 0.01$) for all the characters measured except with unfilled spikelet. These results indicate that 100-grain weight and number of reproductive tillers were found to be correlated indicating that selection for yield under salt stress can be carried out using those agronomic traits. However, the observed negative correlations imply that those traits cannot be used to select for yield under the same environment. This finding is comparable to findings that have been reported by Fiyaz *et al.* (2011) for reproductive tillers and Hassan *et al.* (2016) for 1000-grain weight.

This current study found duration of days to 50% flowering was positively and highly significant to all the other traits under study. Similarly, plant height was significantly and positively correlated with number of tillers per plant, number of panicles per plant, filled spikelet and 100-grain weight. This is an indication that increased plant height would result in an increase in panicle length of plant. This result agrees with the report of Nayak *et al.* (2001). Similar finding was also reported by Zahid *et al.* (2006), showing high positive significant correlation between plant height and 1000 grain weight. Number of sterile spikelets (unfilled grains) was negatively highly correlated with grain weight, this can be due to the increase in the salt level in the field, negatively affecting the grain filling process, which result in increased numbers of unfilled grains as a result of spikelet sterility. Number of tillers was highly but negatively correlated with number of panicles per plant but highly positively correlated with filled grain, unfilled grains and grain weight. Kakar *et al.* (2019) found that salinity tolerance at the seedling stage does not correlate with tolerance at other vegetative and reproductive stages in rice however, it can adversely affect crop yield by negatively affecting yield related components.

6.4.5 Agronomic character as selection criteria for tolerance to salinity in rice

Although selection indices are good tool for selecting tolerant rice genotype under saline conditions, use of agronomic characters as selection criteria could significantly improve efficiency of selection for saline tolerance in rice. In the present study, correlation analysis was performed between agronomic traits and selection indices. The results showed significant correlations between 100-grain yield and the agronomic traits, indicating that the agronomic characters can be used to select for tolerant rice genotypes under saline conditions. In the present study, 50% flowering and number off tillers were found to be the most significant traits that can be used as selection criteria for salt tolerance at vegetative stage while umber of panicle and plant height were significant selection criteria for reproductive stage. Talebi *et al*.(2008) reported that leaf area index (LAI) and sodium-calcium (Na-Ca) selectivity could be used as salt tolerance components for screening salt tolerance in rice. Additionally, their study detected significant correlations between leaf area index (LAI) and yield components in both salt-tolerant and -sensitive genotypes, further confirming the significant contribution of LAI to grain yield. Kakar *et al*. (2019) evaluated rice genotypes under pot-culture at seedling stage and they observed a wide range of variability was showed among genotypes for qualitative traits with root traits being identified as the best descriptors for tolerance to salt stress conditions.

6.4.6 Identification of salt tolerant genotypes using PCA

In order to establish the relationship among distinctive measured traits of promising rice line in differential display, it is necessary to subject them under biplot analysis. The first two vectors (PC1 and PC2) together with the mean values are usually considered for inputting the distribution of the populations in a two-dimensional space thus simplifying the identification of groups. In the current study, a biplot of BC_1F_3 derived inbred lines with the two parents and three checks was plotted with their measured traits and the biplot distributed all genotypes in their corresponding suitable response of traits. The biplot between PC1 and PC2 was plotted by using the variability among salt tolerant line and susceptible lines and to identify relationship between them. As a result, genotypes were assigned into two environments of controlled and salt stress conditions. Since the two major biplot component (PC1 and PC2) together contributed more than 58% of the variability, they were used to separate the genotypes into tolerance, moderately tolerance, moderately susceptible and highly susceptible groups. Under the salt stress conditions, located at the lowermost right corner of the biplot, genotypes 317, 103 and 164 showed the longest vectors towards the salt stress indicating their adaptation in this environment. Genotypes 455, 600 and 591 were found within the lowermost left corner of the biplot. This might imply that the genotypes were moderately tolerant to salt stress.

The present study found that genotypes 455, 168, 291 were localized at the uppermost left corner of the biplot suggesting these genotypes were the most susceptible to salinity within those categories. The genotypes 590, 310 and 314 were assigned within the control environment implying that the three genotypes were the most highly susceptible to salinity. The current study also showed that most of the genotypes were concentrated within the origin of the biplot. This could indicate that large number of the genotypes had moderate performances. Kakar *et al.*, (2019) used principle component analysis and confirmed that genotype FED 473 as salt tolerance and genotype IR85427 as salt sensitive. Generally, the current study showed that genotypes 317, 103 and 164 could were the most tolerant to salt stress.

The grouping of genotypes according to their performances in this study indicates the effects of salt on phenotypic performances of the genotypes. The high performance observed under the control environment could be due to the availability favorable growth conditions. However, under salt stress rice plant is deprived of normal osmotic processes. High salt in the soil affect oil affect movement of nutrients from the soil into the plant due to low salt concentration in the plant tissue. This might have resulted to insufficient water in the rice plant hence reduced rate of photosynthesis due to stomatal closure and other water control mechanisms within the plant system. Kakar *et al.* (2019) observed that high salt stress may cause disruption in ion homeostasis resulting to reduced photosynthesis.

6.4.7 Regression of grain weight and tolerance indices

Correlation gives good information about the relationship between agronomic traits, this can be helpful when using these traits as selection criteria for salt tolerance in salinity prone soils. The correlation between them is expected to produce an agronomic character that has the same ability as the tolerance index in differentiating tolerant and sensitive genotypes and correlates with yield under salinity. Therefore, 100-grain weight, should be considered as a major determinant when screening for salt in both normal and saline conditions. Percentage variability explained by the value provided from the coefficient of determination (R2) for each independent trait can be used to predict the required genotype. The high R^2 valued observed in the present study between 100-grain weight and tolerance indices, suggesting strong relationship between the tolerance indices with 100-grain weight. Therefore, it could be possible to interpolate grain yield using the selection indices in saline induce environments. Conversely, the study found TOL to had negative correlation with grain yield suggesting a yield reduction under salt stress. This same trend was observed in the correlation between TOL and grain yield (Ys) above. This is similar to finding by Anshori *et al*. (2018) who reported that tolerant indices of STI, YSI, GMP, TOL, and MP except TOL, showed high correlation for yield under salt stress environments. The authors added that having a significant tolerant index does not necessarily meaning a correlation to yield of each genotype under stress (Ys). Pandey *et al.* (2015) used high heritability values together with high genetic advance scores such as number of spikelets per panicle, hulling percentage, milling percent and head rice recovery to denote the relevance of additive gene action in the inheritance of the characters. This indicates the possibility of improving such traits using selection in segregating generation. The authors further argued that these characters may serve as an efficient tool for selection breeding programme for rice improvement.

6.5 Conclusion and recommendation

6.5.1 Conclusion

Salt tolerance indices STI, DMP and TSI showed significant segregation among rice populations for yield under saline field conditions. Therefore, these tolerance indices can be utilized as selection indices for confirming tolerant rice genotypes under saline stress environments. Five agronomic traits including days to 50% flowering, plant height, number of tillers, number of panicle and filled-grain showed strong and positive correlations with grain weight hence are good selection indices under salt stress.

6.5.2 Recommendation

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

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

CHAPTER SEVEN

7.0 General conclusion and recommendation

7.1 Conclusion

This study has identified major production constraints including high cost of labour, salinity, lack of credit, lack of access to improved seeds and lack of fertilizer, crabs, pests and diseases as the most important. Agronomic traits including high yield, tall plant height, late maturity, good taste, and high tillering ability were found to be the main traits preferred by the farmers. The study observed poor knowledge by farmers on salinity. However, the farmers showed willingness to adopt salinity tolerant varieties. The study found four distinct groups within the 90 rice accessions. However, further characterization using SNP markers revealed only two main clusters for the accessions under study. The current study detected two major QTL for time to 50% flowering (*qDTF1.1* and *qDTF1.2*) on chromosome one with total PVE% of 10.6. In addition, a major QTL (*qDTF2*) explaining 11.6% PVE was detected for the same trait on chromosome two. The study also found two major QTL (*qPH1.1* and *qPH1.2*) for plant height on chromosome one with a total phenotypic variance of 31.8%. Two QTL were found on chromosome 1 (*qPN1)* and chromosome two *(qPN2*) explaining 8.3% and 8.4% of the phenotypic variance respectively. This study found that salt tolerance indices STI, DMP and TSI had significantly segregated among rice populations for yield under saline field conditions. Similarly, five agronomic traits including days to 50% flowering, plant height, number of tillers, number of panicle and filled-grain showed strong and positive correlations with grain weight. Therefore, these selection indices can provide good tools for selection for salt tolerance under stress environments.

7.2 Recommendations

- The farmers' views gathered in the present study can provide good guidance for designing breeding programs for improvement of rice in mangrove swamps.
- Inclusion of an effective extension program for disseminating useful agricultural information and farmer education on existing problems in the mangrove area
- The various groups of the rice accessions can be useful as gene pools that can be adopted for rice breeding involving crosses.
- More SNP markers should be used to further discriminate the diversity in the rice germplasm
- The QTL identified in this study can provide important tools for marker assisted breeding and section for the important traits in rice. For example, salt tolerance is a polygenic trait controlled by several quantitative loci (QTL) hence pyramiding these QTLs with other major effect QTLs will be crucial in breeding for tolerance to salt stress in rice.
- The seven lines identified as salt tolerant, should be advance and evaluated in multi-environment trials for further selection
- The tolerance indices identified in the present study can be utilized as selection indices for identification of tolerant rice genotypes under saline stress environments.

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APPENDIX

RESEARCH TOPIC: ASSESSING PRODUCTION CONSTRAINTS, FARMERS' PREFERENCES AND SALINITY STATUS IN THE MANGROVE SWAMPS

QUESTIONNAIRE FOR MANGROVE RICE FARMERS

SECTION A: GENERAL INFORMATION

A1	**Questionnaire Information**		**Codes**	
A1.1	Questionnaire ID		/___/___/___/___/___/	
A1.2	Name of Enumerator_____________________________		/___/	
A1.3	Date: /___/___/ /___/___/ /___/___/___/___/ DD/MM/YYYY			
A1.4	Start time: /___/___/ /___/___/ End time: /___/___/ /___/___/			
A2	**Location**			
A2.1	Name of region ________________________		/___/	
A2.2	Name of district ________________________		/___/___/	
A2.3	Name of chiefdom ________________________________		/___/___/___/___/	
A2.4	Name of Village/town : _________________			
A2.5	GPS coordinates	**1. Longitude N(S)** /___/___//___/___//___/___/	**1. Latitude E (W)** /___/___//___/___/___/___/	**2. Altitude/height (m)** /___/___/___/
A3	**Screening Questions**			
A3.1	Are you a farmer?		**1 = Yes** **2 = No >> End interview**	/___/
A3.2	Do you own a rice farm?		**1 = Yes** **2 = No >> End interview**	/___/
A4	**Respondent information**			
A4.1	Name of respondent (Mangrove rice grower)		--	
A4.2	Age		*Years*	---------------------
A4.3	Contact of respondent		*Cell number:*	---------------------
A4.4	Gender		*1= female* *2=male*	/___/
A4.5	Level of education		*0 = None* *1 = Koranic* *2 = Primary* *3 = Junior secondary school* *4 = Senior secondary school* *5 = Tertiary* *6 = Other (specify)*-------------------	/___/
A4.6	Marital status		*1 = Married* *2 = Single* *3 = Widow/widower* *4 = Divorced* *5 = Other (specify)*--------------------	/___/

A5	**Household Information**		
A5.1	Are you the head of the household?	*1= Yes* *2= No*	/___/
A5.2	How many people are in your household *(eating from the same pot)*	/___/___/	
A5.3	How many males *are in the household?*	*1. Adult (> 18 years)*	/___/___/
		2. Youth (12 – 18 yrs)	/___/___/
		3. Children (< 12 yrs)	/___/___/
A5.4	How many females *are in the household?*	*1. Adult (> 18 years)*	/___/___/
		2. Youth (12 – 18 yrs)	/___/___/
		3. Children (< 12 yrs)	/___/___/
A5.5	What is the main source of income for the household?	*1 = Farming* *2 = Business* *3 = Salary from employment* *4 = Others (specify)-----------------*	/___/

SECTION B: MANGROVE RICE PRODUCTION

No	**Questions**		**Response**	**Code**
B1	**Cultivation**			
B1.1	How long have you been growing rice in the mangrove? (Number of years)		--------------------------	
B1.2	Is mangrove rice the main crop that you grow?		1 = Yes 2 = No	/___/
B1.3	If no, what proportion of all your farm land do you allocate to mangrove rice cultivation?		1 = ¼ 2 = ½ 3 = ¾	/___/
B1.4	What is the average farm size of your mangrove swamp (ha)		--------------------------	/___/
B1.5	What is the type of cropping system do you practice?		1 = Sole cropping 2 = Mixed cropping	/___/
B1.6	How do you prepare the land for planting?		1 = 2 = 3 =	/___/
B1.7	Do you normally listen to radio and or television for agricultural information?		Yes No	/___/
B1.8	If no, then how do you get agricultural and marketing information		---	
B.2	**Labour**			
B2.1	What is your major source of labour?		1 = Family 2 = Hired 3 = Both 4 = Others (specify)-------------------	/___/
B2.2	Which category of people perform the following activities (**Gender Desegregation**)	1. Brushing	1 = Adult men (> 18 yrs) 2 = Adult women (> 18 yrs) 3 = Young men (12 – 18 yrs) 4 = Young women (12 – 18 yrs) 5 = Not applicable	/___/
		2. Ploughing	1 = Adult men (> 18 yrs) 2 = Adult women (> 18 yrs) 3 = Young men (12 – 18 yrs) 4 = Young women (12 – 18 yrs)	/___/

			5 = Not applicable	
		3. Planting	1 = Adult men (> 18 yrs) 2 = Adult women (> 18 yrs) 3 = Young men (12 – 18 yrs) 4 = Young women (12 – 18 yrs) 5 = Not applicable	/___/
		4. Weeding	1 = Adult men (> 18 yrs) 2 = Adult women (> 18 yrs) 3 = Young men (12 – 18 yrs) 4 = Young women (12 – 18 yrs) 5 = Not applicable	/___/
		5. Fertilizer application	1 = Adult men (> 18 yrs) 2 = Adult women (> 18 yrs) 3 = Young men (12 – 18 yrs) 4 = Young women (12 – 18 yrs) 5= Not applicable	/___/
		6. Bird Scaring	1 = Adult men (> 18 yrs) 2 = Adult women (> 18 yrs) 3 = Young men (12 – 18 yrs) 4 = Young women (12 – 18 yrs) 5= Not applicable	/___/
		7. Harvesting	1 = Adult men (> 18 yrs) 2 = Adult women (> 18 yrs) 3 = Young men (12 – 18 yrs) 4 = Young women (12 – 18 yrs) 5= Not applicable	/___/
		8. Threshing	1 = Adult men (> 18 yrs) 2 = Adult women (> 18 yrs) 3 = Young men (12 – 18 yrs) 4 = Young women (12 – 18 yrs) 5 = Not applicable	/___/
		9. Winnowing	1 = Adult men (> 18 yrs) 2 = Adult women (> 18 yrs) 3 = Young men (12 – 18 yrs) 4 = Young women (12 – 18 yrs) 5= Not applicable	/___/
		10. Drying	1 = Adult men (> 18 yrs) 2 = Adult women (> 18 yrs) 3 = Young men (12 – 18 yrs) 4 = Young women (12 – 18 yrs) 5= Not applicable	/___/

B3	**Varieties grown**				
B3.1	What is (are) the name (s) of the **most** popular rice varieties that you grow in the mangrove swamps (**AT MOST 3**)?		----------------	---------------	--------------
B3.2	What type of variety (ies) do you grow?	1 = Local 2 = RARC improved 3 = Other improved (Please Specify)--------------------------- 4 = Don't know	/___/	/___/	/___/
B3.3	What is the initial source of the variety (ies) that you grow?	1 = Other farmers 2 = Research station 3 = Seed companies 4 = MAFS; 5 = NGOs	/___/	/___/	/___/

		6 = Others (specify)-------------				
B3.4	Which of these traits do you consider very **important** when selecting variety (ies)? (Please rank in order of importance).	Traits	Rank	1 = Yes 2 = No /___/	1 = Yes 2 = No /___/	1 = Yes 2 = No /___/
		1 = Yield				
		2 = Height		1 = Yes 2 = No /___/	1 = Yes 2 = No /___/	1 = Yes 2 = No /___/
		3 = Grain quality		1 = Yes 2 = No /___/	1 = Yes 2 = No /___/	1 = Yes 2 = No /___/
		4 = Days to maturity		1 = Yes /___/ 2 = No	1 = Yes 2 = No /___/	1 = Yes 2 = No /___/
		5 = Panicle type		1 = Yes 2 = No /___/	1 = Yes 2 = No /___/	1 = Yes 2 = No /___/
		6 = Leaf angle		1 = Yes 2 = No /___/	1 = Yes 2 = No /___/	1 = Yes 2 = No /___/
		7 = Taste		1 = Yes 2 = No /___/	1 = Yes 2 = No /___/	1 = Yes 2 = No /___/
		8 = Grain size		1 = Yes 2 = No /___/	1 = Yes 2 = No /___/	1 = Yes 2 = No /___/
		9= Leaf senescence		1=Yes 2=No /___/	1 = Yes 2 = No /___/	1 = Yes 2 = No /___/
		10 = Tolerance to abiotic stress		1 = Yes 2 = No /___/	1 = Yes 2 = No /___/	1 = Yes 2 = No /___/
		11 = Tolerance to biotic stress		1 = Yes 2 = No /___/	1 = Yes 2 = No /___/	1 = Yes 2 = No /___/
B3.5	What are the traits of the variety (ies) you grow? Use descriptors to assist farmers identify the traits of their variety (ies)					
B3.6	Height	1 = Tall 2 = Medium 3 = Short		/___/	/___/	/___/
B3.7	Thresheability	1 = Good 2 = Medium 3 = Poor		/___/	/___/	/___/
B3.8	Flowering ability	1 = Good 2 =Medium 3 =Poor		/___/	/___/	/___/
B3.9	Taste	1 = Good 2 = Medium 3 = Poor		/___/	/___/	/___/
B3.10	Cooking quality	1 = Faster 2 = Stays longer in the stomach 3 = Sticky 4 = Soft 5 = Keeping quality		/___/	/___/	/___/

B3.11	Leaf angle	1 = Erect 2 = Semi erect 3 = Droopy	/___/	/___/	/___/
B3.12	Grain Size	1 = Long 2 = Short 3 = Big 4 = Small	/___/	/___/	/___/
B3.13	Yield	1 =Low 2 =Medium 3 =High	/___/	/___/	/___/
B3.14	Leaf Senescence	1= 2= 3=	/___/	/___/	/___/
B3.15	Tolerant to drought	1 = Yes 2 = No	/___/	/___/	/___/
B3.16	Tolerant to flooding	1 = Yes 2 = No	/___/	/___/	/___/
B3.17	Tolerant to salinity	1 = Yes 2 = No	/___/	/___/	/___/
B3.18	Tolerant to iron toxicity	1 = Yes 2 = No	/___/	/___/	/___/
B3.19	Tolerant to diseases	1 = Yes 2 = No	/___/	/___/	/___/
B3.20	Yield stability	1 = Yes 2 = No	/___/	/___/	/___/
B3.21	What is your variety's yield/ha?		-----------------	---------------	---------------
B4	**Farmer's Preferred Traits for Mangrove Rice Varieties**				

B4	Please rank these rice traits in order of preference (1 = most preferred to 10 = least preferred)	1. Yield	/___/
		2. Height	/___/
		3. Grain size	/___/
		4. Thresheability	/___/
		5. Cooking quality	/___/
		6. Tolerant to salinity	/___/
		7. Taste	/___/
		8. Tolerance to abiotic stress	/___/
		9. Tolerance to biotic stress	/___/
B5	**Farmer's perception on Mangrove rice cultivation**		
B5.1	Do you normally listen to radio or TV programs for agricultural news?	1 = Yes 2 = No	/___/

	Have you heard about mangrove rice varieties?		1 = Yes 2 = No	/___/
B5.2	Do you get marketing information on mangrove rice varieties either as seeds/as grains?		1 = Yes 2 = No	/___/
B5.3	Do you see any profitability in growing and or marketing mangrove rice?		1 = Yes 2 = No	/___/
B5.4	If no, what do you think should be done in making mangrove rice profitable?		1 = Less 2 = Same 3 = More	/___/
B5.5	How would you evaluate mangrove rice varieties, compared to other varieties?	1. Total yield	1 = Lower 2 = Same 3 = Higher	/___/
		2. Crop duration	1 = Shorter 2 = Same 3 = Longer	/___/
		3. Grain Size	1= Shorter 2= Same 3= Bigger 4= Longer	
		4. Taste	1 = Aromatic 2 = Sweeter 3 = Same	/___/
		5. Cooking quality	1 = Faster 2 = Stays longer in stomach 3 = Sticky 4 = Soft 5 = Keeping quality	/___/
		6. Height	1 = Shorter 2 = Same 3 = Longer	/___/
		7. Flowering ability	1 = Shorter 2 = Same 3 = Longer	/___/
		8.Drought tolerance	1 = Not tolerant 2 = Moderately tolerant 3 = Very tolerant	/___/
		9.Flood tolerance	1 = Not tolerant 2 = Moderately tolerant 3 = Very tolerant	/___/
		10. Pest tolerance	1 = Not tolerant 2 = Moderately tolerant 3 = Very tolerant	/___/
		11.Disease tolerance	1 = Not tolerant 2 = Moderately tolerant 3 = Very tolerant	/___/
B5.6	Who decides whether or not to grow mangrove rice		1 = Husband 2 = Wife 3 = Both	/___/
B5.7	What is the original source of mangrove varieties you grow?		1 = Other farmers 2 = Research institution 3 = NGOs 4 = MAFS extension 5 = Seed companies 6 = Others (Specify)----------- ----------------------------------	/___/

B5.8	Do you have knowledge on the cultivation importance of mangrove varieties?	1 = Yes 2 = No	/___/
B5.9	Please list down some of the importance in order of their relevance 1= most important, 2 = moderately important 3=Least important	1 = 2 = 3 =	
B5.10	What are the threats you are facing in cultivating Mangrove rice	1 = Climate Variability 2 = Insect pests 3 = Animal pests 4 = Diseases 5 = Others (Specify)----------- ----------------------------------	

SECTION C: MANGROVE RICE PROCESSING AND STORAGE

No.	Questions		Response	Codes
	Do you store mangrove rice after harvesting			/___/
C2	If yes, how do you store	2.1 Sacks	1 = Yes	/___/
		2.2 Boxes		
		2.3 Woven baskets		
		2.5 Ceiling of house/hut/kitchen		
		2.6 Others (Specify)----------------		
C3	If no storage, why?		………………………………	
C4	Do you treat rice grains before storage?		1 = Yes 2 = No	/___/
C5	If yes, how (Please explain)		---	
C6	If no treatment, why		---	
C7	Give 3 ways how rice grains deteriorate? In order of level of damage 1= severe, 2 = moderate, 3 = less		1 = 2 = 3 =	/___/

SECTION D: HOUSEHOLD UTILIZATION OF MANGROVE RICE

No.	Questions		Response	Code
D1	Why do you grow mangrove rice?		1 = Food 2 = Cash 3 = Easy access to land	/___/
D2	**Storage roots**			
D2.1	How do you utilize the harvested grains		1 = Food only 2 = Sale only 3 = Both	/___/
D3.1	Marketing of rice product	1. Where and to whom do you sell rice grain?	1 = Take to the market and sell to consumers 2 = Take to market and sell to traders 3 = Sell at farm gate to traders	/___/

			4 = Sell at farm gate to consumers		
		2. If sold in markets, which type of market	1 = Daily community market 2 = Roadside market 3 = Periodic market 4 = Urban markets 5 = Others (Specify) ………………..		/___/
		3. Do your customers have special traits they prefer in rice grains	1 = Yes 2 = No		/___/
		4. If yes, which traits do they prefer?	1. Grain size	1 = Yes 2 = No	/___/
			2. Cooking quality	1 = Yes 2 = No	/___/
			3. Rice grain color	1 = Yes 2 = No	/___/
			4. Taste	1 = Yes 2 = No	/___/
			5. Others (specify) ………………………		
		3. How much do you sell 1 Kg of rice grain	SSL ……………………………		

SECTION E: INSTITUTIONAL FACTORS

No.	**Questions**	**Response**	**Code**
E1	During the past two years, have you attended an agricultural field day or an on-farm demonstration	1 = Yes 2 = No	/___/
E1.1	If yes, who organized the event	1 = Research 2 = MAFS Extension 3 = NGO 4 = University 5= Others (Specify)-------------	/___/
E2	During the past two years, have you contacted any extension officer	1 = Yes 2 = No	/___/
E2.1	If yes, how many times has an extension technician visited you?	1 = None 2 = Once a week 3 = Once every two weeks 4 = Once a month	/___/
E2.2	From which organization?	1 = Research 2 = MAFS Extension 3 = NGO 4 = University	/___/
E2.3	What type of assistance did he/ she provide?	1 = Supply of planting materials 2 = Supply of inputs (tools and agro chemicals)	/___/

No.	Questions	Response	Code
		3 = Extension training 4 = Market information 5 = Others (specify)…………	
E3	During the past two years, have you participated in an agricultural training course?	1 = Yes 2 = No	/___/
E3.1	If yes, who sponsored it?	1 = Research 2 = MAFS Extension 3 = NGO 4 = University	/___/
E3.2	What did you learn from the training?	1 = Improved agronomic practices 2 = IPM 3 = Agribusiness 4 = Other (specify)--------------	/___/
E4	Are you or your spouse, a member of any organisation?	1 = Yes 2 = No	/___/
E4.1	If yes, what type of organization?	1 = FBO 2 = FSA 3 = Social clubs 4 = Others (specify) …………………………..	/___/
E4.2	What are the key benefits you or your spouse derived from the organization?	1 = Financial assistance 2 = Access to agricultural inputs 3 = Exchange of labor 4 = Access to planting materials	/___/

SECTION F: SWOT ANALYSIS

No.	Questions	Response
F1	What are your major strengths? (At most 3)	1. ………………………………………………………..... 2. …………………………………………………….... 3. ………………………………………………………....
F2	What are your major weakness? (At most 3)	1. ………………………………………………………..... 2. …………………………………………………….... 3. ………………………………………………………....
F3	What are your major opportunities? (At most 3)	1. ………………………………………………………....

		2. ……………………………………………………………... 3. ……………………………………………………………....
F4	What are your major threats? (At most 3)	1. ……………………………………………………………. 2. ……………………………………………………………. 3. …………………………………………………………….

General comments and suggestions

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Thank you for your co-operation