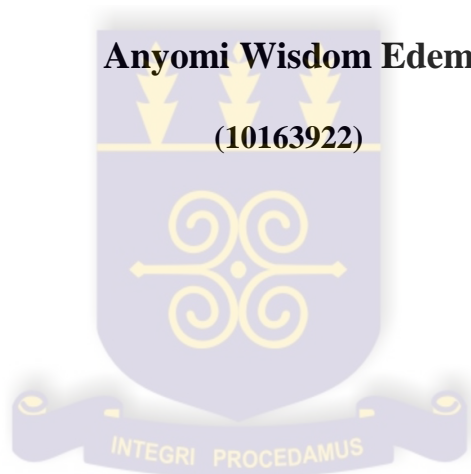


**GENOTYPIC AND PHENOTYPIC VARIABILITY OF SEEDLING
TOLERANCE TO SALINITY OF RICE GERMPLASM**

BY



**THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN
PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF
MASTER OF PHILOSOPHY IN CROP SCIENCE DEGREE.**

JULY, 2015

DECLARATION

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

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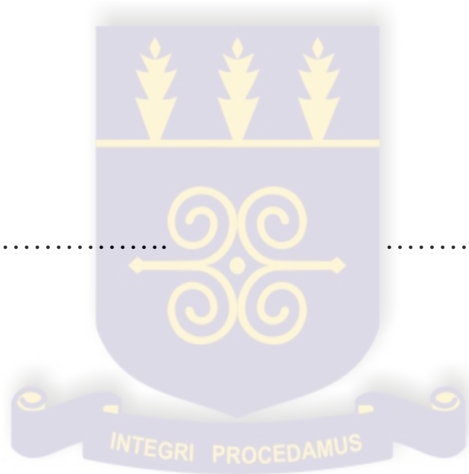
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(SUPERVISOR)

ABSTRACT

Soil salinity is one of the most important factors retarding rice growth and development at both vegetative and reproductive stages. This is becoming a major challenge in the production of rice in Ghana and Africa as a whole. This has necessitated the breeding of salt tolerant lines for the future, since population and hence consumption of rice is always on the increase. Thirty-two (32) rice accessions including four (4) checks were screened for salinity tolerance and also used in diversity studies, in order to select accessions for breeding programs. Pregerminated seeds were sown in Styrofoam hydroponic system. Salinity stress was induced after the third day by replacing the distilled water with nutrient solution with an initial EC of 6 dS/ m. The salinity was increased by adding NaCl to attain an EC of 12 dS/ m by the third day while maintaining the pH of the solution at 5.0 daily. Visual scoring for stress symptoms was done after 12 days with the aid of the checks to separate the accessions.

Shoot samples of each entry were collected and washed carefully with distilled water. They were Oven-dried for 3 days at 70 °C. The dried samples were ground to obtain about 1 g (40 mesh) powder per accession and the Na and K ion concentrations were determined using flame photometer.

Genetic diversity studies was carried with 33 SSR primers, 20 of which were markers for salt tolerance. 28 out of the 33 primers were polymorphic. The PCR products were run and visualized on a 3% agarose gel matrix stained with ethidium bromide. Amplified bands were scored and analyzed with PowerMarker v3.25 and DARwin v5 software. The genetic diversity among the accessions assembled was high ($H_e=0.6$, $I=0.516$, $PIC=0.471$). Saltol

Primers RM10711 and RM10793 were the only primers able to completely discriminate tolerant genotypes from susceptible ones, hence can be used in selections involving the genotypes. Accessions SR1, IR72, Sebotia 337-1, Perfume (Short) type, Anyofula, Local Red, GR18Red, GH1580, GH1528, GH1575, NericaL23, NericaL24 and NericaL27 performed well under salinity stress in this study and were identified to be superior among the accessions used.



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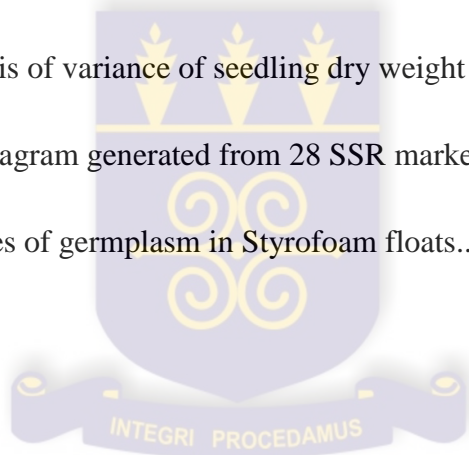


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LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
ARC	African Rice Centre
bp	base pair
Ca	Calcium
CO ₂	Carbon dioxide
CRD	Completely Randomized Design
CRI	Crop Research Institute
CTAB	Cetyltrimethylammonium Bromide
DAS	Days after Sowing
ddi	Distilled deionized
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
EC	Electrical Conductivity
EDTA	Ethylenediaminetetraacetic acid
F ₁	First Filial generation
FAO	Food and Agriculture Organization of the United Nations
GA	Genetic Advance

H	Diversity index
HCl	Hydrochloric acid
IBPGR	International Board for plant Genetic resources
IRRI	International Rice Research Institute
JICA	Japan International Cooperation Agency
K	Potassium
K ⁺	potassium ions
Kb	kilo base
MAS	Marker assisted selection
M	Molar
MgCl ₂	Magnesium Chloride
Mg	Magnesium
Min	minutes
Mt	Metric tones
Mt/Ha	Metric tonnes per Hectare
MOFA	Ministry Of Food and Agriculture
Na	Sodium
Na ⁺	Sodium ions

N_a	Average number of Alleles
NaCl	Sodium Chloride
N_e	Average number of Effective Alleles
NERICA	New Rice for Africa
NPK	Nitrogen Phosphorus Potassium
OD	Optical Density
PAGE	Polyacrylamide Gel Electrophoresis
PC	Principal Component
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PEG	Polyethylene Glycol
PGRRI	Plant Genetic Resource Research Institute
PIC	Polymorphic Information Content
PL	Polymorphic Loci
QTL	Quantitative Trait Loci
RAPD	Random amplified polymorphic DNA
RCBD	Randomized Complete Block Design
RI	Recombinant Inbred

rpm	Revolutions per minute
RFLP	Restriction fragment length polymorphism
SARI	Savanna Agricultural Research Institute
SES	Standard evaluation score
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeat
t/Ha	Tonnes per Hectare
TAE	Tris-acetate-EDTA
TBE	Tris-Borate-EDTA
TE	Tris EDTA
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
UV	Ultra violet
V	Voltage
Vol	Volume
WARDA	West Africa Rice Development Association
WRS	World Rice Statistics
Wt	Weight

CHAPTER ONE

1.0 INTRODUCTION

Rice (*Oryza sativa*, L. and *O. glaberima*, Steudl) is one of the important cereal crops grown worldwide. Rice belongs to the genus *Oryza* L., family Poaceae. It is a small genus of 20–25 species with a pan-tropical and sub-tropical distribution. Two species of the genus are cultivated, namely *Oryza sativa*, the universally cultivated Asian rice, and *O. glaberrima*, the West African cultivated rice. African rice is now only rarely grown in pure stands. It is currently grown in mixture with the Asian rice in various proportions. The extent of even this form of mixed cultivation is diminishing as it is being replaced with ‘pure’ Asian rice (Nayar, 2010).

Rice plays an important role as a staple food crop and is used to feed more than three billion people on a daily calorie intake of 50 to 80% (Khush, 2005; Aggarwal *et al.*, 2002). It is planted on about one-tenth of the earth’s arable land (El-Refaee *et al.*, 2006). Rice is the fastest growing food source in Africa (Nwanze *et al.*, 2006). Africa’s emergence as a big rice importer is explained by the fact that during the last decade rice has become the most rapidly growing food source in sub-Saharan Africa, due to population growth (4% per annum), rising incomes and a shift in consumer preferences in favour of rice, especially in urban areas (Balasubramanian *et al.*, 2007).

West Africa is about 6 million km² in area, and rice occupies about 8% of the total crop area, ranking fifth in area (FAOSTAT, 2009) and its demand for rice is increasing at a rate of 6% per annum, the highest in the world. Rice has become a major source of livelihood

to many smallholder farmers, processors and traders in West and Central Africa (Kijima *et al.*, 2008).

Rice has become a major staple in recent decades with a per capita consumption of 25 kg/annum in Ghana, but most of the consumption is met by imports (MOFA, 2010). In 2009, Ghana imported over 350,000 tons of milled rice worth 600 million US dollars (Duffuor, 2009).

Salinity is one of the major factors limiting the productivity of rice crop in many countries worldwide. Rice crop is very sensitive to salinity especially in early growth stages and it is observed that salinity affects the kernel and aromatic characteristics of rice grains heavily. It also disturbs the antioxidants mechanisms and osmoprotectants balance of the plant (Singh *et al.*, 2007). Salinity is considered as one of the major abiotic stresses that affects crop productivity and quality and has been described as one of the most serious threats to agriculture and the natural status of the environment (Galvani, 2007; Lauchli and Grattan, 2007, Arshad *et al.*, 2012; Chinnusamy *et al.*, 2005, Borsani *et al.*, 2003).

The bulk of Ghana's saline soils occurs within the Coastal Savanna Agro-Ecological Zone of the southeastern corner. In this zone lies the coastal scrub, savannah and mangrove swamp of the Lower Volta Basin in the Greater Accra and Volta Regions within Accra-Ho-Keta Plains. Soils in these areas are highly salty, intolerable to crops, hydromorphic, heavy textured and poor in nutrients. Impenetrable sodium-saturated pan occurs in some of these soils (Agawtaw series) on the uplands. With only 760 mm of unreliable annual rainfall and high potential evapo-transpiration, there is a high water-salt imbalance in the Plains (FAO, 2000). 200,000ha and 118,000ha of Ghana's soils are Solonchaks and

Alkaline respectively (Szabolcs, 1989) while 70, 000 ha and 600, 000 ha are Arenosols and Solonetzs respectively (FAO, 2000).

Increased salinization of arable land is expected to have devastating global effects, resulting in a 30% land loss within the next 25 years and up to 50% by the year 2050 (Wang *et al.*, 2003). Salinity of arable land is one of the most important factors retarding rice growth and development at both vegetative and reproductive stages (Zeng and Shannon, 2000; Zeng *et al.*, 2003). The problem has been approached through better management practices and introduction of salt-tolerant varieties in the affected areas. These improved management practices in salt-affected areas have generally proven to be uneconomical and difficult to implement on a large scale. Thus, genetic improvement of salt tolerance of major cereal crops like rice (*Oryza sativa*), wheat (*Triticum aestivum*), maize (*Zea mays*), and barley (*Hordeum vulgare*) appears to be the most feasible and promising strategy for maintaining stable global food production (Munns, 2002). The success of salt tolerance breeding programs employing traditional screening and selection has been limited in the past decades. Conventional methods of plant selection for salt tolerance are difficult because of the large effects of the environment and low narrow sense heritability of salt tolerance (Gregorio, 1997).

Molecular markers are identifiable DNA sequences, found at specific locations of the genome and associated with the inheritance of a trait or linked gene (FAO, 2004). Thottappilly *et al* (2000), refer to molecular markers as naturally occurring polymorphism which include proteins and nucleic acids that are differently detected. Several molecular markers exist, these include restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPDs), amplified fragment length polymorphisms

(AFLPs), microsatellite or simple sequence repeat (SSR), and single nucleotide polymorphism (SNPs). DNA marker technology, derived from research in molecular genetics and genomics, offers great promise for plant breeding. Owing to genetic linkage, DNA markers can be used to detect the presence of allelic variation in the genes underlying these traits. (Collard and Mackill, 2008).

SSRs are ideal genetic markers for detecting differences between and within species for genes of all eukaryotes (Farooq and Azam, 2002). They consist of tandemly repeated 2-7 base pair units arranged in repeats of mono-, di-, tri-, tetra and penta-nucleotides (A,T, AT, GA, AGG, AAAG) with different lengths of repeat motifs. Some of the prominent features of SSR markers are that they are dominant fingerprinting markers and codominant sequence tagged microsatellites (STMS) markers (Joshi et al, 2011). A number of microsatellite markers have already been developed in rice and their primer sequences have been published (Wu *et al.*, 1993; Akagi *et al.*, 1996; Panaud *et al.*, 1996; Temnykh *et al.*, 2000). Microsatellite markers have been effectively used to identify genetic variation among rice cultivars [Yang *et al.*, 1994; Akagi *et al.*, 1997; Garland *et al.*, 1999]. Similarly Thanh *et al.*, (1999) showed the genetic variation identified by microsatellite markers to be useful in evaluating upland rice accessions.

Yoon *et al.*, (2000) recommended that molecular techniques as a fundamental tool should be exploited to complement morpho-agronomic evaluation because it provides better diversity clarity in plant populations at DNA level. Molecular techniques should be included in taxonomic groupings of accessions to establish their phylogenetic relationship (Fatokun *et al.*, 1993; Kaga *et al.*, 1996). The identification of major gene locus for salt tolerance near a

microsatellite marker can be used by plant breeders to select more efficiently and to better understand salt tolerance, at vegetative and reproductive growth stages (Saqib *et al.*, 2012).

It is often the norm in plant breeding to maximize the genetic diversity between parental genotypes for intercrosses. Genetic diversity between parental genotypes is usually estimated by measurements of physiological and morphological differences of quantitative and economically important traits. The disadvantages of this conventional approach are the cost of time and labor during the measurements, and the influences of environmental factors. (Zeng *et al.*, 2004).

These disadvantages are exacerbated in salt-tolerance breeding. Any change in environment such as temperature, light or humidity can dramatically change the transpirational driving forces and, subsequently, ion uptake (Yeo *et al.*, 1990, Flowers *et al.*, 1997). Such changes may alter salt tolerance among genotypes. It is also important to note that morphological characters are often limited in their numbers and may not adequately represent actual genetic relationships among genotypes. Nonetheless, there must be enough genotypes with high diversity for plant breeding purposes.

The world population is expected to increase rapidly in the near future. Much of this human growth will be concentrated in developing countries, with sub-Saharan Africa (SSA) leading the way, as its population is estimated to double from 770 million in 2005 to 1.5 billion by 2050 (Seck, 2011). Farming, which is the main source of livelihood for millions of poor people, suffer from several local constraints mainly due recently to climatic change whose impact is already being felt in Africa through increased incidences and severity of droughts and floods. Some of these constraints are particularly devastating to Africa's rice production since almost 80% of the region's rice area is rainfed (Mohapatra, 2009).

Fortunately, rice has a significant genetic variation in traits related to local biotic stresses such as: Pests, diseases and abiotic stresses with mainly drought, acidity, iron toxicity, cold and salinity. Indeed, scientists desperately look for useful resistance traits in plant varieties especially for short life cycle duration, favorable root architecture, weed competitiveness and water-use efficiency to incorporate into breeding programs to develop improved, high-yielding and tolerant varieties.

Over 7 million plant germplasm accessions are housed in some 1750 national and international gene banks (FAO, 2010). They are part of a worldwide effort to conserve, characterize, and use plant biological diversity to address problems of global importance.

This collection will serve as a source for the introgression of salt tolerant genes into commercial varieties, to enhance the productivity of rice in Ghana

The main objective of this study is to screen the rice germplasm assembled at the University of Ghana for rice seedlings tolerant to salinity and identify and conserve elite genotypes.

The specific objectives of the study are to;

- (1) screen the rice germplasm under salinized and non-salinized conditions at the seedling stage.
- (2) identify useful microsatellite markers for genotyping the germplasm.
- (3) determine the genetic diversity within the rice germplasm based on their microsatellite marker profile and select salt tolerant genotypes.
- (4) establish an elite collection of salt tolerant lines that will be used in future breeding works to develop salt tolerant varieties.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Botany and economic importance

Rice (*Oryza sativa*, L. and *O. glaberima*, Steudl) genetic resources are widely available worldwide. The crop is cultivated in a variety of ecosystems which include irrigated, rainfed lowland, rainfed upland and flood-prone areas.

Cultivated rice includes two taxonomically distinct species: *Oryza sativa* L., and *Oryza glaberrima* Steudl (usually named African rice). The two species can be distinguished in the field especially by differences in ligule shape and panicle branching (Porteres, 1955; Besançon, 1993). At maturity, lodging and seed dormancy occur within the African rice genotypes. *O. glaberrima* is unique to Africa (Mohapatra, 2010) and was domesticated in West Africa more than 3500 years ago (Portères, 1956; Angladette; 1966). Five wild *Oryza* species occur in Africa, including West Africa namely *O. barthii*, *O. longistaminata*, *O. brachyantha*, *O. eichingeri* Peter and *O. punctata* Kotschy ex Steud (Vaughan, 1994). Among them, *O. barthii* and *O. longistaminata* Cheval and Roehr have the widest and densest distributions. Both occur in extensive stands in aquatic and/or seasonally wet situations, and also as weeds in rice fields. *Oryza barthii* is an annual self-fertilizing species, while *O. longistaminata* is a tall, robust, rhizomatous perennial species. *Oryza longistaminata* occurs in more stable habitats. It is partially self-incompatible and cross-fertilizing (Nayar, 1958, 1967). The remaining three wild species have restricted and scattered distributions.

Rice is a self-pollinating and semi-aquatic plant adapted to submergence. It is classified into three groups depending on their requirement and tolerance to water in highland, lowland (irrigated and rainfed), and deep-water (Takahashi, 1984). Rice grain comes in a variety of colours, including: white rice, brown rice, black rice, purple rice, and red rice (Oka, 1988). Black rice (also known as purple rice or forbidden rice) is a range of rice types, some of which are glutinous rice.

African rice, red rice or rice of Casamance, *Oryza glaberrima* (Steudl) is the main and the only cultivated rice among the African rice species. Poaceous belonging to the tribe Oryzae, and Genus *Oryza*, it is an annual and preferentially selfing crop with $2n = 24$ chromosomes (Besançon and Second, 1984). *O. glaberrima* differs from *O. sativa* in many qualitative and quantitative traits. It is an annual grass, up to 120 cm tall and produces more in upland or irrigated conditions (Besançon, 1993). The rooting system is fibrous. Dry land types possess simple culm often rooting at lower nodes and floating types are often branching and rooting at upper nodes. The stems are without ramifications, except sometimes in floating culture.

Rice has several uses. In parts of West Africa (WA), the grain of the cultivated African rice is a staple food, highly appreciated for its taste and culinary qualities. It is also used in traditional and ritual ceremonies to appease the souls of the ancestors as in the Casamance region in southern Senegal, and the villagers of the Danyi plateau in Togo (Mohapatra, 2010). The finer parts of the bran and broken grains are given as feed to chicken and other livestock. In the Central African Republic, the root is eaten raw to treat diarrhea (www.prota.org). But as a traditional food grain *O. glaberrima* is not traded internationally. It is only distributed within the regions of production. It was estimated that its growing

areas is less than 20% of the total cultivated area allocated to rice in West Africa (WARDA, 1996).

2.2 Genetic diversity and variability among rice

O. glaberrima survived in the African harsh environment with low human interferences. It has therefore developed some resistant characters to its predestinated environment and presents a lot of useful traits to overcome biotic and abiotic conditions (Takeoka, 1965; Second, 1984). It does not readily cross with *O. sativa*, the African rice's greater tolerance to environmental stresses is receiving increasing plant breeding attention (Sano, 1989; Harlan, 1995). Two scientists Sie Moussa and Monty Jones succeeded in breaking the natural barrier that made it difficult for interspecific cross between the two cultivated species and thus reaching the genesis of interspecific varieties trademarked as NERICA (New Rice for Africa). The best NERICA varieties combine the resistance and stress tolerance of *O. glaberrima* and its ability to thrive in harsh environment with the high yielding potential of *O. sativa*. (Somado *et al.*, 2008; Jones *et al.*, 1997; Sié *et al.*, 2005). There are still gaps between the NERICA varieties and *O. glaberrima* in relation to resistance to some local constraints including weeds (Futakuchi *et al.*, 2009).

Mohapatra, (2010), Futakuchi and Sie (2009) and Sarla and Mallikarjuna (2005) reported that CG14, one of the outstanding *O. glaberrima* varieties has consistently proved to be weed competitive with good resistance to iron toxicity, drought, nematodes, water logging, and major African rice diseases and pests. Such multiple resistances to the indigenous

constraints are highly desirable characters for rice cultivation in West Africa's rainfed and lowland ecologies.

(WARDA, (1996) and Jones *et al.*, 1997) also reported *O. glaberrima*, as a rich reservoir of genes for resistance against local stresses. Such useful genetic assets are very important and appropriate for resource-poor farmers, who cannot afford to adopt intensive agronomic measures against the environmental constraints (Futakuchi and Sie, 2009; Rodenburg *et al.*, 2009, Africa Rice, 2010). Notwithstanding the positives of *O. glaberrima*, it has many undesirable traits which result in low yield potential namely, lodging, grain shattering, low panicles primary and secondary branches and long seed dormancy which are the major constraints to its productivity (WARDA, 1993).

According to a USDA report, the world's largest exporters of rice in 2012 were India (9.75 million tonnes), Vietnam (7 million tonnes), Thailand (6.5 million tonnes), Pakistan (3.75 million tonnes) and the United States (3.5 million tonnes). The cultivation of rice extends from dry lands, wetlands to cool climates at altitudes of over 2, 600 m above sea level in the mountains of Nepal, as well as the hot deserts of Egypt. Most of the annual rice production however comes from tropical climate areas (Downing, 1992). Approximately 650,000 ha of rice production land in West Africa are threatened by salinization, particularly within the Sahel (arid or semi-arid region) where rainfed rice production is not feasible (Africa Rice Centre (WARDA), 2007).

Africa's emergence as a big rice importer is explained by the fact that during the last decade rice has become the most rapidly growing food source in sub-Saharan Africa, due to population growth (4% per annum), rising incomes and a shift in consumer preferences in favour of rice, especially in urban areas (Balasubramanian *et al.*, 2007). It is one of the

most important crops in the world, growing on over 1.5 billion hectares of land with overall worldwide production estimated around 747.5 million tonnes (498.4 million tonnes, milled basis) in 2013.(FAO, 2013). Currently rice is grown in 117 countries.

Genetic variability refers to the potential for a given characteristic or genotype to vary within a population when faced with a particular influence. As the genetic variability of a population increases, so does its resistance to environmental and genetic influences and ultimately to extinction. Consequently, genetic variability is directly tied to biodiversity and evolution. Variability is an important factor in evolution as it affects an individual's response to environmental stress and thus can lead to differential survival of organisms within a population due to natural selection for the most fit variants. Genetic variability also underlies the differential susceptibility of organisms to diseases and sensitivity to toxins or drugs. Genetic variation occurs mainly through DNA mutation, gene flow (movement of genes from one population to another) and sexual reproduction.

Knowledge on the nature and magnitude of genetic variation governing the inheritance of quantitative characters like yield and its components is essential for effecting genetic improvement. A critical analysis of genetic variability is a pre-requisite for initiating any crop improvement program and for adopting appropriate selection techniques. High magnitude of variability in a population provides the opportunity for selection to evolve a variety having desirable characters.

Genetic diversity is the variation of heritable characteristics present in a population of the same species. It serves an important role in evolution by allowing a species to adapt to a new environment and to fight off parasites. Genetic diversity is important for two reasons. First of all, when a population of an organism contains a large gene pool, and if the genetic

blueprints of individuals in the population vary significantly the group has a greater chance of surviving and flourishing than a population with limited genetic variability. Genetic diversity also reduces the incidence of unfavorable inherited traits. Genetic diversity can be seen as a defense against problems caused by genetic vulnerability. Traditional farmers built this defense into the genetic structure of landraces through selection over many generations and it may be necessary to introgress such defense mechanisms into modern cultivars to make them sustainable (Chang, 1994). Rice genetic resources available at genebanks can be used for incorporating genetic variability into rice breeding programs, which can potentially generate new cultivars with broadened genetic bases and allow new and useful allelic combinations (McCouch, 2005).

Selection by humans and crop adaptation to diverse environmental conditions have resulted in a large number of genotypes. It has been estimated that about 120,000 rice varieties exist worldwide (Khush, 1997). Rice production doubled between 1966 and 1990 due to the proliferation of highly productive cultivars, but the use of elite germplasm in breeding programs reduced the genetic variability available for selection. This is believed to be the main factor for the leveling off of yield (Rangel *et al.*, 1996). Crosses to broaden the genetic base of rice can promote the preservation of rare alleles that can be incorporated in elite germplasm. The use of adapted rice landraces, as the primary source of variation into which desired characters present in modern cultivars are introgressed may be an effective strategy for producing cultivars adapted to difficult production environments (Hawtin *et al.*, 1997).

According to Lanteri and Barcaccia (2006), landraces have higher genetic variability among the different groups of germplasm, as well as better environmental adaptation, and are an irreplaceable source of highly co-adapted genotypes. As a natural source of genes

for disease and insect tolerance, these materials also have a high potential to increase the genetic bases of elite rice lines and cultivars. The genetic variability found in rice landraces are well documented. The main reasons for their occurrence are the genetic factors involved in changes of allele frequencies acting on each landrace, such as cross pollination and migration, seed mixture, founder effect, genetic drift, or adaptability selection (Tin *et al.*, 2001; Barry *et al.*, 2007).

Determining genetic diversity can be done from morphological, biochemical, and molecular types of information (Sudre *et al.*, 2007; Goncalves *et al.*, 2009). However, molecular markers are advantageous over the other kinds. They show genetic differences on a more detailed level without interferences from environmental factors, and involve techniques that provide fast results detailing genetic diversity (Saker *et al.*, 2005; Goncalves *et al.*, 2008; Souza *et al.*, 2008). Moreover, the discovery of high throughput platforms increases number of data per run, reducing the cost of the data and increasing map resolution.

Claudio *et al.*, (2006) characterized the allelic diversity of 192 traditional varieties of Brazilian rice using 12 simple sequence repeat (SSR or microsatellite) markers. They identified 16 accessions as a mixture of pure lines or heterozygous plants. Analysis identified six clusters of identical accessions with different common names and just one cluster with identical accessions with the same common name. A subset of 24 landraces, representatives of 13 similarity groups plus the 11 accessions not grouped, was the most variable set of genotypes analyzed.

Sandhyakishore *et al.*, (2007) studied genetic divergence for different yield attributing traits in 70 rice genotypes. The analysis of variance revealed significant differences among the genotypes for each character. The genotypes were grouped into nine different clusters. The mode of distribution of genotypes from different eco-regions into various clusters was at random, indicating that geographical diversity and genetic diversity were not related. Similarly, Vaithiyalingan (2005) studied the genetic divergence in twenty nine strains of rice collected from different geographical regions of the world. The genotypes were grouped into four clusters. The clustering pattern was again independent of the geographical distribution. Cluster II included 23 genotypes which could be useful in hybridization to create a wide spectrum of variability. Maximum distance (97.34) was observed between clusters III and IV, plant height, single plant yield and 1000-seed weight were found to be important contributors to genetic divergence. Singh *et al.*, (2006) also studied genetic divergence of 52 traditional lowland rice genotypes from five states of North Eastern Region of India. The genotypes were grouped into six clusters. Genotypes from more than one state were grouped in one cluster, while genotypes from one state were grouped in more than one cluster. Geographical origin was not found to be a good parameter of genetic divergence. Plant height, leaf angle and leaf area, highly contributed (32.43%) to the formation of clusters. Clusters II, IV, and V, which had maximum inter-cluster distances and high values of plant height, days to 50% flowering, panicle length, grain yield/plant and milling percent, were suggested to be used as basis for initiating a hybridization program.

Getachew *et al.*, (2013) assessed the genetic diversity of *Oryza longistaminata* Chev. et Roehr and how this variation is partitioned within and between the eight *O. longistaminata*

populations found in the different geographic regions of Ethiopia using simple sequence repeat markers. Five microsatellite markers in 320 samples generated 64 alleles that revealed the presence of large amount of genetic variability ($H_o = 0.225$; $H_e = 0.768$; $N_a = 7.375$; $N_e = 6.565$ and $P = 0.744$). The genetic diversity results obtained from their data indicated that there were high levels of genetic diversity in the populations of *O. longistaminata* studied and it was higher within than between populations.

Zahida *et al.*, (2010) reported on the genetic polymorphism of 75 rice accessions and improved cultivars using random amplified polymorphic DNA (RAPD) technique. Twenty-eight decamer-primers generated a total of 145 RAPD fragments, of which 116 (80%) were polymorphic. The number of amplification products produced by each primer varied from 3 to 9 with an average of 5.2 alleles per primer. All the 75 genotypes fell into two main groups corresponding to aromatic and non-aromatic types of *indica* rice. Clustering of accessions did not show any significant pattern of association between the RAPD fingerprints and collection sites. This type of analysis grouping different rice accessions in relation to fragrance, a major rice quality determinant, and varietal group is extremely useful to develop a core collection and for gene bank management. Furthermore, the information revealed by the RAPDs regarding genetic variation is helpful to the plant breeder in selecting diverse parents and for future orientation of rice breeding program.

The genetic diversity and phylogenetic relationships of eight different varieties of Rice (*Oryza sativa*) were analyzed by Random amplified polymorphic DNA (RAPD). Eleven RAPD primers were used in a comparative analysis of different varieties of rice. Of the 101 total RAPD fragments amplified, 28 (27.72%) were found to be shared by individuals of all eight varieties. The remaining 73 fragments were found to be polymorphic (72.27%).

The average gene diversity or heterozygosity over all varieties was 0.224. This study offered a rapid and reliable method for the estimation of variability between different accessions which could be utilized by the breeders for further improvement of the local rice varieties. (Skaria et al, 2011). Saker *et al.*, (2005) observed the genetic diversity of seven Egyptian rice genotypes using eight RAPD, six SSR and eight AFLP primer combinations and concluded that some varieties originated from closely related ancestors possessing a high degree of genetic similarity.

Neha and Lal (2012), evaluated sixty nine genotypes of rice (*Oryza sativa* L.) for genetic divergence with respect to thirteen yield attributing characters .The analysis of variance revealed highly significant differences for all the characters studied, indicating that there is ample scope for isolation of promising lines from the gene pool for yield improvement. The genotypes were grouped into nine clusters. The composition of the clusters indicated nonexistence of correspondence between genetic diversity and geographical distribution. Traits like number of spikelets per panicle, plant height and biological yield were the major contributors to genetic divergence.

Kumar *et al.*, (2008) evaluated genetic divergence among 30 genotypes of rice and estimated 12 quantitative traits. The genotypes were grouped into eight clusters. The clustering pattern of genotypes did not follow the geographic origin. The inter-cluster distance was highest between clusters V and VI. This indicated that the genotypes included in the clusters had a broad spectrum of genetic diversity and could be used in hybridization programme to generate high heterosis and possibly produce transgressive segregants in subsequent generations.

Chandra *et al.*, (2007) studied genetic divergence among 49 genotypes of non-scented rice including three checks, for seven quantitative characters. Significant varietal differences were observed for all the characters studied. The genotypes were grouped into eight clusters. Kernel length, kernel breadth, days to 50% flowering and plant height contributed significantly to total divergence. Similarly, Sarawgi and Bisne, (2007), studied genetic divergence among 81 scented rice. The genotypes were grouped into nine clusters. Genotypes from cluster II had desired mean for characters like hulling percentage, milling percentage and head rice recovery as well as panicle length. Cluster VII had high value for kernel length and L/B ratio while cluster V had low value for days to 50% flowering but highest value for grain yield Kg. / ha.

Kiambi *et al.*, (2008) studied the relationship between genetic diversity and eco-geographic variables using *Oryza longistaminata*. They combined hierarchical cluster analysis of both molecular diversity generated using Amplified Fragment Length Polymorphism (AFLP) and climate data available in a GIS software. The study clearly established that there was close relationship between genetic diversity and eco-geographic variables. The study also revealed that genetic diversity is a function of annual rainfall, and that peak diversity occurs in intermediate rainfall areas reflecting the 'curvilinear theory' of clinal relationship between the level of genetic diversity and rainfall. The clear association of genetic diversity with rainfall allows the extrapolation of the potential impacts of global warming on diversity when empirical data on predicted climate models, particularly rainfall, are available. This knowledge would therefore be useful in the development of conservation measures to mitigate the effects of genetic erosion through climate change.

Tereza *et al.*, (2009) evaluated the genetic variability of rice (*Oryza sativa*) landraces collected from Brazilian small farms. Twelve simple sequence repeat (SSR) markers were used to characterize 417 landraces collected in 1986, 1987 and 2003, in the state of Goiás, Brazil. The number of landraces with long and thin grain type increased in the evaluation period. Based on the molecular data, the genetic variability increased during this period and, from the correspondence analysis, most of the accessions were grouped according to the year of collection. The introduction of modern rice cultivars into landrace cultivation areas and the selection carried out by small farmers were the most probable factors responsible for increasing landrace genetic variability, during the evaluation period.

Gaspar *et al.*, (2006), evaluated the molecular variability among a representative sample of rice genotypes. AFLP analysis was applied to 56 rice genotypes (Japanese, Philippines, and Brazilian) from the germplasm bank. Six primer pairs were used and they amplified 249 bands, 205 (82.32%) of which were polymorphic. Three groups of genotypes were detected: one contained five out of seven lowland genotypes, the second contained almost all Brazilian and Philippine genotypes and the third group contained six Japanese genotypes.

2.3 Salinity and rice production

Salinity is a major constraint to irrigated rice production, particularly in semi-arid and arid climates. Irrigated rice is a well suited crop to controlling and even decreasing soil salinity, but rice is a salt-susceptible crop and yield losses due to salinity can be substantial. According to the FAO (2010), over 800 million hectares of land worldwide are severely salt affected and approximately 20% of irrigated areas (about 45 million ha) is estimated to suffer from salinization problems to various degrees (Munns and Tester, 2008). This

constitutes a serious problem since irrigated areas are responsible for a third of world's food production considering that, rice is the most important food crop for over half of the world's population and supplies 20% of daily calories [World Rice Statistics]. It is also mainly grown on irrigated and flooded plains. According to Ashraf (2009), crops grown in salinity prone areas of the world are exposed to growth and yield reduction.

After drought, salinity is the second most widespread soil problem in rice growing countries and a serious constraint to increased rice production worldwide (Gregorio *et al.*, 1997). Salt-affected soils is one of the most serious abiotic stresses that cause reduced plant growth, development and productivity worldwide (Siringam *et al.*, 2011). Water-deficit and salt affected soil are two major abiotic stresses which reduce crop productivity, especially that of rice, by more than 50% world-wide (Mahajan and Tutejan, 2005; Nishimura *et al.*, 2011).

It is known that rice shows variation for salt tolerance (Sabouri *et al.*, 2009, Sabouri and Biabani, 2009, Habib *et al.*, 2013). Screening of rice genotypes at seedling stage is comparatively easier and faster than at reproductive stage. It is very difficult at the reproductive stage (Gregorio *et al.*, 1997). Degree of salt stress can affect different crops differently. For rice, soil salinity beyond EC_e of 4 dS/m is considered moderate salinity while more than 8 dS/m becomes high. Similarly pH 8.8 - 9.2 is considered as non-stress while 9.3 – 9.7 as moderate stress and equal or greater than 9.8 as high stress. Extreme high salt stress conditions kill the plant but the moderate to low salt stress affects the plant growth rate and thereby manifest symptoms which could be associated with morphological, physiological or biochemical alterations. (Garg and Gupta, 1997; Mer *et al.*, 2000; Donahue *et al.*, 1983).

2.3.1 Physical effects of salinity on soil

Salinity through flocculation affects soil physical properties by causing fine particles to bind together into aggregates. This enhances soil aeration, root penetration and root growth. . Although increasing soil salinity has a positive effect on soil aggregation and stabilization, at high levels salinity can have negative and potentially lethal effects on plants growth. High sodium concentrations in the soil cause soil dispersion as a result of breakdown of soil aggregates, which, subsequently, settle into soil pores. Soil dispersion causes soil pore blockage resulting in the reduction of soil permeability. Three main problems caused by sodium-induced dispersion are reduced infiltration, reduced hydraulic conductivity and surface crusting (Frenkel *et al.*, 1978; van de Graaf and Patterson, 2001; Pearson, 2004). Relatively high salt concentration in the soil solution essentially pushes adsorbed cations closer to the surface of the soil particles keeping soil aggregates together (Barbour *et al.*, 1998; Western Fertilizer Handbook, 1995). The relationship between soil salinity and its flocculating effects, and soil ESP (exchangeable sodium percentage) and its dispersive effects, dictate whether or not the soil will stay aggregated or become dispersed under various salinity and sodicity combinations.

Surface crusting of soil may account for the hardened upper layers observed in these soils. The hardened upper layers or surface crust are likely to restrict water infiltration and seed emergence. The primary causes of surface crusting are 1. Physical dispersion caused by raindrops or irrigation water, and 2. Chemical dispersion. Surface crusting due to rainfall is greatly enhanced by sodium induced clay dispersion (Morin *et al.*, 1981). Dispersed clay

particles within the soil solution can clog soil pores when the particles settle out of solution (Buckman and Brady, 1967).

The pore plugging and cement-like structure make it difficult for plants to get established and grow. It also impedes water flow and water infiltration into the soil.

2.3.2 Chemical effects of salinity on soil and plants

The common cations associated with salinity are Na^+ , Ca^{2+} and Mg^{2+} , and the anions are Cl^- , SO_4^{2-} and HCO_3^- . However Na^+ and Cl^- ions are the most important, since Na causes deterioration of physical structure of soil and both Na^+ and Cl^- are toxic to plants (Dudley, 1994; Hasegawa *et al.*, 2000). Historically, soils were classified as saline, sodic, or saline-sodic based on the total concentration of salt and the ratio of Na to Ca and Mg ions in the saturated extract of the soil (Dudley, 1994). Sodium chloride forms the major salt contaminant in most saline soils. The effects of sodium ions are well established as this ion can cause damage to plant cells by both ionic and osmotic effects, leading to growth retardation, low productivity and eventually cell death (Hasegawa *et al.*, 2000; Munns, 2002; Mansour and Salama, 2004; Chinnusamy *et al.*, 2005). High concentrations of salts have detrimental effects on plant growth (Garg and Gupta, 1997; Mer *et al.*, 2000) and excessive concentrations kill growing plants (Donahue *et al.*, 1983). Ashraf and Yousef (1998) established that the excess of NaCl alone can cause more toxicity to the rice plant than mixed salts.

The capacity to tolerate salinity is a key factor in plant productivity (Momayezi *et al.*, 2009).

Addition of salts to water lowers its osmotic potential, resulting in reduced water availability to root cells. Salt stress exposes plants to secondary osmotic stress, which implies that all the physiological responses, which are invoked by drought stress, can also be observed in salt stress (Sairam *et al.*, 2002). Specific effects of salt stress on plant metabolism, especially on leaf senescence, have been related to the accumulation of toxic Na^+ and Cl^- ions and to K^+ and Ca^{2+} depletion (Al-Karaki, 2000). Salinity associated with excess NaCl adversely affects the growth and yield of plants by depressing the uptake of water and minerals and normal metabolism (Akhtar *et al.*, (2001); Akram *et al.*, 2001).

Excessive amount of soluble salts in the root environment causes osmotic stress, which may result in the disturbance of the plant water relations in the uptake and utilization of essential nutrients, and also in toxic ion accumulation (Munns, 2002; Lacerda *et al.*, 2003).

Evidence exist that high salt concentrations cause an imbalance in cellular ions, which results in ion toxicity and osmotic stress, leading to the generation of reactive oxygen species (ROS) which causes damage to DNA, lipids and proteins (Yasar *et al.*, 2006).

Chlorine accumulation in leaf tissues can lead to desiccation. Sodium accumulation can lead to dehydration, reduced turgor, and cell death. Cell membrane integrity can be reduced as sodium displaces calcium, and water and nutrient uptake can subsequently be negatively impacted. Sodium can also reduce protein synthesis and alter hormonal activity. The presence of salt in the water causes plants to exert more energy extracting water from the soil.

2.3.2.1 Na and K Uptake

For plants, sodium ions (Na^+) are harmful, whereas potassium ions (K^+) are essential to reduce the uptake of Na^+ [Kader *et al.*, (2006) and Wu *et al.*, (2009).] The typical mechanism of salinity tolerance in rice is the exclusion or reduction of Na uptake and increased absorption of K to maintain a good Na-K balance in the shoot.

Tolerance of a crop variety is found to be associated with its ability to restrict the uptake of potentially toxic Na^+ ions and with its preferential uptake of balancing ions like K^+ . It is an adaptation for the survival of plants so that the vital metabolic activities are not hampered. There are larger differences in ion (Na^+ and K^+) uptake between the species in comparison to the genotypic differences within a crop species. Although the uptake of Na and K is entirely independent, lower Na/K ratio is considered as desirable trait as it maintains the ion balance. Na is transported to shoot usually through apoplastic pathways (passive transport) while K transport takes place through symplastic pathway, through membrane / plasmalemma (active transport). Younger leaves have relatively lower Na than K as compared to the older leaves, which in turn results in higher Na/K ratio in case of the older leaves. Ray and Islam (2008), observed a low Na/K ratio (1.76) for Pokkali a salt tolerant rice variety and high ratio (2.66) for less tolerant ones like Purbachi and BR29. Thomson *et al.*, (2010) also observed similar results for root Na–K ratio for a set of 39 BC3F5 IR29/Pokkali lines

Morales *et al.*, (2012), determined the growth, yield of photosystem II (PSII), and K^+ and Na^+ concentration in root, stem, old leaves, and young leaves of two Mexican varieties of rice, Tres Ríos and Cotaxtla. In addition, they studied the K^+/Na^+ ratio in stem and root. Plants were grown in a hydroponic solution for 15 days after which some of them were

treated with 100 mM NaCl. They observed that, salt stress caused 20% and 15% reductions in stem and root length, respectively, in the variety Tres Ríos, while in the variety Cotaxtla no significant differences were observed in these variables compared to the control. Dry matter weight decreased by 24% in the variety Tres Ríos. The quantum yield of PSII decreased by 30% at the third day of treatment application, in both varieties. Na⁺ concentration was significantly ($p \leq 0.05$) higher in NaCl-treated plants. In the variety Tres Ríos, the yield of PSII was completely eradicated six days after treatment implementation, while the K⁺ concentration in stem and older leaves also decreased and the lowest K⁺/ Na⁺ ratio in stem was recorded, which could indicate that it is more susceptible to salinity than the variety Cotaxtla. Similarly Asch *et al.*, (2000), subjected Twenty-one rice genotypes to irrigation with moderately saline water (3.5 mS cm⁻¹, electrical conductivity) or irrigation with fresh water. Potassium/sodium ratios of the youngest three leaves (K/Na Leaves) were determined by flame photometry at the late vegetative stage. Grain yield was determined at maturity. The K/Na leaves under salinity was related to grain yield under salinity relative to freshwater controls. There was a highly significant correlation ($p < 0.001$) between K/Na Leaves and salinity-induced grain yield reduction: the most susceptible cultivars had lowest K/Na Leaves and the strongest yield reductions. Although there were major differences in the effects of salinity on crops in both the hot dry season (HDS) and the wet season, the correlation was equally significant across cropping seasons.

Kavitha *et al.*, (2012) in their study, found that the salt-tolerant cultivar Pokkali rice variety maintains a higher K⁺/Na⁺ ratio compared to the salt-sensitive IR20 in the roots as well as in shoots. Using Na⁺ reporter dyes, IR20 root protoplasts showed a much faster

Na⁺ accumulation than Pokkali protoplasts. Membrane potential measurements showed that root cells exposed to Na⁺ in IR20 depolarized considerably further than those of Pokkali. Their results suggest that IR20 has a larger plasma membrane Na⁺ conductance.

A population of recombinant inbred lines (RILs, F_{2:9}) derived from a cross between the salt-tolerant *japonica* rice variety ‘Jiucaiqing’ and the salt-sensitive *indica* variety ‘IR26’, was used by Wang et al. (2012) to determine Na⁺ and K⁺ concentrations in the roots and shoots under three different NaCl stress conditions (0, 100 and 120 mM NaCl). They identified three epistatic QTLs for Na⁺ in roots (RNC), three additive QTLs and two epistatic QTLs identified for Na⁺ in shoots (SNC), four additive QTLs identified for K⁺ in roots (RKC), four additive QTLs and three epistatic QTLs identified for K⁺ in shoots (SKC) and one additive QTL and one epistatic QTL for salt tolerance rating (STR). The identification of salt tolerance in selected RILs showed that a major QTL *qSNC11* played a significant role in rice salt tolerance, and could be used to improve salt tolerance of commercial rice varieties by marker-assisted selection (MAS) approach.

2.3.3 Crop growth and development under saline soil conditions

There are about 380 million hectares of saline soils on the earth’s surface and these are widely distributed in arid and semi-arid as well as seasonally dry coastal areas (Mansuri *et al.*, 2012). Worldwide 20% of all irrigated land is salt-affected (45million out of 230 million ha). Soils in the arid and semiarid regions have excessive concentrations of soluble salts, which adversely affect plant growth (Aref and Rad, 2012). It is known that rice shows variation for salt tolerance (Sabouri *et al.*, 2009, Sabouri and Biabani, 2009, Habib *et al.*, 2013). Rising of salinity level decreases agricultural production by unavailability of fresh

water and soil degradation. Salinity also decreases the terminative energy and germination rate of some plants (Rashid *et al.*, 2004; Ashraf *et al.*, 2002).

The interactions of salts with mineral nutrients may result in considerable nutrient imbalances and deficiencies (McCue and Hanson, 1990). Ionic imbalance occurs in the cells due to excessive accumulation of sodium ion (Na⁺) and Chlorine (Cl⁻) ion and reduces uptake of other mineral nutrients, such as potassium, Calcium, and Manganese (Karimi *et al.*, 2005).

Excess salinity in soil water can decrease plant available water and cause plant stress. Plants regulate water transport under salinity stress because sufficient amount of water is indispensable for the cells to maintain their growth and vital cellular functions such as photosynthesis and other metabolic activities (Horie *et al.*, 2012). Salinity effects varies with different growth stages in rice (Mansuri *et al.*, 2012).

2.3.4 Yield and components of yield under saline soil conditions

Yield is a very complex character which comprises many components. The yield components of rice are related to final grain yield and are also severely affected by soil salinity.

Salinity has been found to negatively impact on a number of yield components of rice including stand establishment; panicles, tillers and spikelets per plant; floret sterility; individual grain size; and even delayed heading (Khatun *et al.*, 1995; Khatun and Flowers, 1995). Maas and Grattan (1999) and Hanson *et al.* (1999) indicated that rice yields decrease by 12 % for every unit (d_{sm}-1) increase in EC (average root-zone EC of saturated soil

extract) above 3.0 dsm-1. Salinity guidelines were first developed by Maas and Hoffman (1977). The major inhibitory effect of salinity on plant growth and yield has been attributed to: i) osmotic effect ii) ion toxicity iii) nutritional imbalance leading to reduction in photosynthetic efficiency and other physiological disorders. Most rice cultivars are severely injured in submerged soil culture on EC of 8-10 dSm-1 at 25 °C. Sensitive genotypes are damaged even at 2 dSm-1 (Mass and Hoffman, 1977).

Clermont-Dauphin *et al.*, (2010) studied the variability of rice yield under water and soil salinity risks in farmers' fields in northeast Thailand. A rice plot was monitored in 24, 16 and 11 farmers' fields during the rice seasons 2005, 2006 and 2007, respectively. The results emphasized that few plots were continuously submerged during the 2005 season, when rainfall was low. Drought significantly affected the rice yield, yield components and the internal efficiency (IE) of the absorbed nutrients, while slight soil salinity had only significant effect of increasing the IE of potassium (IEK). In the heavy rainy 2006 and 2007 seasons, most fields were continuously submerged, and in contrast to 2005, the slight soil salinity that was recorded had significant effects not only on IEK, but also on rice yield, spikelet sterility and 1000-grain weight. The yield decrease due to drought was about 87% and that due to salinity was 20%. When neither salinity nor water was limiting, the soil nutrient supply was high enough to achieve about 80% of the maximum yield reported in the literature for that rice cultivar in the area.

Knowledge of salinity effects on rice seedling growth and yield components would improve management practices in fields and increase our understanding of salt tolerance mechanisms in rice. Hassan *et al.*, (2012) applied four levels of saline irrigation water (2,

4, 6 and 8 dS/m, respectively) to rice varieties at four growth stages (tillering, panicle initiation, panicle emergence and ripening), with the aim of determining the effect of salinity levels on some agronomic characters of rice. The study showed that increase in salinity levels of irrigation water significantly decreased length of filled panicle, number of filled grains per filled panicle, number of spikelets per filled panicle and total number of spikelets per panicle but effect of different salinity levels on percentage or ratio of filled panicle number to tiller number and percentage or ratio of yield to straw weight was not significant. The least of these yield components were observed at the highest salinity level (8 dS/m). At different growth stages of rice, all yield components were different. Final growth stages, panicle emergence and ripening showed less sensitivity to salinity but primary stages, tillering and panicle initiation were more sensitive to salinity.

Salinity sensitivity of rice was studied to determine salinity effects on seedlings and yield components. Plants of rice cultivar M-202 were grown in a greenhouse in sand and irrigated with nutrient solutions of control and treatments amended with NaCl and CaCl₂ and 11.5 dS/m electrical conductivity. Seedling growth was significantly reduced by salinity at the saline treatment, 1.9 dS/m. At 1.9 and 3.4 dS/m, significant reduction of seedling growth occurred at longer cumulative thermal time than at higher salt levels. Seedling survival was significantly reduced when salinity was 3.40 dS/m and higher. Highly significant, linear responses of grain weight per plant, grain weight per panicle, spikelet number per panicle, and tiller number per plant to salinity were observed. There was a common lowest salt level for fertility and pollen germination beyond which they were significantly reduced by salinity. (Zeng and Shannon. 2000)

2.4 Methods of overcoming salinity as a constraint in rice production

The strategy for overcoming salt related problems is based on three approaches

The first is an agronomic approach which involves changing the growing environment to make it normal and suitable for the normal growth of plants. This approach involves major engineering and soil amelioration processes which need a lot of resources often out of the reach of small and marginal farmers.

The second biological approach is to select or change genetic architecture of the plant so that it could be grown in saline areas that is breeding crop varieties with in-built salt tolerance. This is less resource consuming, economical and socially acceptable approach. Hence the ability of the plant to tolerate salt stress is of paramount importance to manage the resources optimally. This is the reason to develop tailored crops with higher salt tolerance suited to salt stress environments.

A third approach termed a hybrid approach is a combination of both environment modification and biological approach. It is highly productive, less resource consuming and economically viable approach. Currently major soil reclamation programs involve both biological and hybrid approaches to combat the salt problem.

(www.knowledgebank.irri.org)

2.4.1 Agronomic methods

This approach is normally by taking morphological data of several parameters among varieties, this helps to identify which varieties performs better in the saline condition. Though there is no single definite morphological marker available for salt tolerance or

sensitivity in any crop, a combination of criteria give a good indication for the salt response of crop plants. Therefore, several parameters are used jointly for effective and reproducible screening for salt tolerance. Parameters like germination studies, survival of the plant, injury score, phenotypic expression, grain yield and mean tolerance index (MTI) give an indication of the ability of the species to tolerate salinity stress. Tolerant plants can then be selected. (www.knowledgebank.irri.org).

Another way to alleviate salinity hazards in crop production is to remove the salts from the root zone by leaching. Salt leaching requires adequate irrigation management, which is based on adding sufficient amounts of water beyond the water requirement for meeting evapotranspiration demands, in order to leach the excess salt from the root zone (Russo *et al.*, 2009). Evaluation of genotypes on farmer's field is however not reliable due to heterogeneous conditions.

2.4.2 Genetic methods

Conventional methods of plant selection for salt tolerance are difficult because of the large effects of the environment and low narrow sense heritability of salt tolerance (Gregorio, 1997). Molecular markers can provide useful information to select divergent parents for developing both breeding and mapping populations. A molecular marker is a genetic tag that identifies a particular location within a plant's DNA sequences. Markers can be based on either DNA or proteins. DNA markers are naturally occurring tags attached to specific segments of a chromosome, which in turn are associated with specific phenotypes. A marker can either be located within the gene of interest or be linked to a gene determining a trait of interest.

DNA markers that differentiate genotypes are more reliable and convenient than physiological or morphological characters in the identification and characterization of genetic variation. They differ in a variety of ways – such as their technical requirements; the amount of time, money and labor needed; the number of genetic markers that can be detected throughout the genome; and the amount of genetic variation found at each marker in a given population.

2.4.2.1 SSR Markers

SSR or microsatellite markers are stretches of 1 to 6 nucleotide units repeated in tandem and randomly spread in eukaryotic genomes. SSR are very polymorphic due to the high mutation rate affecting the number of repeat units. Their polymorphisms can be easily detected on high resolution gels by running PCR amplified fragments obtained using a unique pair of primers flanking the repeat (Weber and May 1989).

SSRs have several advantages over other molecular markers, (i) They allow the identification of many alleles at a single locus, (ii) they are evenly distributed all over the genome, (iii) they are co-dominant, (iv) little DNA is required and (v) their analysis can be semi-automated and performed without the need of radioactivity. SSRs presented a higher level of polymorphism and greater information content, as assessed by the expected heterogosity, than AFLPs and RAPDs. (Belaj *et al.*, 2003).

The usefulness of SSR markers for rice germplasm characterization has been described in several studies on landraces, cultivars and different species of *Oryza*. Junjian Ni *et al.*, (2002) suggested that a relatively small number of microsatellite markers could be used for

the estimation of genetic diversity and the identification of rice cultivars. Garris *et al.*, (2004) used SSRs to sort *Oryza sativa* into five groups.

The multivariate nature of SSR markers has the unambiguous advantage of discriminating genotypes more precisely (Prabakaran *et al.*, 2010). The number of alleles detected by a single SSR locus varied from 1 to 31 depending upon the fingerprinting techniques and materials used in the studies (Jayamani *et al.*, 2007).

Microsatellites markers that are efficient and cost-effective to use. Compared with other markers, they are abundant, co-dominant, highly reproducible and interspersed throughout the genome. Microsatellite markers based on SSRs have been developed in many crop species, including rice, tomato, soybean and others. These markers detect simple sequence length polymorphism (SSLP) and are rapidly replacing RFLPs and others for genetic studies, due to their technical simplicity, the small amount of starting DNA required, the relatively low cost to the user, and the level of allelic diversity as well as its high power of resolution. Additionally, it is a PCR based assay making it easy to detect in gel electrophoresis. Microsatellite marker analysis is promising for identification of major gene loci for salt tolerance that can be helpful to plant breeders for developing new cultivars (Jonah *et al.*, 2011). In the last ten years, a rapid progress has been made towards the development of molecular marker technologies and their application in linkage mapping, molecular dissection of the complex agronomical traits and marker-assisted breeding. Kumar *et al.*, (2011).

Claudio *et al.*, (2006) characterized the allelic diversity of 192 traditional varieties of Brazilian rice using 12 simple sequence repeat (SSR or microsatellite) markers. The

germplasm was divided into 39 groups by common name similarity. A total of 176 alleles were detected, 30 of which (from 23 accessions) were exclusive. The number of alleles per marker ranged from 6 to 22, with an average of 14.6 alleles per locus. They identified 16 accessions as a mixture of pure lines or heterozygous plants. A subset of 24 landraces, representatives of the 13 similarity groups plus the 11 accessions not grouped, was the most variable set of genotypes analyzed.

A set of 36 microsatellite markers distributed over 12 chromosomes of rice were used to assess genetic diversity in 33 medicinal rice genotypes. Behera *et al.*, (2012) reported a total of 166 polymorphic alleles, of the total 169 amplified. All the rice genotypes showed the presence of multiple alleles. Genetic similarities among genotypes varied from 0.239 to 0.827 with an average of 0.5. All the genotypes included in the study could be uniquely distinguished from each other.

Emon *et al.*, (2015) used a total of 160 SSR markers to evaluate 5 rice genotypes, they revealed 209 alleles among them. 4 SSR markers RM105, RM125, RM178 and RM549 with highest PIC (0.67) and high level of genetic diversity value (0.72) were found and recommended to be used to pyramid major and minor salt stress related genes via marker assisted selection in rice.

2.5 Identification of salinity-resistant genotypes

2.5.1 Molecular methods- SSR

Microsatellite markers have been ideal for identification and purity checks of rice varieties (Nandakumar *et al.*, 2004). They have proved to be conducive and effective for making

genetic maps [Islam, 2004; Niones, 2004)], for assisting selection and studying genetic diversity in germplasms. Progress in rice breeding for salt tolerance requires the identification of the major loci conferring salt tolerance at different growth stages. With recent developments in the field of molecular marker analysis, it is now feasible to analyze both the simply inherited traits and the quantitative traits and to identify the individual genes controlling salinity tolerance which could facilitate selection in rice for this trait with low heritability. SSR markers are playing important role in identifying genes for salt tolerance that can be helpful for plant breeders to develop new cultivars.

SSR markers revealed varying degrees of genetic similarity among the accessions of cultivated and wild species of rice (Jayamani *et al.*, 2007). Mehede *et al.*, (2014) used three SSR markers in their evaluation of 27 rice genotypes to determine salinity tolerance in the genotypes. The markers identified seven genotypes as tolerant but ten of them were rated susceptible by all three markers compared to two checks. Six genotypes were tolerant in both phenotypic and SSR screening. Kabir *et al.*, (2008) used twelve SSR markers for parental survey, three polymorphic SSR markers OSR34, RM443 and RM169 were selected to evaluate 26 F₃ rice lines for salt tolerance. Marker OSR34 identified 15 lines as salt tolerant, 9 lines as susceptible and 2 lines as heterozygous. Marker RM443 identified 3 tolerant, 14 susceptible and 9 heterozygous rice lines. Marker RM169 identified eight tolerant, 11 susceptible and 7 heterozygous lines. Thus the tested markers could be efficiently used for tagging salt tolerant genes in marker-assisted breeding program.

Salinity screening was done at the seedling stage using hydroponic system for 22 selected F₃ lines of a cross, Jangliboro × BRRI Dhan40 (Ahmed *et al.*, 2010). They were then

genotyped with SSR markers. Four lines, 07, 17, 26 and 40 were found to be salt tolerant, five were moderately tolerant, ten were susceptible and rest of the lines were highly susceptible. Parental polymorphism survey was done with seven SSR primers and out of them three primers, RM18, RM127 and RM169 were selected to characterize the F₃ lines for salt tolerance. With respect to RM18, eleven lines were found to be salt tolerant and eleven lines were susceptible. Primer RM127 revealed 8 tolerant, 7 susceptible and 7 heterozygous lines while primer RM169 identified 6 tolerant, 11 susceptible and 5 heterozygous lines. Line 17 was tolerant and lines 08 and 12 were susceptible in comparison with salt tolerant parent BRRI Dhan40 and salt susceptible parent Jangliboro based on the three selected markers used. Under salt stress at seedling stage, line 17 was tolerant and line 8 was susceptible, similar to marker analysis, but line 12 showed different result. It was moderately tolerant at seedling stage but susceptible according to the marker survey.

A study designed to characterize the genetic diversity within a subset of rice germplasm with different adaptations to saline soils using microsatellite markers was carried out by Zeng *et al.*, (2004). Salt tolerance was then analyzed among molecularly characterized genotypes. Plants of 33 genotypes were grown in sand tanks under greenhouse condition and irrigated with nutrient solution. Two salt treatments were imposed with electrical conductivities of 0.9 dSm⁻¹ (control) and 6.5 dSm⁻¹ (6:1 molar ratio of NaCl and CaCl₂). A total of 123 alleles were generated among the 33 genotypes at 25 microsatellite loci. Genotypes of japonica rice grouped into three clusters and those of indica rice grouped into two clusters based on microsatellite markers. Thirty percent of the alleles detected in 20

breeding lines were not identified in the cultivars analyzed. These alleles may provide favorable allelic combinations if the breeding lines are used for intercrosses. Physiological and morphological characters under salt stress were significantly different among microsatellite clusters. These results indicate that the adaptation of rice to saline soils is different among genotypes with diverse genetic backgrounds.

2.5.2 Agromorphological methods

Mehede *et al.*, (2014) screened 27 rice genotypes for salinity at the seedling and reproductive stages respectively, in the hydroponic system and in sustained water bath. They phenotyped the germplasm at EC 12dS/m and 6dS/m, at seedling and reproductive stages, respectively. They found eight genotypes to be salt tolerant, four were moderately tolerant and the rest 15 were susceptible. At the reproductive stage, six genotypes were tolerant to EC 6dS/m whereas eleven of them were susceptible. Salil *et al.*, (2009) evaluated 11 rice genotypes for salt tolerance at the seedling stage and in hydroponic system using salinized (EC 12 dS/m) nutrient solution. Large variation in salinity tolerance among the rice germplasms were detected. They also observed that plant height and total dry matter of tolerant lines were reduced by 19.0% and 40.6%, respectively under salt stress (EC 12 dS/m), whereas, those of susceptible lines were reduced by 46.0% and 73.5%, respectively

Shereen *et al.*, (2005) evaluated the effect of different levels of salinity (0, 50, 75 mM NaCl) on the growth, yield and yield components of different inbred rice lines. Studies at seedling stage under salinity showed significant growth reduction in terms of leaf mortality

and shoot fresh weight in all tested lines. However, the rate of reduction varied among different lines. Variable responses to salinity were observed at vegetative and reproductive stages. There was significant reduction in characters like fertility, tiller numbers, panicle number and panicle length under salinity. Sterility was found to be the major cause of yield losses under saline conditions.

Emon *et al.*, (2015) also studied 5 rice genotypes under two salt stress treatments at seedling, vegetative and at reproductive stages. Na^+/K^+ uptake ratio were surveyed to evaluate salt stress effects. At vegetative stage, all the genotypes survived. However, at reproductive stage all the genotypes were affected by salt stress except Binadhan-10 that survived the whole life cycle.

2.6 Utilization of resistant genotypes

Markers can be used in transferring a single gene into a new cultivar or in testing plants for the inheritance for many genes at once.

Marker-assisted selection can be defined as selection for a trait based on genotype using associated markers rather than the phenotype of the trait (Foolad and Sharma, 2005).

Marker-assisted or molecular-assisted breeding is the use of molecular markers to track the genetic makeup of plants during the variety development process. It improves the efficiency with which breeders can select plants with desirable combination of genes.

Molecular markers could be used to tag QTL (tag quantitative trait loci) and evaluate their contributions to the phenotype by selecting for favorable allele at those loci in marker assisted selection (MAS) scheme aimed at accelerating genetic advancement in rice which is faster, more efficient and cost-efficient than conventional screening under saline field

conditions [Gregorio and Senadhira (1993), Aliyu *et al.*, 2011]. Advanced backcross QTL analysis can be used to evaluate mapped donor introgression in the genetic background of an elite recurrent parent (Tanksley and Nelson 1996).

QTLs for salt tolerance in rice via RFLP markers and microsatellites reported by Lang *et al.*, (2000) and Tuan *et al.*, (2000) were based on recombinant inbred population of Tesanai 2/CB, and backcross inbred lines (BC1F8) of Nipponbare/Kasalath, respectively.

In rice, important traits such as salt tolerance are controlled by polygenes with additive and dominant effects that are described by quantitative trait loci (QTLs) (Gregorio and Senadhira 1993).

Saltol QTL is considered as a main QTL for salt tolerance and was previously identified and detected on the chromosome of the salt tolerant Pokkali rice cultivar. This QTL confers salinity tolerance at the vegetative stage and accounts for 64% to 80% of the phenotypic variance (Bonilla *et al.*, 2002). Ren *et al.*, 2005), Takehisa *et al.*, 2004) and Islam *et al.*, (2013) also detected the same QTL in some other rice cultivars.

Le Hung *et al.*, (2012), used marker-assisted backcrossing (MABC) to improve rice salt tolerance in BT7 cultivar. FL478 was used as a donor parent to introgress the *Saltol* QTL conferring salt tolerance into BT7. Three backcrosses were conducted to successfully transfer positive alleles of *Saltol* from FL478 into BT7. The selected lines that carried the *Saltol* alleles were screened in the field for their agronomic traits. All improved lines had *Saltol* allele similar to the donor parent FL478, whereas their agronomic performances

were the same as the original BT7. In the study, MABC accelerated the development of superior qualities in the genetic background of BT7.

Kabir *et al.*, (2008) made crosses between high yielding salt susceptible BINA varieties (Binadhan-5) with salt tolerant rice landrace (Harkuch) to identify salt tolerant rice lines. Thirty six F₃ rice lines of Binadhan-5 x Harkuch were tested for salinity tolerance at the seedling stage in hydroponic system using nutrient solution. In F₃ population, six lines were found to be salt tolerant and 10 lines were moderately tolerant based on phenotypic screening at the seedling stage.

In a genetic study of salinity tolerance in F₁, F₂ parents and backcross generations of six crosses, segregation analysis indicated partial dominance for salinity tolerance. Estimation of genetic parameters indicated the importance of additive effects in the inheritance of salinity tolerance. Highly significant additive gene action in Pokkali/BR29 and both additive and dominance gene action in Nonabokra/BR29 without interaction were observed. The non-additive interactions with duplicate type of epistasis were observed in the crosses with moderately tolerant and susceptible parents. (Ray and Islam, 2008).

Salil *et al.*, (2009) used three SSR markers viz., RM7075, RM336 and RM253 to evaluate 11 rice genotypes for salt tolerance. The SSR markers showed polymorphism and were able to discriminate salt tolerant genotypes from susceptible. The SSR markers (RM7075, RM336 and RM253) identified 8, 9 and 7 salt tolerant genotypes, respectively. Through phenotypic and genotypic study, three genotypes viz., Pokkali, TNDB-100 and THDB were identified as salt tolerant rice cultivars. These SSR markers might have sequence

homology with salt tolerant rice genotypes and consequently the markers were able to identify salt tolerant rice genotypes from susceptible

Lui *et al.*, (2013) in developing new rice lines with salinity tolerance and high yield, applied markers assisted selection (MAS). They used 21 SSR primers in the *Saltol* QTL region to screen the two parent varieties to identify polymorphic primers for screening the *Saltol* QTL region of the breeding population. The individual plants in BC1, BC2, and BC3 generations of the Q5DB/FL478 were analyzed to evaluate the introgression of *Saltol* fragment into Q5DB cultivar. After screening of 3 BC generations, the best individual plants of BC3F1 of the plant numbers QF-3-1, QF-3-2, QF4-3-3, QF6-3 with almost all the recipient alleles were selected.

2.7 Role of core collection of rice

A core collection is a representative set of accessions covering maximum diversity with minimum repetition and consisting of ecologically and genetically distinct accessions according to Frankel (1984). Besides reducing conservation costs and increasing management efficiency, it promotes effective and sustainable use of genetic diversity by facilitating rapid and precise identification of germplasm sources for improvement of desired traits. For description of a core collection, the extent of diversity to be covered and number of accessions to be included in the set must be established. A core collection can be made more dynamic with the integration of molecular techniques to increase the number of distinct alleles, remove duplicates and create scope for the addition of new accessions whenever identified as different from the present ones.

For a crop like rice with a large gene pool, the development of core collections will facilitate easy accessibility to and effective use of the genetic diversity preserved. Great

effort is needed to evaluate and optimize genetic resources in national, regional and global rice improvement programs (FAO, 2002). Genetic enhancement of significant economic traits needs particular attention. It is essential to re-orient future breeding research to improve and sustain genetic diversity, broadening the genetic base for important economic traits.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Site

Screening of the genetic resources for salinity tolerance was carried out in Styrofoam floats in a greenhouse at the Sinna garden, Crop Science Department of the School of Agriculture, University of Ghana, Legon. Molecular screening for salt tolerance markers and diversity in the germplasm was carried out at the laboratory of the Biotechnology Center of the School of Agriculture between January and June 2014

3.2 Experimental materials

Plant materials were obtained from the Plant Genetic Resource Institute and Savanna Agricultural Research Institute (SARI), both of the Council for Scientific and Industrial Research (CSIR), Ghana. NERICA rice genotypes were also obtained from Africa Rice Center's, Sahel Station- Senegal, and from farmers' fields in Ghana. Table 1 shows the list of accessions and their sources.

FL478 an International salt-tolerant accession and IR29, an international sensitive genotype obtained from Africa Rice Center were used as checks, Accession 'CG14' from Africa Rice Center was included as check for *O. glaberrima*, in the accessions from African countries. A total of 36 germplasm were investigated. (Table 1).

Table 1: The germplasm studied and their sources

Accession Number	Name	Source
1	GH 1593	PGRI - CSIR, Ghana
2	GH 1575	PGRI - CSIR, Ghana
3	GH1585	PGRI - CSIR, Ghana
4	GH1598	PGRI - CSIR, Ghana
5	GH1571	PGRI - CSIR, Ghana
6	GH 1533	PGRI - CSIR, Ghana
7	GH 1528	PGRI - CSIR, Ghana
8	GH1545	PGRI - CSIR, Ghana
9	GH 1580	PGRI - CSIR, Ghana
10	GH 1599	PGRI - CSIR, Ghana
11	GH 1837	PGRI - CSIR, Ghana
12	GR 18 Red	SARI -CSIR, Ghana
13	Anyofula	PGRI - CSIR, Ghana
14	Matigey	PGRI - CSIR, Ghana
15	Viwornor	PGRI - CSIR, Ghana
16	Abidjan	Local farmer, Ghana
17	Local Red	Farmer collection, E/R
18	SR-1	ARC- Senegal
19	IR-29 (Susceptible check)	ARC- Senegal
20	CG14	ARC- Senegal
21	FL478(Resistant check)	ARC- Senegal
22	Nerica L23	ARC- Senegal
23	Nerica L9	ARC- Senegal
24	Nerica L24	ARC- Senegal
25	Nerica L27	ARC- Senegal
26	Sebota 33	Cameroun
27	Sebota 337-1	Cameroun
28	Sebota 41	Cameroun
29	Sebota 281-2	Cameroun
30	Sebota 68	Cameroun
31	IR 72 (Ph)	IRRI, Philippines
32	Basmati 122	IRRI, Philippines
33	Local Basmati-2	IRRI, Philippines
34	Good and New (JP)	Japan
35	Koshihikari	Japan
36	Perfume (Short type)	Thailand

3.3 Evaluation of rice genotypes at the seedling stage

Floats were constructed by boring 3 cm diameter holes in Styrofoam containers, the under part which comes into contact with the nutrient solution was covered with a fine nylon net, this also served as an anchor for the suspended roots of the seedlings. Plate.1 shows the styrofoam float setup for salinity screening.

The salinity screening was done in a hydroponic setup using IRRI standard protocol (Gregorio, 1997) at seedling stage. Two treatments were given, salinized and non-salinized nutrient solutions with three replications using Randomized Complete Block Design. Seeds were nursed in petri dishes lined with moist filter paper. Pre-germinated seeds were sown in the hydroponic system in distilled water. After three days, the distilled water was replaced with nutrient solution (Yoshida *et al.*, 1976). There were eight record plants per accession. Each Styrofoam tray included the tolerant and sensitive checks.

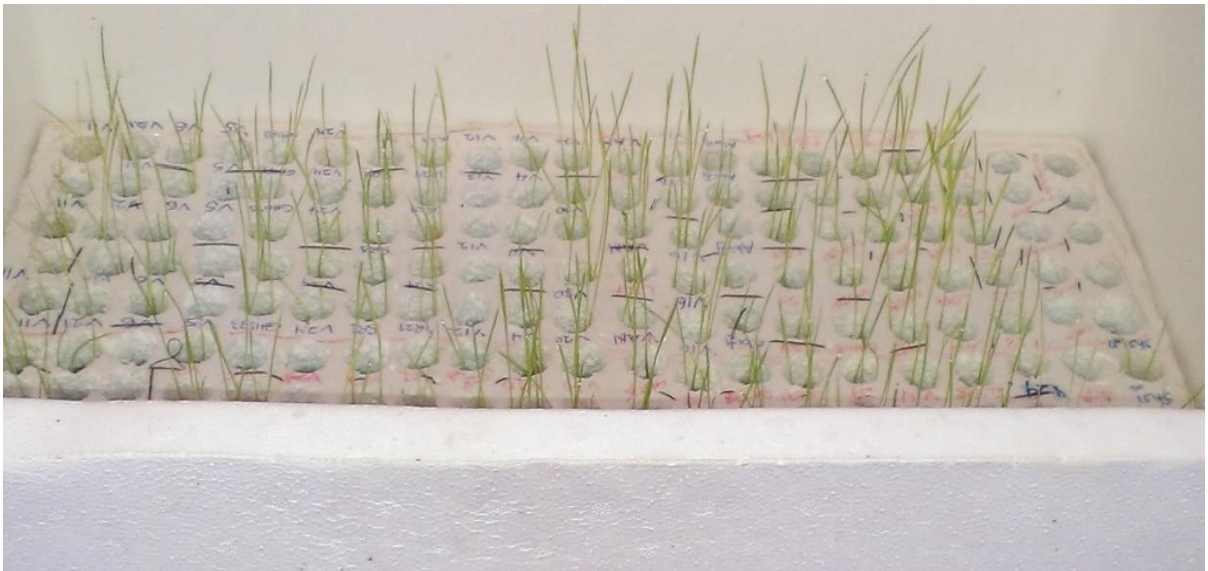


Plate 1. Styrofoam float with germinated rice seedlings

3.3.1 Stock Solutions

The stock solutions consisted of macro and micro nutrients prepared separately to be used in composing the working solutions for the growth of the rice seedlings. For the macronutrient stock solutions, the required amount of reagent was weighed and transferred into a 1000-mL beaker, the initial mixing was done with about 750 mL distilled water. The mixture was then poured into the 2-L volumetric flask, and distilled water added to make up a volume of 2 L. The mixture was stirred for 15 min using a magnetic stirrer bar then transferred to a stock solution bottle. Table 2 shows the components and concentrations of chemicals used in preparing the stock solutions.

Each reagent of the micronutrient solution listed was dissolved separately. 50 mL distilled water was used to dissolve each reagent except for ferric chloride, which was dissolved in 100 mL distilled water. All solutions were mixed together in 1 L distilled water using 2.0-L capacity volumetric flask. The ferric chloride solution was added to the mixture just before addition of the citric acid and stirred for 15 min using a magnetic stirrer. Finally 100 mL sulfuric acid was added to the mixture and made up to volume of 2.0 L. It was stirred for another 10 min and stored in a dark glass bottle. All stock solutions were properly labeled and kept in separate bottles.

The working nutrient solution was made by adding distilled water to measured stock solution, the pH was adjusted to 4.5. The micro nutrient stock was then added and the final pH adjusted to 5.0.

Initial salinity was at EC of 6 dS/ m. This was achieved by adding 3g/L NaCl to the solution. Three days later, salinity was increased to EC of 12 dS/ m by adding more NaCl

to the nutrient solution. The solution was renewed every eight days and pH was maintained at 5.0 daily.

Table 2: Constitution of stock solution

Stock No.	Chemical	Amounts (g or ml)/ 4 litres
1	NH ₄ NO ₃	365.6
2	NaH ₂ PO ₄ H ₂ O	142.4
3	K ₂ SO ₄	285.6
4	CaCl ₂	469.4
5	MgSO ₄ 7H ₂ O	1,296.0
6	MnCl ₂ 4H ₂ O *	6.0
	(NH ₄) ₆ Mo ₇ O ₂₄ 4H ₂ O *	0.296
	H ₃ BO ₃ *	3.736
	ZnSO ₄ 7H ₂ O *	0.140
	CuSO ₄ 5H ₂ O *	0.124
	FeCl ₃ 6H ₂ O *	30.800
	C ₆ H ₈ O ₇ H ₂ O *	47.600
	1M H ₂ SO ₄ *	250ml

* Micronutrients

Test entries were rated at 12 and 16 days after transfer into the salinized solution. Air temperature ranged from 23°C to 33 °C during the day and 17°C to 23 °C during the night. Relative Humidity ranged from 40 to 70% in the green house.

The modified standard evaluation score (SES) of International Rice Research Institute, IRRI. (Gregorio *et al.*, 1997) was used to assess the visual symptoms of salt toxicity, the assessment criteria used is presented in Table 3.

Table 3: Modified standard evaluation score (SES) for visual salt injury at seedling stage

Score	Observation	Response category
1	Normal growth with no leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant
5	Growth severely retarded; most leaves rolled; only a few are elongating	Moderately tolerant
7	Complete cessation of growth; most leaves dry; some plants dying	Susceptible
9	Almost all plants dead or dying	Highly susceptible

Source: Gregorio *et al.*, 1997

For phenotypic observation, seedling height and total dry matter were recorded for salinized and non-salinized conditions. Plants response to salinity via phenotypic responses to stress was used to aid scoring. Eight plants per accession were scored.

For tissue analysis, shoot samples from each entry were collected and washed carefully with distilled water especially the lower part where it made contact with the salinized culture solution. They were subsequently oven-dried for 3 days at 70 °C. The dried samples were ground to obtain about 1 g (40 mesh) powdered form from each sample. The Na and K concentrations were analyzed using a flame photometer.

0.1g of the paste was weighed into 100ml conical flask, 5ml of concentrated sulphuric acid (98%) was added and allowed to stay overnight.

The solution was then digested with 1ml of 30% hydrogen peroxide on a heating mantle. It was allowed to cool and 20ml of distilled water were then added and filtered into a 100ml volumetric flask and made up to the mark with distilled water. The final solution was shaken to ensure homogeneity. Measured volume of the stock solution was added to distilled water to make up the working solution

3.4 DNA Extraction for Molecular Studies

DNA was extracted using E.Z.N.A.™ SP Plant DNA Mini Kit.

Approximately 0.03g of leaf samples were frozen in liquid nitrogen and ground in a microfuge tube. 400µl of buffer SP1 was immediately added followed by 5µl of RNase A solution after which the samples were incubated at 65°C for 10 minutes. 140 µl of buffer SP2 was added to each sample and mixed vigorously by vortexing followed by incubation on ice for 5 minutes and centrifugation at 14000 rpm for 10 minutes. The supernatant that resulted was carefully aspirated into an Omega® Homogenizer Column placed in 2 ml collection tube and centrifuged at 14000 rpm for 2 minutes. 500 µl of the clear lysate that resulted were transferred into a 1.5 ml tube. Binding conditions of the sample were then adjusted by pipetting 750 µl of buffer sp3/ ethanol mixture directly onto the clear lysate.

650µl of the resulting mixture were transferred into a Hiband® DNA Mini Column placed in a 2mL collection tube and centrifuged for 1 minute at 14000 rpm after which the flow through was discarded. This was repeated for the remaining mixture.

The columns were then placed into a new 2ml collection tube and 650µl of SPW Wash Buffer diluted with ethanol was added. This was centrifuged at 14000 rpm for 1 minute

and the flow through discarded. This step was repeated with the sample volume of SPW wash buffer. The empty column was centrifuged at 14000 rpm for 2 minutes.

The Hiband® Mini column was then transferred into a sterile 1.5ml tube and 100µl of pre-warmed (65°C) elution buffer was added. This was then centrifuged at 14000 rpm for 1 minute to elute the DNA.

3.5 Polymerase chain reaction (PCR) amplification

Thirty SSR primers (Table.4), 15 of which were markers for salt tolerance were selected for the PCR.

15 µl PCR reaction was composed of 1X Taq buffer, 2mM MgCl₂, 1U Taq DNA polymerase, 0.2 mM of each dNTP's and 0.4 µM SSR primer pair. Deionized water was used to make up the volume to the final PCR reaction volume.

The thermal cycling conditions were as follows; 94°C for 5 mins followed by 35 cycles of 94°C for 1 min, 1min for annealing temperature (55°C-67°C) depending on the primer used and 72°C for 2 min, and a final extension at 72°C for 5 min.

Table 4: SSR primers used in PCR, their annealing temperatures and flanking sequences

PRIMER	ANN. TEMP	FORWARD SEQUENCE (5'-3')	REVERSE SEQ. (5'-3')
RM20	55	ATCTTGTCCTGCAGGTCAT	GAAACAGAGGCACATTTTCATTG
RM307	55	GTACTACCGACCTACCGTTCAC	CTGCTATGCATGAACTGCTC
RM5	55	TGCAACTTCTAGCTGCTCGA	GCATCCGATCTTGATGGG
RM552	55	CGCAGTTGTGGATTTTCAGTG	TGCTCAACGTTTGACTGTCC
RM19	55	CAAAAACAGAGCAGATGAC	CTCAAGATGGACGCCAAGA
RM454	55	CTCAAGCTTAGCTGCTGCTG	GTGATCAGTGCACCATAGCG
RM11	55	TCTCCTCTTCCCCGATC	ATAGCGGGCGAGGCTTAG
RM518	55	CTCTTCACTCACTCACCATGG	ATCCATCTGGAGCAAGCAAC
RM334	55	GTTCAGTGTTTCAGTGCCACC	GACTTTGATCTTTGGTGGACG
RM237	55	CAAATCCCGACTGCTGTCC	TGGGAAGAGAGCACTACAGC
RM259	55	TGGAGTTTGAGAGGAGGG	CTTGTTGCATGGTGCCATGT
RM474	55	AAGATGTACGGGTGGCATTG	TATGAGCTGGTGAGCAATGG
RM178	67	TCGCGTGAAAGATAAGCGGCGC	GATCACCGTCCCTCCGCTGCTG
RM489	55	ACTTGAGACGATCGGACACC	TCACCCATGGATGTTGTCAG
RM312	55	GTATGCATATTTGATAAGAG	AAGTCACCGAGTTTACCTTC
RM253	55	TCCTTCAAGAGTGCAAAACC	GCATTGTCATGTCTGAAGCC
RM336	55	CTTACAGAGAAACGGCATCG	GCTGGTTTGTTCAGGTTCCG
RM10655	55	AGTACCGTTGAATCCGATATGC	TGGTTGAGGTGCTGAATTGG
RM10696	60	CCTTCGACTCCATGAAACAAACG	TCTCTTTGCCCTAACCCATATGTC
RM10711	55	GCTTCGATCGATGAGAAAGTAGAGG	GAATCTCCCATCCTTCCCTTCC
RM10713	60	ATGAACCCGGCGAACTGAAAGG	CTGGCTCCCTCAAGGTGATTGC
RM10748	60	CATCGGTGACCACCTTCTCC	CCTGTCATCTATCTCCCTCAAGC
RM10722	60	GCACACCATGCAAATCAATGC	CAGAAACCTCATCTCCACCTTCC
RM10793	60	GACTTGCCAACTCCTTCAATTCG	TCGTGAGTAGCTTCCCTCTTACC
RM10800	60	CGTACGCCCTCACATCACCTTCC	CTCTCCGGGAGCTCACTTGTCG
RM10825	60	GGACACAAGTCCATGATCCTATCC	GTTTCCTTTCATCCTTGTTGC
RM10843	60	CACCTCTTCTGCCTCCTATCATGC	GTTTCTTCGCGAAATCGTGTGG
RM10852	60	GAATTTCTAGGCCATGAGAGC	AACGGAGGGAGTATATGTTAGCC
RM10864	60	GAGGTGAGTGAGACTTGACAGTGC	GCTCATCATCCAACCACAGTCC
RM10890	60	GCTTCGGCTCTTCATTCAGTGG	GCGATTATAGGAGCGCTATGTGG
RM10927	60	TGGATCCCACTAATCCAAATGC	GAAAGACTCCTTCCAATGTTAGGC

3.6 Gel electrophoresis of amplified products

For a 120 ml electrophoresis casting tray, 3.6g of agarose was weighed into 120 ml of TAE buffer. The initial weight was noted. This was then melted on a hot plate after which distilled water was used to make up the weight difference. The melted gel was then cooled under running water after which 8 μ l of ethidium bromide was added and swirled gently to mix. The agarose gel was then poured into the casting tray and combs set in place. This was allowed to solidify for 40 minutes. The casting tray together with the solidified agarose was then transferred into the electrophoretic tank and submerged with TAE buffer and the combs gently removed.

10 μ l of PCR amplicons was mixed with 3 μ l of 6X loading dye and carefully loaded into wells created by removing the combs. The leads of the electrophoretic tank were connected to electrophorese amplicons at 100V for 1 hour after which the gel was viewed with the aid of a UV transilluminator.

3.7 Data Collection and Analyses

3.7.1 Phenotypic screening for Salt tolerance

The modified standard evaluation score (SES) of IRRI (Table 3) was used to assess the visual symptoms of salt toxicity. Scoring started on the twelfth day after salinization treatment till the final day (when the susceptible variety IR29 scored 9). The scores from the three replications were averaged to get the mean score for the accession.

Plant height was measured at first day of scoring that is the twelfth day after salinization. It was measured from the point of entry of the stem into the nylon net on the Styrofoam to the tip of the longest leaf.

Dry weight was measured by oven drying the leaves and the roots of the samples at 70°C for 3 days and weighing. The average of 8 record plants was found and their percentage reduction from the controls calculated

$$\% \text{ Reduction} = \frac{\text{Mean of non-salinized} - \text{mean of salinized}}{\text{Mean of non-salinized}} \times 100$$

3.7.2 Determination of Na and K

Determination was done using the flame photometer

Calculation

$$\text{K (ppm)} = \frac{\text{R} \times \text{Ext Vol} \times \text{dilution factor}}{\text{Wt}}$$

$$\text{K (\%)} = \frac{\text{R} \times \text{Ext Vol} \times 100 \times \text{dilution factor}}{\text{Wt} \times 10^6}$$

Where R is spectrophotometer reading

Ext Vol is extraction volume (100ml)

Wt is the sample weight

The same calculation was used for both sodium and potassium ion concentration

3.7.3 Allele scoring and data analysis

The polymorphic bands were scored for each of the microsatellite primer pairs in each genotype based on presence 1 or absence 0 for bands to generate a matrix of 1 and 0. The

size (in nucleotide base pair) of the amplified band for each SSR marker was determined based on its migration with comparison to a known molecular weight marker (1Kb DNA Ladder). Allele numbers, gene diversity, heterozygosity and polymorphic information content (PIC) were calculated with PowerMarker v3.25 software (Liu and Muse 2005). Between samples, genetic distances were assessed through simple matching index as implemented in DARwin v5 software (Perrier *et al.*, 2003, Perrier *et al.*, 2006). A dendrogram was constructed based on the unweighted pair-group method with arithmetic averages (UPGMA) using the neighbour-joining (NJ) method as implemented in the same software. Polymorphic information content (PIC) values were calculated with the following formula (Anderson *et al.*, 1993):

$$n \text{ PIC}_i = 1 - \sum_{j=1}^n P_{ij}^2$$

Where, n is the number of marker alleles for marker i and P_{ij} is the frequency of the j^{th} allele for marker i .

CHAPTER FOUR

4.0 RESULTS

4.1 Phenotypic screening at seedling stage for salt tolerance

Table 5 shows the salinity scores of various genotypes used in the study. None of the entries screened could be scored as highly tolerant. The two checks, one tolerant and the other susceptible behaved as expected. Five of the entries scored as tolerant but ten and fourteen of them showed moderate tolerance and susceptibility respectively. Only five entries were highly susceptible. Among the entries from PGRRI, four showed tolerance, three moderately tolerant and five were susceptible. None of them was highly susceptible. Most of the Nerica materials showed moderate tolerance whereas the Sebota entries were mostly susceptible or highly susceptible, except Sebota 337-1. All the Basmati entries from the Philippines' were highly susceptible.

Table 5. Salinity reaction of seedlings of rice genotypes in salinized hydroponic system

Genotype	Salinity score	Salinity tolerance (phenotype)	Genotype	Salinity score	Salinity tolerance (phenotype)
FL478 (TC)	3	Tolerant	GH 1837	7	Susceptible
SR-1	3	Tolerant	GH1545	7	Susceptible
GH 1580	3	Tolerant	GH 1533	7	Susceptible
GH 1599	3	Tolerant	GH 1593	7	Susceptible
GH1571	3	Tolerant	GH1585	7	Susceptible
Anyofula	3	Tolerant	GH1598	7	Susceptible
Perfume S	5	Moderately tolerant	Local Red	7	Susceptible
Abidjan	5	Moderately tolerant	Matigey	7	Susceptible
GH 1575	5	Moderately tolerant	Viwornor	7	Susceptible
GH 1528	5	Moderately tolerant	Koshihikari	7	Susceptible
GR 18 Red	5	Moderately tolerant	CG14	7	Susceptible
Nerica L24	5	Moderately tolerant	Sebota 41	7	Susceptible
Nerica L23	5	Moderately tolerant	Sebota 281-2	9	Highly susceptible
Nerica L27	5	Moderately tolerant	Sebota 33	9	Highly susceptible
Sebota 337-1	5	Moderately tolerant	Sebota 68	9	Highly susceptible
IR 72 (Ph)	5	Moderately tolerant	LocalBasti-2	9	Highly susceptible
Nerica L9	7	Susceptible	Basmati 122	9	Highly susceptible
Good/New	7	Susceptible	IR-29 (SC)	9	Highly susceptible

* 1 = highly tolerant, 3 = tolerant, 5 = moderately tolerant, 7 = susceptible and 9 = highly susceptible. TC: Tolerant check; SC: susceptible check

4.2 Plant growth and accumulation of sodium and potassium in rice seedlings

4.2.1 Seedling height

Seedling height of the genotypes reduced in the salinized setup compared to the non-salinized (Table 6). The degree of stunting varied greatly among the different genotypes. The tolerant check FL478 recorded a height reduction of 29.3% while the susceptible check IR29 reduced by 34.9%.

The germplasm from PGRRI and Ghana showed varying performance; the degree of reduction ranged from a highest of 46% in GH1585 to the lowest of 20.9% in GR 18 Red. Abidjan and Local Red which were farmers grown accessions reduced by 39.3% and 41.8% respectively. In all, five of the Ghanaian entries recorded reductions lower than the tolerant check. Nine of them also recorded reductions higher than the susceptible check. Among the Nerica's, L27 had the highest reduction of 32.5% and L9 the lowest of 26.1%. The Sebota entries recorded a reduction range of 29.2% to 38.6%. Both Basmati's recorded reduction higher than the susceptible check. One of the two Japanese entries (Koshihikari) recorded the second lowest reduction of 17.2% in the experiment. The lowest reduction in the setup was observed in the only entry from Thailand, Perfume short type which recorded a reduction of 8.4%. The known glaberrima in the experiment CG14 had a reduction of 29.3%

Table 6. Seedling height of rice genotypes under normal and saline conditions

Genotype	Normal	Saline	Probability	%Red.	Genotype	Normal	Saline	Probability	%Red.
FL478	32.24	22.78	0.005972	29.3	SR-1	25.99	19.16	0.001417	26.3
GH 1593	38.93	23.33	0.005036	40.1	CG14	32.36	22.88	2.34E-05	29.3
GH 1575	30.48	22.02	0.000218	27.7	Nerica L23	28.73	20.78	0.000346	27.7
GH1585	37.19	20.09	0.005762	46.0	Nerica L9	25.38	18.75	3.8E-05	26.1
GH1598	35.33	23.08	0.002803	34.7	Nerica L24	29.89	21.76	0.000246	27.2
GH1571	27.46	19.73	0.001084	28.2	Nerica L27	29.64	19.99	0.0008	32.5
GH 1533	35.73	21.84	0.004366	38.9	Sebota 33	27.80	17.07	0.004688	38.6
GH 1528	33.15	20.08	9.91E-05	39.4	Sebota 337-1	30.10	20.36	0.009179	32.4
GH1545	34.06	22.73	0.000374	33.3	Sebota 41	28.50	19.36	0.00334	32.1
GH 1580	33.49	23.55	2.64E-07	29.7	Sebota 281-2	32.00	20.42	4.29E-06	36.2
GH 1599	47.89	30.67	6.17E-05	36.0	Sebota 68	30.09	21.30	0.00023	29.2
GH 1837	46.30	27.81	0.000107	39.9	IR 72 (Ph)	28.96	20.29	0.000322	29.9
GR 18 Red	24.89	19.68	0.002627	20.9	Basmati 122	35.76	21.63	0.001672	39.5
Anyofula	41.76	27.03	0.001677	35.3	Local Basmati-2	33.90	21.64	0.00336	36.2
Abidjan	38.86	23.59	0.004563	39.3	Good and New	35.80	22.74	0.000963	36.5
Local Red	37.56	21.84	0.001981	41.8	Koshihikari	25.54	21.14	0.002819	17.2
Matigey	38.74	25.89	0.001967	33.2	Perfume	22.39	20.51	0.023198	8.4
Viwornor	32.43	23.83	0.004798	26.5	IR-29	23.26	15.14	0.004876	34.9

4.2.2 Plant dry matter

Plant dry matter was reduced in the salinized medium (Table 7). The tolerant check FL478 reduced by 10.9% while the susceptible check IR29 reduced by 25.7%. The glaberrima CG14 reduced by 55.2%. The PGRI and Ghana showed varying degrees of reduction GR18 Red recorded the lowest reduction of 12% while GH1598 recorded the highest reduction of 55.4%. The two farmer's entries also recorded high reductions. None of the entries recorded a reduction lower than the tolerant check. The Nerica entries had low reduction (similar to the tolerant check) except Nerica L23 which had a reduction of 30.9%. The Sebota entries had moderate reductions in seedling height except Sebota 281-2 which had a reduction of 66.2%, the highest in the study. Both Basmati's recorded very high reductions. Similarly both entries from Japan also had very high reductions, all higher than the susceptible check.

Table 7. Seedling dry weight of rice genotypes under normal and saline conditions

Genotype	Dry matter			% Red	Genotype	Dry matter			% Red
	normal	saline	Probability			normal	saline	Probability	
FL478(TC)	0.12	0.11	2.919986	10.9	SR-1	0.09	0.07	0.010705	23.9
GH 1593	0.10	0.05	0.015844	48.5	CG14	0.15	0.07	0.000517	55.2
GH 1575	0.10	0.06	0.00116	34.7	Nerica L23	0.10	0.07	0.001002	30.9
GH1585	0.08	0.05	0.018859	36.6	Nerica L9	0.07	0.06	0.004943	12.1
GH1598	0.14	0.06	0.00609	55.4	Nerica L24	0.07	0.06	0.000615	10.2
GH1571	0.09	0.07	3.37E-06	22.0	Nerica L27	0.07	0.06	0.085062	11.8
GH 1533	0.06	0.04	0.024411	20.1	Sebota 33	0.07	0.05	0.074409	20.2
GH 1528	0.08	0.05	0.025666	33.7	Sebota 337-1	0.09	0.07	0.033167	24.8
GH1545	0.11	0.05	0.000634	54.4	Sebota 41	0.06	0.04	0.013793	38.6
GH 1580	0.09	0.08	0.014499	18.4	Sebota 281-2	0.10	0.03	0.001079	66.2
GH 1599	0.14	0.09	1.62E-05	33.8	Sebota 68	0.07	0.04	0.00309	39.3
GH 1837	0.16	0.08	0.000147	51.6	IR 72 (Ph)	0.07	0.06	0.007571	19.4
GR 18Red	0.07	0.06	0.047506	12.0	Basmati 122	0.11	0.05	0.001444	59.2
Anyofula	0.12	0.08	0.015624	32.3	LocalBasmti-2	0.10	0.06	0.000607	41.8
Abidjan	0.09	0.06	0.004834	35.9	GoodNew (JP)	0.12	0.07	0.002298	45.2
Viwornor	0.08	0.05	0.065099	30.3	Koshihikari	0.06	0.04	0.000282	38.4
Matigey	0.12	0.06	0.000265	52.9	PerfumeStype)	0.08	0.07	0.060943	18.4
Local Red	0.09	0.05	0.009526	45.1	IR-29 (SC)	0.05	0.04	0.014201	25.7

4.2.3 Sodium and Potassium concentrations

Sodium ions (Na^+) absorption increased in the saline setup compared to the control. The concentrations of sodium in the control setup were similar in most genotypes (Table 8). The susceptible genotypes recorded higher Na^+ concentrations compared to the tolerant genotypes. The tolerant check FL478 had Na^+ concentration of 16ppm in the control and 18 ppm in the saline setup respectively. IR29 the susceptible check, had Na^+ concentration of 15 ppm in the control setup but very high concentration (24.5) in the saline setup. The highest concentration of Na^+ in the salinized setup was recorded in GH 1593 which had 33.5 ppm. Thirteen of the Ghanaian entries recorded Sodium concentrations lower than the tolerant check in the control setup. While only two entries had higher concentrations, Gh1580 and Abidjan (an entry from a farmer's field). All the entries however recorded higher sodium concentrations than the tolerant check in the saline setup. Three of the Nerica entries had sodium concentrations (15 ppm) lower than the tolerant check, but the same value as the susceptible check in the control setup. All four Nerica entries had higher sodium concentration scores in the saline setup than the tolerant check. Two of the Nericas had concentration lower than the susceptible check while the other two had Na concentrations higher than the susceptible check in the saline medium. All the Sebota entries had lower Sodium concentration than the tolerant check in the control setup and higher concentration than the same check in the saline setup. In the control medium one Sebota entry (Sebota 281-2) had the same concentration of sodium as the susceptible check. The remaining three Sebota accessions had lower concentration than the susceptible check. All four entries however had Na concentrations higher than the susceptible check in the saline setup. The two Basmati's had sodium concentrations lower than both checks in the

control and higher than both checks in the saline setup. One of the two entries from Japan (Good and New JP) had Na concentration higher than the susceptible check in the control setup and the other lower, but both entries had higher concentrations than checks in the saline setup. CG14 had concentrations equal to the susceptible check in both setups. The tolerant genotypes had lower sodium concentration in their tissues in the saline setup while the susceptible ones had higher concentrations.

Table 8. Na⁺ concentrations in seedling leaves (ppm)

Genotype	Normal	Saline	Genotype	Normal	Saline
FL478(TC)	16	18	SR-1	14	19.5
GH 1580	17	23	CG14	15	24.5
GH 1599	14	20.5	Nerica L23	17	23.5
GH 1533	15	21	Nerica L9	15	27
GH 1593	16	33.5	Nerica L24	15	26
GH 1575	13	26.5	Nerica L27	15	24
GH 1528	13	28	Sebota 33	13	27.5
GH1585	14	23.5	Sebota 337-1	14	25.5
GH1598	15	25	Sebota 41	13	26.5
GH1571	15	24.5	Sebota 281-2	15	32
GH1545	15	27.5	Sebota 68	14	30.5
GH 1837	16	27.5	IR 72 (Ph)	13	25.5
GR 18 Red	15	25.5	Basmati 122	14	31
Abidjan	17	25.5	Local Basmati-2	13	27.5
Anyofula	14	25	Good and New	16	27.5
Local Red	15	26	Koshihikari	15	28.5
Matigey	15	29.5	Perfume(Short)	16	23.5
Viwornor	14	25.5	IR-29 (SC)	15	24.5

TC = tolerant check and SC =susceptible check

The potassium ion (K⁺) concentration in the seedling leaves was generally very high in the control setup compared to the saline setup. It was on the average twice as high as in the

saline setup (Table 9). The tolerant check FL478 had very high K concentration of 21.6 ppm in the saline setup. The susceptible IR29 had very low (K^+) concentration of 9.65 ppm in the saline setup. Among the entries from Ghana had GH1599 recorded the highest K concentration (20.25 ppm) in the saline setup. Seven of the Ghanaian entries had concentrations lower than the tolerant check in the control set up. Six other Ghanaian entries including one of the farmer's accessions (Abidjan), had concentrations higher than the tolerant check in the control setup. GH1593 however recorded the lowest K^+ concentration 9.3 ppm among the genotypes studied in the saline medium. In the saline setup, all the Nerica entries recorded lower potassium ion concentration in their tissues than the tolerant check but higher than the susceptible check. Two of them had higher K^+ concentrations in the control setup than both checks. SR1 had the highest potassium ion concentration of 65 and 23.55 under the control and saline setup respectively. All Sebota entries had high concentrations in control setup but performed averagely in the saline setup compared with the tolerant check. Only Sebota 337-1 recorded a high concentration (19.6ppm) in the saline setup. Both Basmatris performed averagely as compared to the tolerant check. The two Japanese entries recorded higher values in the control setup than the tolerant check, but averagely in the saline setup compared to the tolerant check. IR72 from the Philippines recorded higher values in the control setup than the checks and averagely in the saline setup. CG14, the glaberrima, recorded low concentration in the saline setup. Generally, the tolerant genotypes had higher potassium concentration in the saline setup than the susceptible ones.

Table 9. K⁺ concentration in the seedling leaves (ppm)

K ⁺ Content			K ⁺ Content		
Genotype	Normal	Saline	Genotype	Normal	Saline
FL478(TC)	40	21.6	SR-1	65	23.55
GH 1593	35	9.3	CG14	40	13.45
GH 1575	45	17.25	Nerica L23	35	10.75
GH1585	40	18.15	Nerica L9	45	11.75
GH1598	40	13.05	Nerica L24	50	12.25
GH1571	40	16.75	Nerica L27	50	15.5
GH 1533	25	15.35	Sebota 33	40	16.05
GH 1528	25	13.05	Sebota 337-1	45	19.6
GH1545	25	11.15	Sebota 41	40	9.95
GH 1580	45	18.5	Sebota 281-2	35	10.15
GH 1599	40	20.25	Sebota 68	40	13.1
GH 1837	45	13.55	IR 72 (Ph)	45	17.75
GR 18 Red	45	12.55	Basmati 122	40	10.4
Abidjan	50	13.45	Local Basmati-2	40	16.2
Anyofula	35	18.05	Good/ New (JP)	40	12.45
Local Red	35	14.95	Koshihikari	45	16.7
Matigey	45	11.8	Perfume (S type)	45	15.7
Viwornor	35	15.1	IR-29(SC)	45	9.65

TC = tolerant check and SC =susceptible check

The Na/K ratio was lower generally in the control setup compared to the saline setup (Table 10). FL478 recorded the lowest ratio of 0.40 in the control setup as compared to 0.83 (which was the lowest in the study) in the saline setup. IR29 had a ratio of 0.33 in the control setup and 2.54 in the salinized media. Tolerant varieties absorb less Na⁺ and more K⁺, but the sensitive ones show the reverse tendency. Nevertheless, all the genotypes under

salinity stress intake Na^+/K^+ more than under the control. It is understandable that plants under salinity stress absorb more sodium ion.

SR1 recorded the lowest ratio in both setup. The entries from Ghana had a range of 1.01 (GH1599) to 3.6 in the highest (GH1593). The Nerica entries recorded average Na^+/K^+ ratio between the two checks except Nerica L27 which had quite a low ratio. Sebota 281-2 had the highest ratio of 3.15 among the entries from Sebota with, while Sebota 337-1 recorded the lowest among the Sebota accessions. Both Basmati's as well as both Japanese entries recorded average ratios.

Table 10. Na^+/K^+ ratio of seedlings

Genotype	Na+/ K+ Ratio		Genotype	Na+/ K+ Ratio	
	Normal	Saline		Normal	Saline
FL478(TC)	0.4	0.83	SR-1	0.22	0.83
GH 1593	0.46	3.6	CG14	0.38	1.82
GH 1575	0.29	1.54	Nerica L23	0.49	2.19
GH1585	0.35	1.29	Nerica L9	0.33	2.3
GH1598	0.38	1.02	Nerica L24	0.3	2.12
GH1571	0.38	1.46	Nerica L27	0.3	1.55
GH 1533	0.6	1.37	Sebota 33	0.33	1.71
GH 1528	0.52	2.15	Sebota 337-1	0.31	1.3
GH1545	0.6	2.47	Sebota 41	0.33	2.66
GH 1580	0.38	1.24	Sebota 281-2	0.43	3.15
GH 1599	0.35	1.01	Sebota 68	0.35	2.33
GH 1837	0.36	2.03	IR 72 (Ph)	0.29	1.44
GR 18 Red	0.33	2.03	Basmati 122	0.35	2.98
Abidjan	0.34	1.9	Local Basmati-2	0.33	1.7
Anyofula	0.4	1.59	Good and New	0.4	2.21
Local Red	0.43	1.74	Koshihikari	0.33	1.71
Matigey	0.33	2.5	Perfume (Short	0.36	1.5
Viwornor	0.4	1.69	IR-29(SC)	0.33	2.54

TC = tolerant check and SC =susceptible check

4.3 Microsatellite variations of the rice accessions

4.3.1 Salt tolerance SSRs

Twenty saltol SSR markers were screened, 16 produced polymorphic bands. Primers RM10711 and RM10793 showed bands that differentiated the tolerant and susceptible checks.

Primer RM10711 was able to separate ten Ghanaian, three Nericas, the two Basmatis and three Sebota entries as susceptible. It also distinguished Koshihikari as susceptible. The primer recognized three entries as tolerant. Accessions Perfume (Short type), Sebota 41, Good and New (JP), GH 1528, Sebota 337-1, local Red, GH1545, GH 1580, SR-1 and Gh1585 did not share any bands with the two checks.

Primer RM10793 showed that accessions GH1599, SR-1, GH1571, GH1533, Nerica L9, Nerica L27, Perfume (Short type), GH1837, Matigey, Basmati 122, Sebota 281-2, Sebota 68 shared a common band with IR29 the susceptible check. GH 1575, GH1585, GH1598, GH 1528, GH1545, GH 1580, CG14, Nerica L23, Nerica L24, Sebota 33, Sebota 41, Local Red, Good and New (JP), IR 72 (Ph), Local Basmati-2, Koshihikari , Viwornor all had similar bands to FL478 the salt tolerant check. GH1593, Sebota 337-1, Anyofula, and GR 18 Red did not share bands with the two checks in relation to primer RM10793.

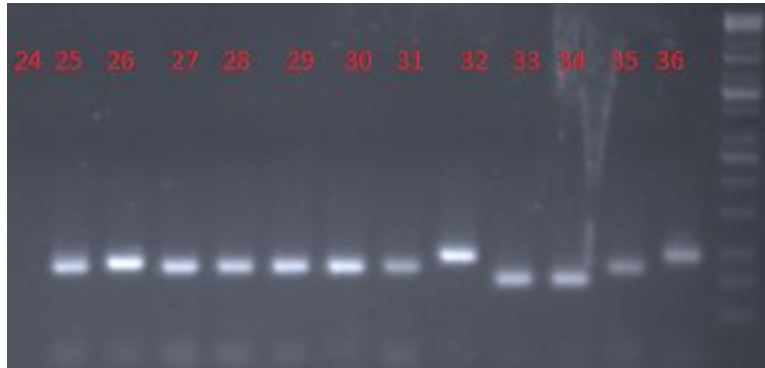
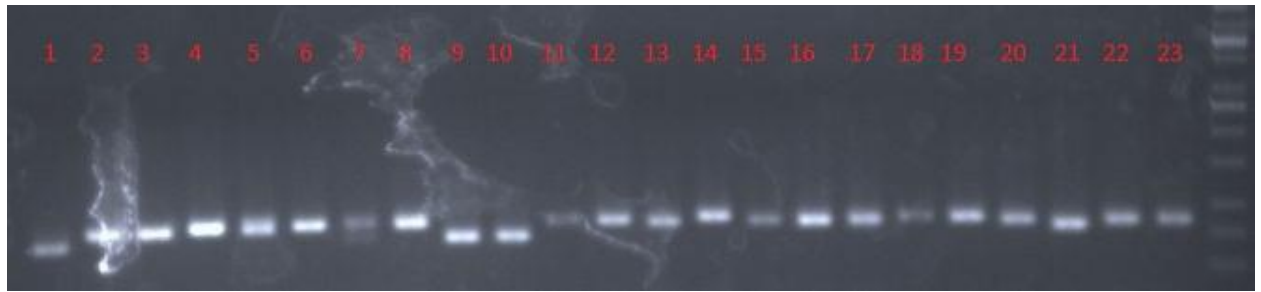


Plate.2 A gel image of the banding pattern of the genotypes with Primer RM10711. 1= Local Red, 2= Sebota 41, 3= Perfume (Short type), 4= Sebota 337-1, 5= Sebota 33, 6= Nerica L27, 7= Nerica L24, 8= Nerica L9, 9= Nerica L23, 10= FL478, 11= CG14, 12= IR-29, 13= SR-1, 14= GH1599, 15= GH1580, 16= GH1545, 17= GH1528, 18=GH1533, 19= GH1571, 20= GH1598, 21= GH1585, 22= GH1575, 23= GH1593, 24= Abidjan, 25= Sebota 68, 26= Sebota 281-2, 27= Viwornor, 28= Koshihikari, 29= Local Basmati-2, 30= GR 18 Red, 31= Basmati 122, 32= Matigey, 33= GH 1837, 34= IR 72 (Ph), 35= Good and New (JP), 36= Anyofula

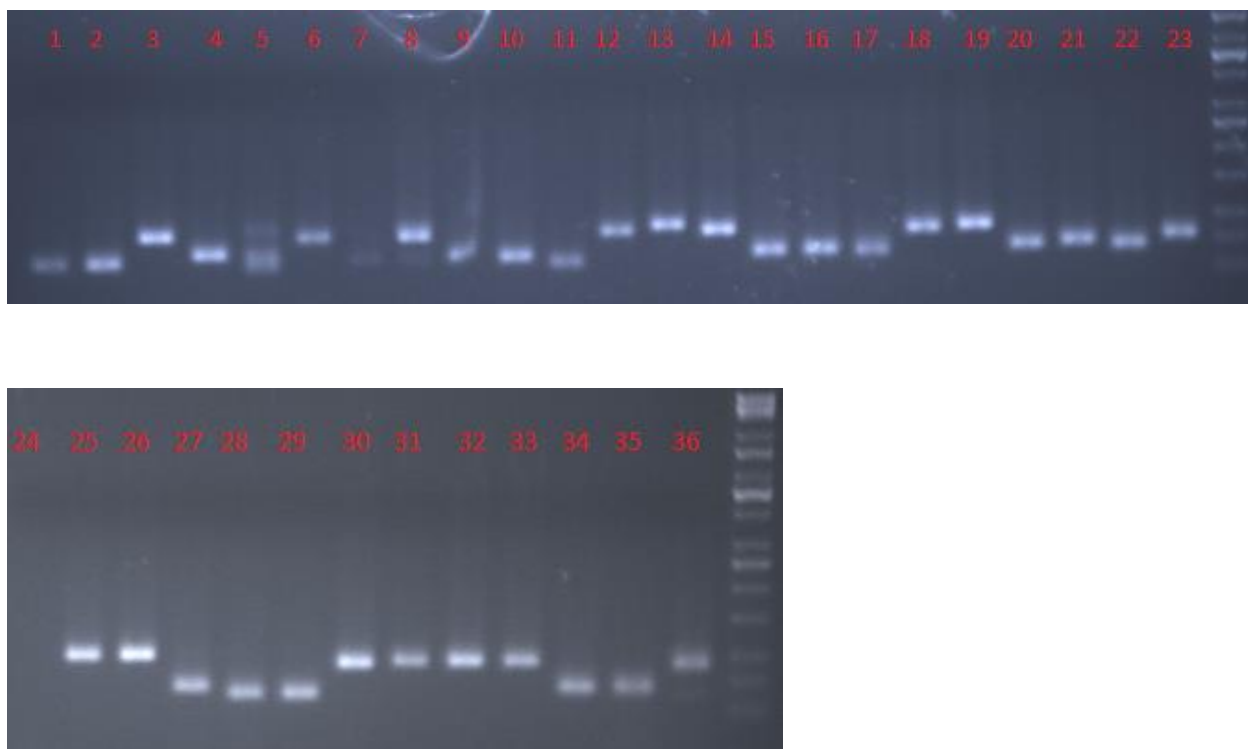


Plate.3 A gel image of the banding pattern of the genotypes with primer RM10793. 1= Local Red, 2= Sebota 41, 3= Perfume (Short type), 4= Sebota 337-1, 5= Sebota 33, 6= Nerica L27, 7= Nerica L24, 8= Nerica L9, 9= Nerica L23, 10= FL478, 11= CG14, 12= IR-29, 13= SR-1, 14= GH1599, 15= GH1580, 16= GH1545, 17= GH1528, 18=GH1533, 19= GH1571, 20= GH1598, 21= GH1585, 22= GH1575, 23= GH1593, 24= Abidjan, 25= Sebota 68, 26= Sebota 281-2, 27= Viwornor, 28= Koshihikari, 29= Local Basmati-2, 30= GR 18 Red, 31= Basmati 122, 32= Matigey, 33= GH 1837, 34= IR 72 (Ph), 35= Good and New (JP), 36= Anyofula

4.3.2 Diversity among accessions

Out of the 33 SSR primers used, 28 produced polymorphic bands representing 84.8%. Fourteen out of the 28 SSR primers were located at the saltol loci of the rice genome, the remaining 14 were spread throughout the entire genome. A total of 116 alleles with an average of 4.14 alleles per locus were generated by the 28 primers (Table 11).

The highest allele frequency was 100% produced by primer RM454 and the lowest allele frequency was (30%) by primers RM10748, RM10864 and RM20. The overall average allele frequency was 60%. The Polymorphic Information Content, PIC, of the primers

among the 36 rice genotypes was observed in the range of 0.053 to 0.785, with the average of 0.471. The genetic diversity within the population was 51.6% but RM20 had the highest diversity of 84.6% and RM454 the lowest of 5.4%

Primer RM20 had the highest PIC of 0.829 followed by RM10864 and RM10793 respectively with RM454 having the least PIC of 0.053. Primer RM20 had the highest diversity discrimination of 84.6% followed by RM10864 with 78.5% and RM10793 with 75% respectively while RM454 had the lowest diversity of 5.4%.The population had an average diversity of 51.6%

Table 11. SSR primers used with their parameters for diversity

Marker	Major Allele Frequency	No of alleles	Gene Diversity	PIC
RM336	0.4	5	0.690	0.639
RM10655	0.4	5	0.708	0.660
RM10696	0.7	5	0.511	0.470
RM10711	0.4	5	0.719	0.668
RM10713	0.8	4	0.336	0.317
RM10722	0.4	6	0.728	0.689
RM10748	0.3	4	0.742	0.694
RM10793	0.4	7	0.750	0.711
RM10800	0.5	4	0.642	0.583
RM10825	0.5	4	0.640	0.592
RM10843	0.7	4	0.444	0.409
RM10852	0.5	4	0.625	0.568
RM10864	0.3	7	0.785	0.755
RM10890	0.7	2	0.444	0.346
RM10927	0.7	2	0.424	0.334
RM253	0.8	3	0.323	0.285
RM518	0.9	2	0.153	0.141
RM312	0.9	2	0.105	0.099
RM489	0.8	3	0.403	0.363
RM474	0.5	5	0.660	0.604
RM259	0.8	4	0.412	0.383
RM11	0.6	3	0.537	0.441
RM454	1.0	2	0.054	0.053
RM19	0.7	3	0.415	0.349
RM5	0.6	2	0.486	0.368
RM20	0.3	9	0.846	0.829
RM307	0.8	5	0.298	0.287
RM522	0.6	5	0.576	0.536
Mean	0.6	4	0.516	0.471

4.3.3 Genetic Divergence of Rice Population as revealed by the Dendrogram

A dendrogram was constructed based on the unweighted pair-group method with arithmetic averages (UPGMA) using the neighbor-joining (NJ) method .The dendogram constructed

grouped the accessions into clusters, indicating the diversity that existed between the accessions. The accessions were separated into three major clusters (Fig 4.2)

Major Cluster 1 comprised Nine Ghanaian entries, all four Nerica's, one Sebota entry, one Thailand entry, one Japanese entry and the two checks.

Major cluster 2 comprised eight Ghanaian entries including a collection from a farmer's field, four Sebota entries, the two Basmati entries from the Philippines, one Japanese entry and two entries from ARC Senegal.

Major cluster 3 had only IR72 (Ph) from the Philippines

Major cluster 1 had two sub clusters. Sub cluster I and II, sub cluster I had two sub clusters, the first contained three Nerica entries and five Ghanaian entries together with the checks, the second cluster consisted of four Ghanaian entries only. All Nericas were separated from each other in this sub group. Sub cluster II had only two entries, a Nerica and a Ghanaian entry

Major cluster 2 had two sub clusters III and IV. Sub cluster III had two lower clusters, the first comprised six Ghanaian entries, one Japanese and Philippine entry. The second contained only one entry from Sebota. Sub cluster IV had two sub groups, the first consisted of three Sebota entries, two Ghanaian entries and one Philippino entry. The second group had one entry from ARC Senegal.

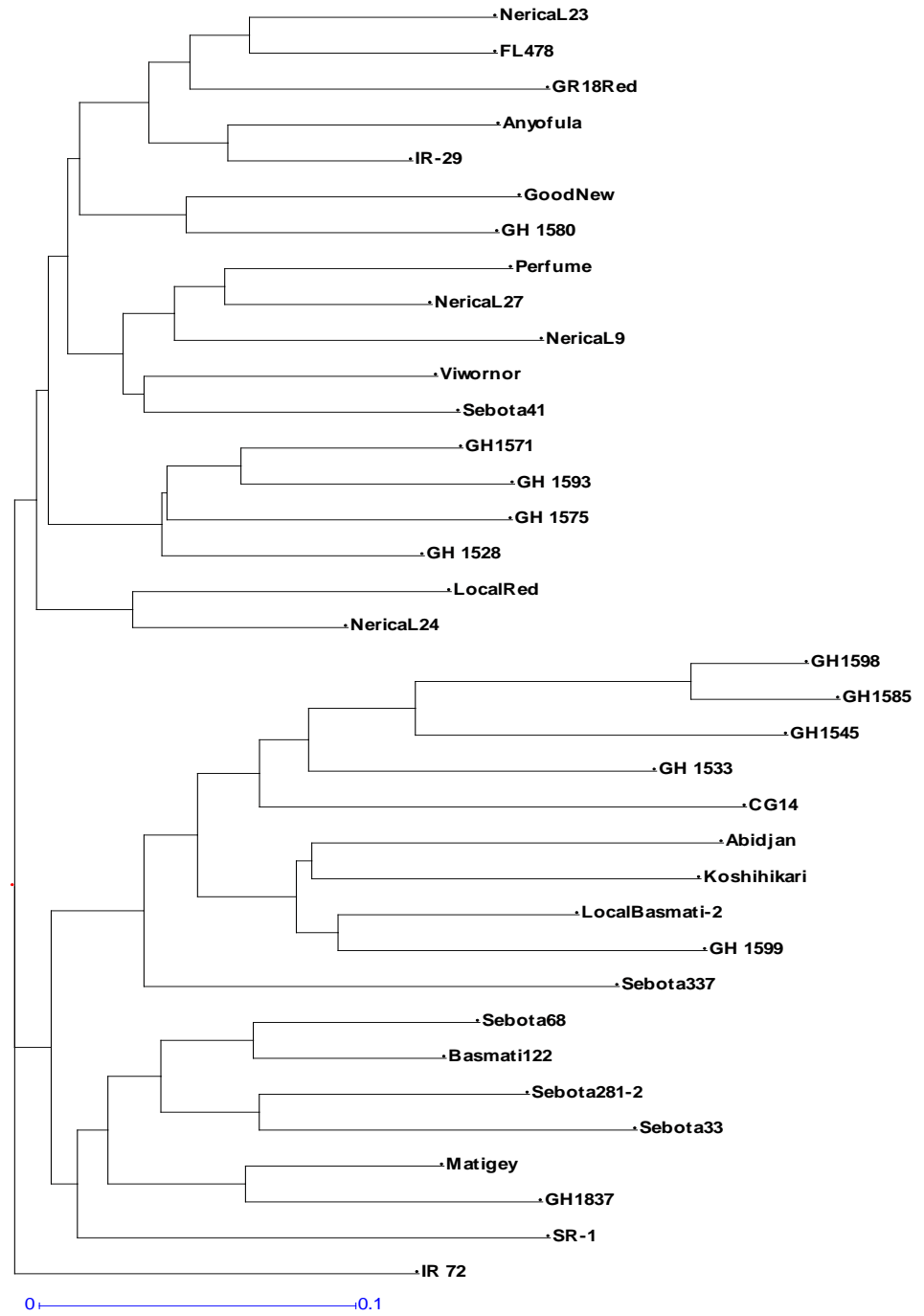


Figure.1 Dendrogram of the clustering of the genotypes with the SSRs markers

CHAPTER FIVE

5.0 DISCUSSION

5.1 Variability in tolerance of seedlings of rice genotypes to salinity

To counteract the problems posed by increasing salinization of arable lands, there is the need to carefully incorporate salinity tolerant genes into farmers' varieties and new varieties being produced. In line with this it is important to identify tolerant germplasm phenotypically.

Based on the SES, which represents a phenotypic screening approach, accessions SR-1, FL478, GH1580, GH1599, Anyofula and GH1571 were tolerant varieties. These accessions were able to produce high total dry matter under the saline condition by maintaining active photosynthetic cells

Perfume (Short type), Nerica L23, Sebota 337-1, GH 1575, Nerica L27, Nerica L24, GH 1528, GR 18 Red, IR 72 (Ph) and Abidjan were moderately tolerant to salt stress, though their growth reduced, they were able to maintain cells active enough to keep them alive and growing.

The two groups showed varied degrees of tolerance to high salinity levels in the growing environment, some were affected more than others. These varieties proved to be more tolerant to salinity than the rest of the accessions hence they could be incorporated in plant breeding works to produce tolerant commercial varieties, which can withstand the threat posed to rice production by salinized soils.

According to Wu *et al.*, (2009), the Na / K ratio measures the plant's ability to keep a good balance of nutrients needed for its upkeep and proper growth. Tolerant varieties keep their

leaves relatively free of the toxic ions (Na) besides having assured K supply. This attribute together with the higher number of leaves maintained by the plants and higher leaf area contributes to their success under high salt concentration. Tolerance to salinity in rice is associated with Na⁺ exclusion and increased absorption of K⁺ to maintain a good Na /K balance in the shoot under saline condition. It is considered that damage of plant leaves under saline conditions is attributed to accumulation of Na⁺ from the root to the shoot in external high concentration (Aref and Rad, 2012).

In the salt sensitive lines, the reduction in growth became more pronounced with the passage of time. Salinity injury symptoms like leaf rolling, tip whitening and reduction in leaf area are the first symptoms. Salinity suppresses the growth of leaves in the plants causes' complete cessation of plant growth and premature senescence of leaves (Sohrawardy *et al.*, (2008); Islam *et al.*, 2008).

The tolerant varieties SR-1, FL478, GH1580, GH1599, Anyofula and GH1571 maintained lower Na⁺ concentrations besides maintaining high K⁺ concentrations under high sodicity. On the other hand, the sensitive rice varieties IR29, Sebota 281-2, Basmati 122, Local Basmati-2, Sebota 68 and Sebota 33, Nerica L9, GH 1533, GH 1837, GH1545, Viwornor, CG14, Koshihikari, GH1585, GH1598, Matigey, Good and New (JP), GH 1593, Local Red, Sebota 41 were unable to effectively prevent both the accumulation of Na⁺ in their cells and the depletion of K⁺ from their cells. The success of the tolerant varieties in gaining higher fresh and dry weights widened the differences between them as well as the differences in their Na⁺ concentrations. This supports the findings of Ray and Islam (2008) and Thomson *et al.*, (2010) who both had lower Na/K ratio in salt tolerant accessions and higher ratios in the susceptible accessions. Emon *et al.*, (2015) also reported similar results

when they screened five (5) promising rice genotypes under salinity stress. They observed that all the genotypes had the Na^+/K^+ ratio of the tolerant genotypes and moderately tolerant genotypes had lower Na^+/K^+ ratios than those of the sensitive genotypes. Also the difference in the ratio of Na^+/K^+ under normal condition for all genotype was not significant indicating equal performance of salinity tolerant and sensitive genotypes in the absence of salinity challenge.

5.2 Identification of useful microsatellite markers for genotyping germplasm.

Progress in rice breeding for salt tolerance, constitutes the identification of the major locus conferring salt tolerance at different growth stages. Microsatellites are efficient and cost-effective to use. Compared with other markers, they are abundant, co-dominant, highly reproducible and interspersed throughout the genome. In particular, microsatellite markers have been widely applied in rice genetic studies as they are able to detect high levels of allelic diversity. SSR markers are playing important role in identifying genes for salt tolerance that can be helpful for plant breeders to develop new cultivars. Molecular markers could be used to tag QTL and evaluate their contributions to the phenotype by selecting for favorable alleles at those loci in marker assisted selection (MAS) scheme with the aim to accelerate genetic advancement in rice. This is faster, more efficient and cost-efficient than conventional screening under saline field conditions [Gregorio, 1997; Aliyu et al, 2011]

Out of the 33 SSR primers used, 28 produced polymorphic bands representing 84.8%. Fourteen out of the 28 SSR primers were located at the saltol loci of the rice genome, the

remaining 14 were spread throughout the entire genome. A total of 116 alleles with an average of 4.14 alleles per locus were generated by the 28 primers. These primers were useful and informative in genotyping the germplasm used in the study. High PIC value of a marker indicates high probability to detect the number of alleles among cultivars. A PIC value higher than 0.50 indicates high degree of polymorphism. Based on this RM20, RM10864, RM10793, RM10748 and RM10722 were very good primers for this diversity study. These results show that the markers used in this study are revealing and good for genetic diversity studies in rice. The total number of alleles generated by the 28 primers compares to Zeng *et al.*, (2004) who observed a total of 123 alleles among 33 rice genotypes with an average of 4.9 alleles per locus. The number of alleles per locus ranged from 2 to 9. The PIC values for the microsatellite loci ranged from 0.06 to 0.85 with an average of 0.57. Low PIC values were observed for 5 primers and the PIC values for the remaining 17 microsatellite loci were all above 0.50. Lapitan *et al.*, (2007) also obtained PIC ranges from 0.18 to 0.91 with an average 0.68 per marker, making them very useful for genotypic studies. Prabakaran *et al.*, (2010) had a total of 11 alleles detected by 5 SSR primers and the number of alleles per locus ranged from 2 to 3 with an average of 2.2 per locus. Among the primers used, RM 481 identified higher number of alleles and average PIC was 0.43. Behera *et al.*, (2012) observed a wider range of PIC, between 0.24 and 0.956 with a higher average of 0.811 per locus than was obtained in this study.

5.3 Genetic diversity and selection of salt tolerant genotypes.

The SSR primer pairs used produced 116 alleles with an average of 4.14 per locus. The average allele frequency was 0.6. The PIC ranged from 0.053 to 0.829, with an average of

0.471. The genetic diversity within the population was 51.6%, the highest diversity was 84.6% and the lowest 5.4%. Similar results were recorded by Lang *et al.*, (2008) where 95% of SSR markers for genetic diversity were reported to be polymorphic in IR64 variety. The results is also in tune with Deepti *et al.*, (2013), who reported a higher average PIC of 0.67 for 26 SSR markers with a range of 0.50 to 0.89. The number of alleles obtained per locus was 7.1, which was higher than values in this work. Mahalingam *et al.* (2013) on the other hand reported an average PIC value of 0.44 which was lower than what was obtained in this study. Their highest PIC value reported was greater than 0.60 and the lowest was 0.035 which are both lower than what was obtained in this experiment. The present estimate of PIC was also larger than reported by Hashimoto *et al.*, (2004) in a Japanese rice population comprising 171 cultivars used in brewing of Japanese rice wine which had a diversity of 0.33.

Singh *et al.* (2011) in their genetic diversity study of rice genotypes using 30 SSR markers, noted fewer alleles (83) with a lower average of 2.76 alleles per marker, but they had a high PIC value varying from 0.54 to 0.96. Studies by Chakravarthi *et al.*, (2006) revealed that primer RM20 on chromosome 12 had seven alleles. In the present study, primer RM20 had 9 alleles, indicating that it is very polymorphic and suitable for diversity studies. El-Malky *et al.*, (2007) used 14 microsatellites to generate a total of 122 alleles with an average PIC of 0.782 and a range of 0.438 to 0.891. All their diversity parameters were higher than those obtained in this work.

Islam *et al.*, (2012) detected a total of 168 alleles, the number of alleles per locus ranged from 2 to 6 which was lower than what was obtained in this work (2 to 9), but they had an average of 4.2 alleles per locus which was slightly higher than the value obtained in this

study. Polymorphic information content (PIC) value varied from 0.21 to 0.76 with an average of 0.57 also higher than this study of 0.471. Lapitan *et al.*, (2007) reported higher parameters than obtained in this study. They had a total of 176 alleles. Their number of alleles per marker was high ranging from 6 to 22, with an average of 14.6 alleles per locus. Their primers were thus very useful in distinguishing the germplasm used. Roychowdhury *et al.*, (2013) also detected a total of 122 alleles which was higher than obtained in this study but the primer used had a lower allele range of 2 to 5 alleles compared to this studies (2 to 9). They also reported a lower average of 3.21 alleles per locus but the PIC value was 0.524 which was higher than the results from this study. Emon *et al.*, (2015) reported a total of 209 alleles among 5 rice genotypes using 160 SSR markers. They had a lower PIC of 0.32 and also a lower diversity of 0.37. Ram *et al.*, (2007), reported number of alleles per locus to vary from 3 to 8, with average number of alleles per locus at 4.86, indicating almost same magnitude of diversity with reference to the markers used in this investigation. Behera *et al.*, (2012) observed a total of 169 alleles, of which 166 were polymorphic from a set of 36 microsatellite markers. Their number of alleles per locus ranged from 2 to 9 with an average of 4.69 alleles per locus. Their (PIC) ranged between 0.24 and 0.956 with an average of 0.811 per locus, which were all higher than reported in this work

Dendrogram generated by SSR primers further grouped the germplasm into three major clusters. Cluster 1 had IR29 and FL478 clustering together, it also had the Nerica's at different sub clusters. Nerica L9 is a cross between TOG5681 and 3 rounds of backcross to IR64, Nerica L-23 and Nerica L-24 are crosses between TOG5681 and 2 rounds of backcross to IR64 followed by crosses to IR31851-96-2-3-2-1, Nerica L-27 crosses

between TOG5681 and 4 rounds of backcross to IR64. This probably explains why they were in the first cluster together, even though at different sub clusters. FL478 is a salt tolerant variety developed from a cross between Pokkali and IR29. The genotypes that clustered closely to these checks were similar to them.

Major cluster 2 had GH1598, GH1585, GH1545, GH 1533, clustering with CG14, a glaberrima and native to Africa, at a lower clustering level. This means the varieties in this group could be indigenous landraces from Africa, and may carry lots of unexploited genes for rice breeding. The glaberrima carries genes for tolerance to lots of natural stresses, from environmental to biotic stresses (Takeoka, 1965; Second, 1984). Abidjan, Koshihikari, Local Basmati-2 and GH 1599 also formed a cluster, Koshihikari is a known Japanese elite variety, with cold tolerant genes and the Basmatias are known for their aroma. Sub clustering under this group showed SR-1 singly and separately clustering with the group, Sebota 68, Basmati 122, Sebota 281-2, Sebota 33, Matigeey, and GH 1837. SR-1 seed shattering tendency when mature, this may imply that the group clustering with it could behave similarly. There is also the possibility that SR-1 is the only one with the shattering ability, hence on a different branch. Shattering will however be a negative trait in rice breeding.

Major cluster 3 had only IR72 which had high amylose content and popular among Cambodian farmers because it produces higher yields with superb grains of good quality, long grain and good taste, it can grow in dry season too. This accession did not cluster with any of the accessions indicating how unique and diverse it was from the rest.

The germplasm from PGRRI showed high degree of variability indicating how widely diverse they are genetically and how rich the germplasm is. This is good for rice breeding as it indicates a rich array of genes which could be useful for improving the crop.

This results further highlighted the divergence of the population studied, this diversity can be explored in breeding to improve local rice cultivars.

Microsatellite markers were able to distinguish between salt tolerant and susceptible entries. Out of Twenty saltol primers screened, only primers RM10711 and RM10793 were able to discriminate tolerant genotypes from susceptible. Based on Primer RM10711, Nerica L23, Local Red, IR 72 (Ph) were tolerant to salinity stress, CG14, Nerica L9, Nerica L24, Nerica L27, Sebota 33, Anyofula, Matige y, Basmati 122, GR 18 Red ,Local Basmati-2, Koshihikari, Viwornor, Sebota 281-2, Sebota 68, GH 1593, GH 1575, GH1598, GH1571, GH 1533, GH 1599 were however sensitive to salinity stress. With regard to RM10793, GH1575, GH1585, GH1598, GH1528, GH1545, GH 1580, CG14, Nerica L23, Nerica L24, Sebota 33, Sebota 41, Local Red, Good and New (JP), IR 72 (Ph), Local Basmati-2, Koshihikari , Viwornor were tolerant and GH 1599, SR-1, GH1571, GH 1533, Nerica L9, Nerica L27, Perfume (Short type), GH 1837, Matige y, Basmati 122, Sebota 281-2, Sebota 68 were susceptible accessions. This indicates that the primers could be used in marker assisted selection involving these genotypes. Aliyu *et al.*, (2011) used RM10793 on a collection of 150 diverse rice genotypes with a tolerant salt variety Pokkali and found the marker very informative. Deepti *et al.*, (2013) also found the primer very informative in their study on salt tolerance in some rice accessions. Huyen et al. (2012) used RM10793, RM10711 in Introgressing salinity tolerance QTLs *Saltol* into AS996 rice variety with Five hundred BC2F1 individuals. Kabir *et al.*, (2008) also used twelve SSR

markers for parental survey and among them three polymorphic SSR markers, OSR34, RM443 and RM169 were selected to evaluate 26 F₃ rice lines for salt tolerance. With respect to marker OSR34, 15 lines were identified as salt tolerant, 9 lines were susceptible and 2 lines were heterozygous. Several SSR primers (RM21, RM51 and RM127) were used by Sohrawardy *et al.*, (2008) for the identification of salt tolerant rice lines of PNR-519 x IR29 in F₃ population. Islam *et al.*, (2008) selected different SSR primers to evaluate F₂/F₃ rice lines for salt tolerance and identified 15 rice lines as salt tolerant by using RM231 and RM24 primers.

5.4 Establishing a core collection of salt tolerant lines to be used in future breeding works

In crop improvement and plant breeding, availability of genetic resources adapted to different environmental stresses is important in providing useful genes for producing new improved species.

Salinity caused a significant reduction in seedling growth with varying degree of variability among the accessions. It is always important to make a phenotypic/agro-morphological as well as genotypic assessment of the germplasm for crop improvement, to obtain a complete information on their potential contribution to the breeding program. Combining the performance of the accessions under the two screening methods, accessions SR1, IR72, Sebota 337-1, Perfume (Short) type, Anyofula, Local Red, GR18Red, GH1580, GH1528, GH1575, NericaL23, NericaL24 and NericaL27 performed well under salinity stress and have been identified to be superior among the accessions studied. These germplasm are presumed to have genes that confer various degrees of tolerance to salinized soils.

Accessions GH1545 and GH1585 were distinguished as carrying the tolerant genes by the markers used. However phenotypically they were susceptible whiles GH1599 and GH1571 were phenotypically tolerant but none of the markers used could confirm it. The remaining accessions did not perform well enough under salinized condition though some were separated by the Saltol SSR markers as tolerant.

CHAPTER SIX

6.1 CONCLUSIONS

6.1.1 Screening the rice germplasm under salinized and non-salinized conditions at the seedling stage

- SR-1, GH1580, GH1599, Anyofula and GH1571 were tolerant while Perfume (Short type), Nerica L23, Sebota 337-1, GH 1575, Nerica L27, Nerica L24, GH 1528, GR 18 Red, IR 72 (Ph) and Abidjan were moderately tolerant
- The genotypes used in this study exhibited high genetic variability. This indicates a positive genetic base for rice improvement in Ghana. Accessions SR1, IR72, Sebota 337-1, Perfume (Short) type, Anyofula, Local Red, GR18Red, GH1580, GH1528, GH1575, NericaL23, NericaL24 and NericaL27 performed well under salinity stress and have been identified to be superior among the accessions used in this study. These accessions were tolerant to various degrees of salinity in the growth medium. Eight (Anyofula, Local Red, GR18Red, GH1580, GH1571, GH1599, GH1528 and GH1575) of them were collected from the PGRRI and are indigenous accessions.

6.1.2 Identification of useful microsatellite markers for genotyping germplasm.

- RM336, RM10655, RM10696, RM10711, RM10713, RM10722, RM10748, RM11, RM253, RM10793, RM10800, RM10825, RM10843, RM10852, RM10864, RM10890, RM1092, RM518, RM312, RM489, RM474, RM259, RM454, RM19, RM5, RM20, RM307 and RM522 were useful in diversity studies and were able to separate the germplasm.

6.1.3 Determination of the genetic diversity with microsatellite markers and the selection of salt tolerant genotypes.

- The PIC value of a marker which reflects marker allele diversity and frequency among the cultivars was very high for some of the primers. High PIC value of a marker indicates high probability to detect the number of alleles among cultivars. Based on this RM20, RM10864, RM10793, RM10748 and RM10722 were very good primers for this diversity study. These results show that the markers used in this study are revealing and good for genetic diversity studies in rice
- Primers RM10711 and RM10793 were very useful in detecting salt tolerance in the rice accessions.
- The studies indicated that the materials used were diverse especially the collection from Ghana

6.1.4 Establishing an elite collection of salt tolerant lines to be used in future breeding works

- Accessions SR1, IR72, Sebota 337-1, Perfume (Short) type, Anyofula, Local Red, GR18Red, GH1580, GH1528, GH1575, NericaL23, NericaL24 and NericaL27 performed well under salinity stress and have been identified to be elite lines among the accessions studied

6.2 RECOMMENDATIONS

The following recommendations were;

- Plant Genetic Resource Institute holds a lot of rice germplasm that are yet to be characterized. More screening work should be done on these materials to have a wide range of germplasm for rice improvement programs.
- The germplasm used in this study should be subjected to full crop cycle under salinized condition, so that their characteristics will be fully investigated. This will ensure full knowledge on their agronomic attributes as well as their salt tolerance at reproductive stage. GH 1580, GH 1599, Anyofula and GH1571 should be included in MAS in improving salt tolerant commercial rice varieties since they performed very well in the current study.
- Primers RM10711 and RM10793 were very useful in detecting salt tolerance in the rice accession, therefore they should be used in marker assisted breeding for salinity tolerance in rice.
- Accessions GH1545, GH1585, GH1599 and GH1571 should be subjected to further studies with other saltol markers because GH1545 and GH1585 were tolerant with the markers used in this study but phenotypically susceptible. GH1571 and Gh1599 was also susceptible with the markers used in this study but phenotypically tolerant.

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APPENDIX**Appendix 1. Analysis of variance of seedling plant height**

Analysis of variance

Variate: Plant_Height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	99.188	49.594	40.30	
Rep.*Units* stratum					
Variety	35	3732.802	106.651	86.66	<.001
Treatment	1	6422.423	6422.423	5218.30	<.001
Variety.Treatment	35	692.480	19.785	16.08	<.001
Residual	142	174.767	1.231		
Total	215	11121.660			

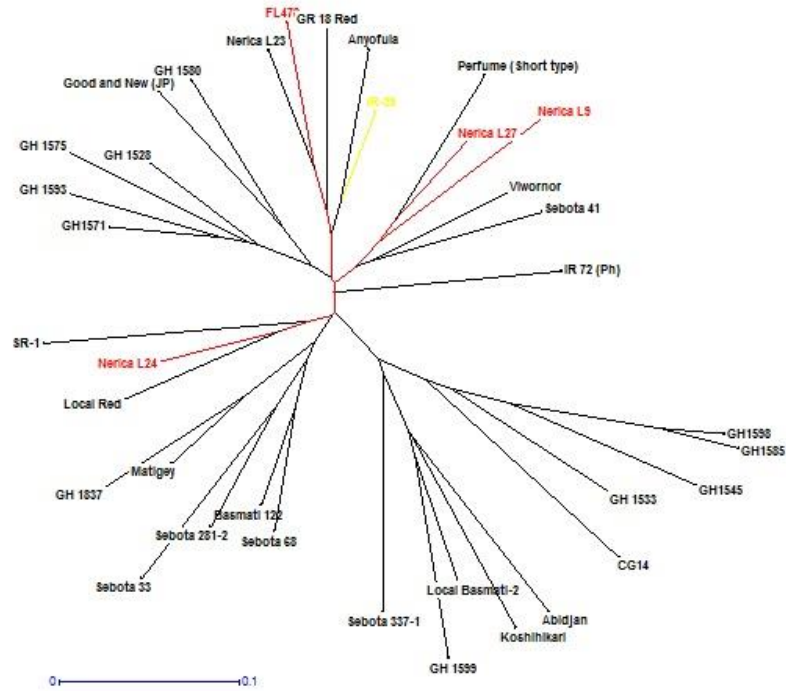
Appendix 2. Analysis of variance of seedling dry weight

Analysis of variance

Variate: Dry_Matter

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	7.6614	3.8307	4.83	
Rep.*Units* stratum					
Variety	35	31.0241	0.8864	1.12	0.318
Treatment	1	22.0650	22.0650	27.81	<.001
Variety.Treatment	35	32.4864	0.9282	1.17	0.258
Residual	142	112.6665	0.7934		
Total	215	205.9035			

Appendix 3. Tree diagram generated from 28 SSR markers



Appendix 4. Samples of germplasm in Styrofoam floats

