GENETIC STUDIES ON SALT TOLERANCE IN RICE (*O. sativa*) USING CONVENTIONAL AND MOLECULAR METHODS

By

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DECLARATION

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

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ABSTRACT

Niger's rice production has not been able to match growth in demand. The slow growth in domestic rice production has been attributed to salinity, non-adapted germplasm, and low farmer adoption of improved varieties. This study was carried out to: determine farmers' perception on influence of salinity in rice production and their preference for rice varieties in salt affected areas; identify potential sources of genes for salt tolerance from local and exotic rice germplasm; determine genetic control of salt tolerance in rice; and evaluate yield performance of early segregating generations of rice from crossing diverse parental genotypes.

Participatory rural appraisal (PRA) was conducted in three regions of Niger involving 197 farmers from 11 villages. The results showed that rice is the most preferred crop. Farmers identified lack of fertilizer, diseases, salinity, and lack of good varieties as the main production constraints. Ninety (90) percent of the farmers were aware of salinity problem and use manure and/or ashes, straw, and the avoidance of urea as coping strategies. The farmers preferred high yielding rice varieties with tolerance to diseases and salinity, medium height and high tillering ability.

Laboratory analyses were carried out on soils from irrigated and non-irrigated fields as well as irrigation water. Most of the irrigated rice fields were clayey (40 to 50% clay). The non-irrigated ones were mostly sandy (50 to 90% of sand). The irrigated soils were characterized by pH which varied from 3.2 to 6.8, an electrical conductivity (EC) above 4, a sodium adsorption ratio (SAR) below 13 and an exchangeable sodium percentage (ESP) below 15. The water analysis indicated the sodium adsorption ration (SAR), the potassium adsorption ration (PAR) the total dissolved solids (TDS), and sodium content of irrigation water varied from site to site. The total sodium

quantity estimated to be deposited per hectare per year varied from 87 kg/ha/year to 218 kg/ha/year.

Fifteen exotic and five local varieties were evaluated in a greenhouse under four salt levels and a control without salt. Significant genotype by salt concentration effect was observed. Significant variability among varieties (probability level of 0.001) across and within salt levels was present for all the traits measured. Four salt tolerant genotypes with high selection index were identified.

One hundred and twenty F_3 families derived from a diallel cross of 4 parents were evaluated in salt affected farmer's fields in two sites. High heritability was observed for almost all the traits. Additive effects for tiller number, panicle number, and panicle weight, additive maternal effects, and partial dominance effects for height and duration were observed. Yield potential varied significantly (Probability level of 0.001) among F_3 families ranging from 2.52 to 4.17 t/ha. Correlation analysis among traits showed that yield was significantly and positively associated with height, tiller and panicle number, and panicle weight.

Leaf samples of the four parents used in the diallel were genotyped by LGC genomics, (United Kingdom) using 1896 SNPs. The results indicated that all the parents were homozygote and genetically differed from each other in terms of substitution pattern, sequences disparity and divergence and base composition. Two hundred SNPs were polymorphic and were selected for early generations screening for salt tolerance.

DEDICATION

To our lovely sons Ismael and Ishaaq

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LIST OF ABBREVIATIONS

EC: Electrical conductivity

EPS: exchangeable sodium percentage

FAO: Food and Agriculture Organization of the United Nations

IRRI: International Rice Research Institute

K+: Potassium ion

NaHCO3: Sodium carbonate

NaCl: Sodium chloride

ONAHA: National office for irrigated schemes management

PRA: Participatory rural appraisal

PAR: Potassium adsorption ration

QTL: Quantitative traits loci

SAR: Sodium adsorption ration

TDS: Total dissolved solids

WACCI: West Africa Centre for Crop Improvement

CHAPTER ONE

1.0. GENERAL INTRODUCTION

Rice (*Oryza sativa L.)* has been cultivated as a major crop for over 10,000 years, and it sustains nearly one-half of the world population (Joseph *et al.*, 2010). Rice is the third most cultivated cereal after millet and sorghum in Niger. Niger has a large suitable area (about 270 000 Ha) for rice growing meanwhile only one fifth of this area is exploited. However, rice has passed millet and sorghum in terms of consumption of urban households. Rice is grown in the valley of Niger River and on the edges of Komandougou. Three major types of rice cultivation occur in Niger, namely i) traditional rice growing under flooding in the Niger River edge and pond; ii) Small private rice growing; and iii) rice growing on irrigated perimeter with total control of water.

Rice production in Niger is limited by factors such as low soil fertility, weeds, diseases, extreme temperatures and salinity (Sido, 2010). Salinity is a major problem limiting rice production in West Africa, particularly within the Sahel where rainfed rice production is not feasible (Manneh *et al.*, 2007). It is the most serious abiotic constraint of rice production in Niger. Up to 20% of irrigated fields are affected by salt problems (Guero, 2000).

Salinity has a negative impact on a number of yield components of rice including stand establishment; panicles, tillers and spikelets per plant; floret sterility; individual grain size; and even delayed heading. Maas and Grattan (1999) have estimated that rice yields decrease by 12 % for every unit (dsm^{-1}) increase in EC above 3.0 dSm^{-1}. This accounts for drastic yield losses and hence loss of income for rice farmers (Gregorio *et al.*, 2002; El-Bably, 2002).

Research to overcome salt related problems is based on two approaches: (i) either change the growing environment to make it suitable for the normal growth of plants or (ii) select the crop and/or change genetic architecture of the plant so that it could be grown in such problem areas. The first approach involves major engineering and soil amelioration process which need lot of resources which are often out of the reach of small and marginal farmers. The second approach involves breeding crop varieties with in-built salt tolerance is realized as the most promising, less resource consuming and socially acceptable. Improvement of salt stress tolerance in rice varieties would increase grain yield of rice under slight salt-stress condition, and may also extend rice growing to regions with moderate salt content in soils (Ming-Zhe *et al.*, 2005).

The varietal development program for salt tolerance in rice has progressed very slowly worldwide (Singh *et al.*, 2010a). This has been attributed to: high genotype and environment interaction effect associated with rice, a lack of precise and repeatable phenotyping facilities and limited knowledge on mechanism for salt tolerance.

Crosses made involving some salt-tolerant landraces or their selections before 1970s have led to development of varieties. However, the resulting varieties were not accepted by farmers at large, because of their insufficient level of salt tolerance and/or poor grain quality.

In the mid-1970s, attempts were made to transfer the salinity tolerance from highly tolerant traditional varieties such as ‘Pokkali’ and ‘Nona Bokra’ to an improved genetic background but the recombinants generated were either not equally tolerant or carried many linked undesirable traits from donors. However, with the development of better screening techniques in the late 1980s, many salt tolerant rice varieties in improved backgrounds were developed in different countries (Gregorio, 2002; Gregorio *et al.*, 2003). The first high-yielding, salt-tolerant, and early maturing rice variety; CSR10 was developed in India and was released in 1989 for sodic and

inland saline soils (Mishra *et al.*, 1992). Other varieties with high salt tolerance and good agronomic characteristics have been released in the Philippines, India, Bangladesh, Vietnam and Egypt (Singh *et al.*, 2010a).

Nevertheless, no studies have been conducted in Niger to breed rice for tolerance to salinity. The development of improved salt-tolerant materials would directly benefit farmers in salt-affected lands by increasing their harvest. Rice production would also expand to include marginal lands which have salinity problems. Furthermore, rice production would be increased in all the rice fields along the banks the Niger river that are especially affected by salinization processes because of excessive irrigation over decades under strong evaporative conditions. Ninety percent (90%) of rice produced in Niger is under irrigation. Unfortunately, irrigation is strongly linked with salinization (Ghassemi *et al.*, 1995). Thus, the primary value of developing salt tolerance rice would be an impetus to expansion of irrigated rice production and sustainability.

The strategies adopted for this work were: i) Implication of farmer upstream of the breeding programme. Farmers need to become a part of the varietal development process in order to consider their preferences, which are crucial for the adoption of new varieties. Based on this concept, participatory rural Appraisal (PRA) was conducted in the rice production area. ii) Germplasm collection, screening and selection of parental genotypes under salt conditions, iii) creation of genetic variability through hybridization of farmer's preferred varieties and exotic salt tolerant, iv) the ensued generations were advanced to F_3 that were evaluated on farmer's fields under salt conditions for genetic studies and agronomic performances. The most performed F3 families were selected and will be advanced for line fixation.

The broad objective of the study was to appreciate farmer's concern of the problem of salinity and to integrate their preferences of rice varieties to develop rice varieties that combine high tolerance to salinity with good agronomic characteristics.

The specific objectives were to:

i. determine farmers' perception on influence of salinity in rice production and their preference for rice varieties.
ii. identify potential sources of genes for salt tolerance from local and exotic rice germplasm.
iii. determine genetic control of salt tolerance in rice.
iv. evaluate yield performance of early segregating generations of rice.
v. determine molecular characteristics of parental genotypes used in the crossing.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. Taxonomy and origin of rice

Chang (1976) classified rice as follows

ORDER: Poales

FAMILY: Gramineae

TRIBE: Oryzeae

GENUS: Oryza

SPECIES:

- *Oryza glaberrima*
- *Oryza sativa*

There are 22 wild species of genus Oryza. Nine of the wild species are tetraploid. The remaining wild species and the two cultivated are diploid (Vaughan, 1994; Subudhi *et al.*, 2006). *O. sativa* is the most widely grown of the two cultivated species. *O. glaberrima* however, is grown solely in West African countries (Linares, 2002). It can be distinguished from Oryza sativa because of its short, roundish, tough ligules and the small number of secondary branches on its panicles (Morishima, 1984). *O. sativa* has a relatively small (430 million base pairs) diploid genome (2n = 24). This is the smallest genome of all crops and approximately 50% of the genome is composed of repetitive sequences (Chang, 2003).

The common rice, *Oryza sativa*, and the African rice *Oryza glaberima*, are thought to be examples of parallel evolution in crop plants (Khush, 1997). Asian rice (Oryza sativa L.) was domesticated around 10,000 years ago from the wild annual *O. rufipogon*. It includes two main

subspecies: *indica* (adapted to tropical and subtropical floating, lowland and irrigated agrosystems) and *japonica* (adapted to temperate and tropical upland ecosystems) (Chang, 1984; Khush, 1997). In a parallel evolution path, *O. glaberrima* was domesticated from annual *O. breviligulata*, which in turn evolved from perennial *O. longistaminata* (Khush, 1997).

2.2. Ecology of rice

The rice plant is an annual grass with round, hollow, jointed culms, flat leaves, and a terminal panicle. It is the only cultivated cereal plant adapted to growing in both flooded and non-flooded soils. Rice is grown under a wide range of climatic and geographical conditions on all five continents (Toriyama *et al.*, 2005). Rice is grown in widely diverse production environments. Five major rice growing environments can be broadly identified based on water regime: irrigated, rainfed lowland, tidal wetland, deepwater, and upland (Khush, 1984).

2.3. Characteristics of salt affected soils

Salinity refers to the increase in the soil surface of dissolved salts, mostly sodium chloride or common table salt, but calcium, magnesium, sulphates and bicarbonates are also implicated in soil contamination by salts (Mba *et al.*, 2007). Oldeman *et al.* (1991) had estimated that 19.5 percent of irrigated land was salt-affected soils and that 2.1 percent of dryland agriculture, was salt-affected soils. The estimate of Flowers (2004) puts the figure of salt polluted soils at 9×10^8ha. All estimates indicate a disturbing trend for this major constraint to irrigated and dry land agriculture. A distinction can be made between primary salinization that involves salt accumulation through natural processes and secondary salinization processes caused by human interventions such as inappropriate irrigation practices (Gergely, 2012). There are 2 main categories of salt affected lands, the saline, sodic (alkali). This classification is based on the

electrical conductivity (EC); soil pH; and exchangeable sodium percentage or sodium adsorption ratio (SAR) (Lamond and Whitney, 1992; Flowers and Flowers, 2005).

2.3.1. Saline soils

Saline soils are dominated by neutral soluble salts consisting of chlorides and sulphates of sodium, calcium and magnesium. The pH of saturated soil paste is less than 8.2 and an electrical conductivity (EC) of the saturated soil extract of more than 4 dS m^{-1} at 25 °C. Sodium is generally the dominant soluble cation and the soil solution also contains appreciable quantities of divalent cations. In the presence of excess neutral soluble salts the clay fraction is flocculated and the soils have a stable structure. The permeability of soils to water and air and other physical characteristics are generally comparable to normal soils (FAO, 1988). Saline soils tend to dominate in arid and semi-arid regions.

2.3.2. Sodic soils

Sodic soils tend to dominate in semi-arid and sub-humid regions. These soils contain sodium salts capable of alkaline hydrolysis, mainly Na_2CO_3. The pH of the saturated soil paste is more than 8.2. The exchangeable sodium percentage (ESP) is about 15 or more. The electrical conductivity of the saturated soil extract is generally less than 4 dSm^{-1} at 25°C. Sodium is the dominant soluble cation and High pH of the soils results in precipitation of soluble Ca and Mg such that their concentration in the soil solution is very low (FAO, 1988). High sodium concentrations in soil generally cause soil dispersion. The later causes soil pore blockage resulting in the reduction of soil permeability (Frenkel *et al.*, 1978; Van De Graaff and Patterson, 2001; Pearson, 2004; Allotey *et al.*, 2008).

2.4. Effects of soil salinity on plant growth

Most crops are adversely affected by salinity. In general, cereals are more tolerant than legumes (Reynolds *et al.*, 2005). Many wild relatives of crop plants show greater tolerance than their domesticated descendents (Blum, 2004). Salt has both osmotic and salt-specific effects on plants, (Munns, 2002; Munns, 2005), impacting at different times (Shannon, 1997). Rapid changes can occur in field salinity (Richards, 1984). The effects of these changes are increased by plants preferentially extracting water from less saline areas of the root zone, by drought in rainfed environments (Srivastava and Jana, 1984), and by water logging in irrigated environments. This can be compounded by additional stresses which vary with time (Gregorio *et al.*, 2003). Among cereals, rice (*Oryza sativa*) is the most sensitive (Munns and Tester, 2008).

The general effect of salinity is to reduce the growth rate resulting in smaller leaves, shorter stature, and sometimes fewer leaves (Munns and Termaat, 1987; Jacoby, 1994). Rajendran *et al* (2009) reported that salinity stress affects crop growth, yield and productivity. The reduction in shoot growth occurs in two phases: a rapid response to the increase in external osmotic pressure, and a slower response due to the accumulation of Na^+ in leaves (Munns and Tester, 2008).

Roots are also reduced in length and mass but may become thinner or thicker. Maturity rate may be delayed or advanced depending on species (Shannon and Grieve, 1999). Salinity effects on plants are complex (Greenway and Munns, 1980). The initial and primary effect of salinity, especially at moderate salinity concentrations, is due to its osmotic effects (Munns and Termaat, 1986). At the whole plant level, ion concentrations in plant tissues increase as a result of salinity stress. The measurable or visible effects of salinity on plants can include reduced growth rate, damage of meristems in growing shoots, reductions in yield components, or typical symptoms of nutritional disorders under osmotic and ionic stress. Grain yield reduction of rice under stress of

root zone salinity can be caused by injuries at both seedling and maturity stages. In most commonly cultivated rice cultivars, young seedlings were very sensitive to root zone salinity (Zeng and Shannon, 2000a).

The degree to which growth is reduced by salinity differs greatly with species and to a lesser extent with varieties within a species. The severity of salinity response is also mediated by environmental interactions such as relative humidity, temperature, radiation and air pollution (Shannon *et al.*, 1994).

2.5. Salt-affected soils management for rice Production

2.5.1. Physical and hydro-technical amelioration

Physical amelioration means giving mechanical treatment to improve physical nature of saline and alkaline soils. This involves deep plowing, sub-soiling, sand filling and profile turning. These treatments improve permeability of the soil to water by loosening and breaking the hard clay pan found in subsoil of alkaline soils (FAO, 1988). After the suitable mechanical treatment of the soil, leaching and drainage with good quality water are carried out.

2.5.2. Biological amelioration

All the growing plants and their residues or any other organic residues have ameliorative effect on saline and alkaline soils. Roots of growing plants intercept ascending seepage water, thus, prevent the upward movement of salts. Shading effect of plant foliage reduces evaporation from the soil surface and, therefore, discourages upward movement of salty water from deep soil layers. Dead plant and animal residues undergo the process of decomposition and carbon dioxide

(CO2) is released. This CO2 dissolves in soil water and forms an acid that counter- act salinity and alkalinity of the soil and push the soil reaction towards neutrality (FAO, 1988).

2.5.3. Chemical amelioration

For alkali soils in which sodium swarms around each and every soil particle, it is necessary to flush out sodium from soil particles to soil solution before leaching the soil. Application of a soluble calcium compound such as gypsum or any other acidifier like pyrite and sulphur must be made before hydro-technical treatment of soil (FAO, 1988).

2.6. Factors affecting salt tolerance of rice

2.6.1. Agronomic factors

Many agronomic factors besides genetic potential affect the salt tolerance of plants. These include water regime, water quality, fertility level, land leveling, sowing/transplanting methods, crop rotation and ontogenic drift. The latter is the change in genotypic expression with plant development. Rice is considered as a salt-sensitive crop, with a salinity threshold of as low as 3 dSm^{-1} (Maas and Hoffman, 1977). But this sensitivity varies with plant growth stage (Makihara *et al.*, 1999; Rao *et al.*, 2008; Singh *et al.*, 2010b).

2.6.2. Climatic Factors

Temperature and relative humidity are the most important climatic factors affecting salt tolerance. Temperature regime greatly influences the growth duration and growth pattern of almost all crop plants under normal soil conditions. Crop plants have critical temperatures for different stages. Temperatures higher than the critical temperature greatly affect plant growth,

especially under salt stress (Singh *et al.*, 2005). Therefore, a tolerant plant under a normal temperature regime may behave as sensitive under a hot and dry environment. The relative humidity, on the other hand, also plays an important role under salt stress. Dry weather and very low humidity increase the evapo-transpiration rate of plants while the reverse is true with high humidity. Similar to the conditions under high temperature, very low humidity in a stress environment is detrimental to plant growth because of higher ion uptake (Singh *et al.*, 2005).

2.6.3. Rainfall and soil physical properties

Soil texture and structure greatly affect the expression of plant traits. A genotype can behave differently with its inherent salt tolerance in different soil textural classes at a particular stress level. Heavy clay soils impose more stress on a plant than sandy soils (Singh *et al.*, 2010b).

A good rainfall lowers salt stress in the soil to a certain extent. Soluble salts are washed out in saline soils and sodic soils and stress also become less severe due to the dilution effect from good quality rainwater. A crop such as rice that favors water stagnation is benefited most by a well distributed rainfall pattern (Singh *et al.*, 2010b).

2.7. Participatory research technology development and transfer

Crop management research is increasingly involving farmers in evaluating new technologies, identifying adoption constraints and opportunities for improving farm performance to produce more sustainable impact (Rusike *et al.*, 2006). The term "participatory research" is a collection of approaches that enable participants to develop their own understanding of and control over the processes and events being investigated (Ashby, 1997). But the basic idea of participatory research is that farmers and professional researchers have different knowledge and skills, which may complement each other and that by working together the two groups, may achieve better

results than by working alone. Ideally, the strengths of one group would compensate for any constraints and limitations of the other group (Hoffmann *et al.*, 2007).

The process of farmer participatory research requires scientists to welcome farmers as partners in developing new technologies. In that way, these methods bring together two sorts of "experts" into the research process. One group of experts is the scientists, whose knowledge grounded in empirical experimental research can contribute new technical procedures for growing and managing crops. The other group of experts is the farmers who have an intimate knowledge of the 'day-to-day' conditions under which the technologies will be used (Stephen, 2008).

Traditional approaches aimed at adoption of integrated plant nutrient systems and integrated pest management have fallen short of anticipated outcomes in respect of plant nutrition and plant health. Furthermore, intended beneficiaries have been passive recipients of the prescribed technological packages (FAO, 2006). Several farmer participatory methods are commonly applied in research projects. These are:

2.7.1. Participatory rural appraisal (PRA)

This is an exercise to be conducted early in a project for purposes of characterizing a village's natural social and economic environment where on-farm experiments will occur. PRA involves focus group discussions, key informant interviews, and the collection of secondary data, in order to describe the farming system, existing indigenous and new technologies, production problems, and social/poverty categories, as articulated by the farmers. Researchers use this information for designing interventions appropriate for the local context. PRA methodologies facilitate the capture of the perspectives of farmers and other key actors to inform the management of soil and plant nutrients, as well as plant health. PRA provides scope for all involved to learn from each

other and stimulates self-propelled initiatives. This methodology permits the application of a set of tools and techniques that allow for the transformation of knowledge and shared experiences into actions that are oriented towards economically justifiable, socially acceptable and environmentally sound production system(s) (FAO, 2006). As technologies are developed under the controlled experimental conditions of a research station, there is a need to understand the actual circumstances under which farmers would use them. The variable environments of soil types, weather, and stresses that farmers confront, and the distribution of labor in the household farming system, may not be easily replicated on the station (Chambers, 1997).

2.7.2. Participatory varietal selection

This is a process by which farmers evaluate new crop varieties/lines under on-farm conditions. Participatory varietal selection (PVS) involves an initial round of researcher-managed on-farm "mother trials," from which farmers choose preferable materials, which are then evaluated in farmer-managed "baby trials," to give them actual experience in testing new germplasm. In both trials, visiting farmers vote their preferences, which is followed up with a group discussion to generate their criteria for their selections (Chambers, 1997).

2.7.3. Participatory research and varietal development in rice

Scientists work with farmers to develop new crop management practices that may require households to considerably modify labor allocation, input levels, and the timing of seasonal activities. On-farm experiments are challenging because farmers do not observe the immediate results of the technology, as in the case of adopting new varieties. Furthermore, new "off-the-shelf" management practices may have to be modified considerably before farmers achieve the intended benefits of labor and cost savings (Chambers, 1997).

2.8. Screening methodologies for salt tolerance in rice

Several methods were used for rice screening. These are:

Field screening: It is the most ideal method for identifying adapted and tolerant genotypes because salt tolerance is a complex phenomenon. However, spatial variability (Singh *et al.*, 2010a) and Rapid changes (Richards, 1984) in the field makes escape possible. Hence, field screening becomes less reliable (Singh *et al.*, 2010a) because of stress heterogeneity, the presence of other soil-related stresses and the significant influence of climatic factors (Xie *et al.*, 2001; Gregorio *et al.*, 2002).

Screening in Microplots: This method is used to screen mostly early segregating populations and also stabilized populations in addition to genetic studies (Singh *et al.*, 2010a).

Salinity Screening in Solution Culture: The solution culture technique is used in two ways: first, for screening up to seedling stage; second, for screening up to maturity.

Screening in Trays: This method is employed for large-scale screening of varieties at germination/seedling stage (Singh *et al.*, 2010a).

Screening in Pots: For more precise studies of individual plant response under constant stress, round porcelain or plastic pots of 20–30 cm diameter, with a capacity of 4 or 16 kg soil and a provision to allow or prevent leaching from the bottom portion, are used. Genetic and physiological studies on salt tolerance that require precision are mostly done in pots (Singh *et al.*, 2010a).

2.9. Variance components and heritability estimates in rice

2.9.1. Means, variances and correlations for quantitative traits

The variation among genotypic values in a breeding population is the genotypic variance (σ_G^2). Genetic variance is the sum of the additive, dominance and epistatic genetic variance components: $\sigma_G^2 = \sigma_A^2 + \sigma_D^2 + \sigma_I^2$ (Hallauer and Miranda Fo, 1988). The breeding value of one individual assesses its usefulness in selection. It is determined by the mean of its progeny and is associated with additive effects. Breeding value in one individual is twice the mean deviation of its out crossed progeny from the population mean, which is equal to the sum of the average effects of the alleles it carries. The variation among breeding values is attributed to the additive effects of genes and is called additive genetic variance (δ^2_A). Dominance deviation, D, is the difference between the genotypic value (G) and the breeding value (A) for a given genotype in the absence of epistasis. The dominance deviations are due to within locus interaction between the different alleles. Variation among genotypes for dominance deviations is the dominance genetic variance (σ_D^2). The variation associated with differences among genotypes for epistatic interactions is the epistatic variance (σ_I^2).

2.9.2. The environment and its interaction with genotypes in plant breeding

The environment affects the expression of quantitative traits, and different environments can affect genotypes differently. Phenotypic values are classically divided into genotypic (G), environmental (E) and genotype × environmental interaction (G×E) effects: P = G + E + G×E. Likewise, phenotypic variance is divided into genotypic, environmental and G×E variance components.

2.9.3. Concept of Heritability

Heritability is the relative importance of genetic and non-genetic factors in the expression of phenotypic differences among genotypes in a population (Fehr, 1988). Heritability is used to estimate expected response to selection and to choose the best breeding approach to improve the target trait(s) (Betrán *et al.*, 2009). Heritability is responsible for the resemblance among relatives due to the transmission of traits from parents to offspring and also for accuracy of the prediction of the genetic gain from the selection. However, heritability estimates are always based on the target population in both space and time, and the environment where it is estimated (Singh *et al.*, 2010b). There are two basic types of heritability: broad-sense heritability and narrow-sense heritability (Nyquist, 1991; Holland *et al.*, 2003).

Heritability in the broad sense (H) is the proportion of the phenotypic variance of family means that is due to all genetic effects (Falconer and Mackay, 1996; Holland *et al.*, 2003).

$H = \sigma_G^2 / \sigma_P^2$.

Broad-sense heritability can be estimated from standard analysis of variances (Betrán *et al.*, 2009).

Heritability in the narrow sense (h^2) is the proportion of phenotypic variance among individuals in a population that is due to heritable genetic effects (Nyquist, 1991; Holland *et al.*, 2003).

$h^2 = \sigma_A^2 / \sigma_P^2$

Narrow sense heritability can be estimated from variance components or from parent-off-spring regression (Betrán *et al.*, 2009).

Heritability of a trait can be estimated using the amount of genetic gain that is realized by selection within a population (Falconer and Mackay, 1996). This is known as realized heritability and can be estimates a posteriori as: h^2=R/S, where R = response to selection and S = effective selection differential applied in selection.

2.9.4. Response to selection

The theoretical response to selection can be defined as $\Delta G = Sh^2$, where S is the selection differential (the difference between the mean of the selected individuals and the mean of the whole population) and h^2 the heritability of the target trait(s). S is determined by the intensity of selection (i), which is the number of genotypes selected relative to the total number under evaluation. Intensity of selection is the standardized selection differential:

$$i = \frac{S}{\sigma P}$$

Where σ_P is the square root of the phenotypic variance.

2.10. Genetics of salt tolerance in rice

The first reports on inheritance of salt tolerance came from Akbar and Yabuno (1972; 1975) in which it was inferred that inheritance of panicle sterility under salinity stress is controlled by a small number of dominant genes, but their studies were not extended to later segregating generations. Akbar and Yabuno (1977) reported that rice sterility in saline conditions, is determined by at least three genes. Most inheritance studies indicated a normal distribution of the trait in different populations, indicating its polygenic inheritance. Moeljopawiro and Ikehashi (1981) did one of the earliest studies in rice using two crosses between two moderately tolerant parents and between two tolerant parents rather than the contrasting parents. They found a low

genetic response to selection and a high degree of environmental fluctuations. Shannon (1985) suggested that there is evidence of a genetically complex trait, showing heterosis, dominance and additive effects. In diallel analysis the effects of salinity on the seedling stage and on sterility suggested both additive and dominance effects, some with high heritability (Moeljopawiro and Ikehashi, 1981; Akbar *et al.*, 1985). Another inheritance study for salinity tolerance in rice by Mishra *et al.* (1998) also inferred polygenic inheritance. It was also inferred that the salinity tolerance trait also lacked maternal influence. A similar inheritance study for sodicity tolerance was conducted involving the same populations. Results indicated that sodicity tolerance is also a polygenic trait acting both additively and with interactions between the alleles at some loci (Singh et al., 2001).

Evidence of dominance in salt tolerance is also seen with pigeon pea (*Cajanus cajan)* (Subbarao *et al.*, 1990). There is also evidence of dominance in the salt tolerance of sorghum. Diallel analysis, based on assessing tolerance to NaCl as relative root length in salt-treated as compared with control plants, showed that there were both additive and dominance effects of NaCl (Azhar and Mcneilly, 1988; Ashraf *et al.*, 2001).

2.10.1. Association Studies

Association studies are important for indirect selection for a desired trait(s) using associated traits if phenotyping of the latter is relatively easy. The association could mainly be due to two reasons: either the traits are closely linked or the gene has pleiotropic effects. Salt stress affects most growth parameters; therefore, by selecting the most closely associated trait, a desirable genotype can be selected. An altered effect due to salt stress is much more evident in salt-sensitive genotypes than in tolerant ones. Therefore, traits such as the proportion of

filled/unfilled grains, degree of deviation for grains per panicle, spikelet fertility, plant height, fertile tillers and flowering in comparison to non stress are good indicators for selecting tolerant genotypes (Mishra, 1994; Gupta, 1999).

2.10.2. Gene Action and Heritability

According to Lin *et al*. (2004) and Hu *et al*. (2012), salt tolerance of rice is the genetics of quantitative characters, which is controlled by multiple genes, with the additive and dominant effects, the former playing a major role (Moeljopawiro and Ikehashi, 1981; Gregorio and Senadhira, 1993; Gu *et al*., 1999). Akbar *et al.* (1985) reported that the dry matter weight of rice seedling under salt stress was affected by at least two groups of genes with additive effect, and no epistatic effect was detected. Thi Lang et *al*. (2010) reported that a dominant gene controls resistance to salt stress in the allelism test. Gregorio and Senadhira (1993) observed that there were two groups of genes involved in the sodium and potassium uptake in rice, one group for sodium exclusion and the other for potassium absorption.

2.11. Breeding Methodology for tolerance to salinity in rice

2.11.1. Conventional Approaches

2.11.1.1. Mass selection

This method selects and bulks a few hundred to a few thousand superior plants on the basis of phenotype. It is used to improve old local varieties or purify existing varieties. Varieties are maintained through mass selection. Only those varieties that show genetic variation can be improved through mass selection (IRRI, 2007).

2.11.1.2. Pureline selection

Pureline is the progeny of a single, homozygous, self pollinated plant. With this method you select a large number of plants whose individual progenies are tested. The best progeny is then released as a variety. It is used to develop a variety from local selections, introductions and old pureline varieties (IRRI, 2007).

2.11.1.3. Pedigree Method

The pedigree method has been the most widely used and successful in rice improvement. The method requires much time to periodically evaluate lines throughout the growing season and to keep records on which selection at maturity is based (Jennings *et al.*, 1979).This is a classical method in which the lineage of the plant selection in the segregating generation is maintained until it is stabilized in the F7 or F8 generation. But, due to cumbersome procedures and the involvement of more resources, breeders are modifying this method and not adhering to it strictly (Singh *et al.*, 2010b).

2.11.1.4. Bulk Pedigree Method

In the case of self-pollinated crops this method seems particularly suitable to breeding for resistance to abiotic stresses (Grando and Ceccarelli, 2009). It has also proved to be ideal for use in participatory breeding programmes with self-pollinated crops (Ceccarelli and Grando, 2007). After producing the F1 and the F2 on station, three years of multi-location yield testing and selection of the bulks are carried out in the target environment(s). Selection is done between bulks by identifying the best populations for either yield or other characters. In parallel with the field testing of the bulks, a within-bulks selection is conducted only in those bulks that are selected for the next level of field testing. The families deriving from the populations that

maintained their superiority for three cropping seasons will enter yield testing (Grando and Ceccarelli, 2009).

The method is based on the basic assumptions that (i) a superior bulk is made by a large number of superior genotypes, and (ii) that if the superiority is maintained for a period of three cropping seasons in a highly variable environment; the probability is small that the superiority is associated with heterosis (Grando and Ceccarelli, 2009). The method is also based on the exploitation of the genetic variance between populations (Vb) because estimates of Vb are comparatively easy and economical to obtain, while estimates of within-population variance (Vw) are more expensive and much less precise because of interaction and competitive effects (Simmonds, 1991).

2.11.1.5. Modified Bulk Pedigree Method

A combination of pedigree and bulk breeding methods, this is almost as effective as the pedigree method, with relatively less use of resources. It has flexibility and is useful for less heritable traits, with the individual F2 plants harvested in bulk up to the F4 or F5 generations, followed by panicle selection and handling of the population as in the pedigree method. However, for highly heritable traits, the individual plants are selected in the earlier generations (F2 or F3), followed by bulking for a few generations and ultimately single-plant or panicle selection in the F5 or F6 generation (Singh *et al.*, 2010b).

2.11.1.6. Shuttle Breeding

Since most abiotic stresses are very location specific, the varieties developed need to fulfill specific plant-type requirements. In the shuttle breeding approach, pre-breeding or advanced breeding materials are evaluated at different locations for their adaptability and the best adapted

materials are again crossed and evaluated at different target sites in replicated trials. The latter step functions like the multi-location testing of advanced breeding materials (Mishra, 1994; Singh *et al.*, 2010b).

2.11.1.7. Backcross method

The F1 generation and the progenies in the subsequent generations are repeatedly backcrossed to one of the original parents used in the cross. The objective of backcrosses method is to improve one or two specific defects of a high yielding variety. The backcross method has not been used extensively because of a lack of suitable recurrent parents (Khush, 1978).

2.11.2. Non-conventional Approaches

2.11.2.1. F1 Anther Culture Technique

F1 anther culture has the twin advantages of increasing speed and improving breeding efficiency. Thus, it has become an effective tool to attain homozygosity of recombinants within the shortest possible time (Singh *et al.*, 2010b). Following a conventional cross, it takes a minimum of four to five years before complete or 100% homozygosity is reached, as only two generations can usually be advanced in a single year. The use of anther culture overcomes this problem by regenerating F1 pollen into homozygous plants (Singh *et al.*, 1992; Singh and Mishra, 1995).

2.11.2.2. Marker assisted selection in rice breeding

The development of gene identification technologies using the tools of biotechnology provides ample opportunities for scientists to further improve modern cultivars (Jena and Mackill, 2008). Genomics tools and approaches, particularly the QTL mapping and comparative mapping offer new possibilities for crop improvement (Paterson, 2012; Saito *et al.*, 2012). Significant progress

in rice functional genomics is being made since the completion of the international rice genome sequencing project (Chen *et al.*, 2011) which offers tremendous opportunities for breeders to improve this important crop by molecular breeding. Many molecular and genetic analyses have been performed on rice different traits such as: heading date (Izawa *et al.*, 2003; Izawa, 2007; Shibaya *et al.*, 2011), spikelet sterility (Ikehashi and Araki, 1986; Sawamura and Sano, 1996; Zhang and Lu, 1996; Wan *et al.*, 1998; Wang *et al.*, 1998; Yan *et al.*, 2000; Ji *et al.*, 2005; Zhao *et al.*, 2006; Zhang *et al.*, 2011), leaf size (Li *et al.*, 1998; Shen *et al.*, 2003; Kobayashi *et al.*, 2006; Yue *et al.*, 2006; Tong *et al.*, 2007; Farooq *et al.*, 2010) and yield components traits (Li *et al.*, 1998; Erik *et al.*, 2002; Ishimaru, 2003; Thomson *et al.*, 2003; Yoon *et al.*, 2006).

A major QTL (Saltol) derived from the salt-tolerant cultivar Pokkali has been located on chromosome 1. This QTL confers salinity tolerance at the vegetative stage and explains 64 to 80% of the phenotypic variance (Bonilla *et al.*, 2002); it has also been detected in other varieties (Takehisa *et al.*, 2004). A gene for salt tolerance at the vegetative stage has been identified in a similar position in the cultivar Nona Bokra and positionally cloned (Ren *et al.*, 2005).

Markers associated with tolerance for a variety of environmental stresses rank as important targets for molecular MAS in cereal breeding because these complex traits are often prohibitively difficult to screen using classical selection techniques. Efforts to identify QTL associated with tolerance to drought, salt and mineral deficiencies or toxicities (Champoux *et al.*, 1995; Flowers *et al.*, 2000; Gregorio, 2002; Kamoshita *et al.*, 2002; Nguyen *et al.*, 2002; Price *et al.*, 2002) in a number of genetic backgrounds represent an important first step towards achieving this goal. Additional studies have specifically addressed the problems associated with G x G and G x E (Hittalmani *et al.*, 1995; Zheng *et al.*, 2000; Hittalmani *et al.*, 2003; Li *et al.*, 2003). In the area of biotic stress, several genes have been cloned and characterized for resistance to major

diseases such as bacterial blight and blast (Song *et al.*, 1995; Yoshimura *et al.*, 1998; Bryan *et al.*, 2000; Sun *et al.*, 2004) and many other genes for disease resistance have been tagged with linked markers (Valent *et al.*, 2001).

CHAPTER THREE

3.0. RICE PRODUCTION ENVIRONMENT, FARMERS' PERCEPTION OF SALINITY PROBLEM, EFFECTS, AND PREFERRED VARIETIES IN WESTERN AND SOUTH-WEST NIGER

3.1. Introduction

Niger is a landlocked country in the heart of West Africa with a total area of 1,267,000 sq km, but only half of this is habitable due to adverse climatic or soil conditions (Hohenheim, 2000). Agriculture is the mainstay of the country's economy, with most families relying on subsistence farming and livestock breeding for their survival. Millet, sorghum and rice are the main crops (FAO, 2011). Rice production is estimated to be 10% of the total cereal production. Irrigated rice is about 80 to 90% of national rice production (Issaka, 2000). However, the potential for irrigation is yet to be exploited and rice production cannot satisfy the growing demand. Irrigated production schemes are highly criticized for their high investment costs, deterioration of the infrastructure, stagnating yields and the non-competitiveness of local rice (FAO, 2004). The national average yield is decreasing from 3.96 t/ha in 2000 to 1 t/ha in 2012 (Figure 3.1). This led to a decrease of national total production from 78400 t in 2000 to 5,400 t in 2012. Sido reported in (2010) that about 210000T of rice was imported in 2005 to meet the national consumption.

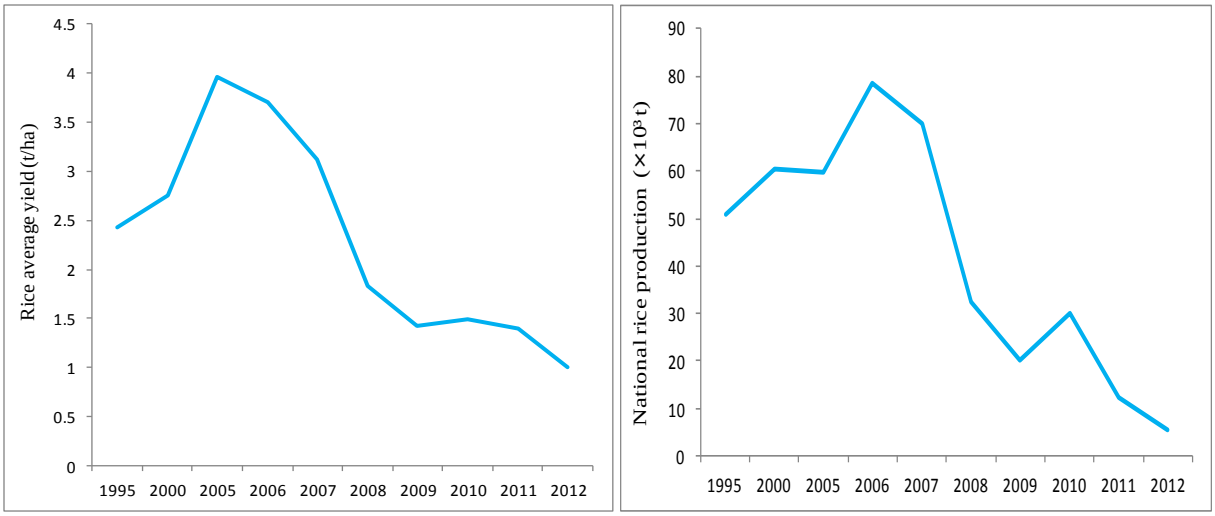

Figure 3.1: The national rice average yield and total production from 1995 to 2012 (source (INS and Mda, 2012)).

Increased production of rice will increase food availability and food security. There is also the attendant increase in employment expected and improvement of livelihood of farmers. Increasing yield will help to significantly reduce importation, and boost national economy.

Information on characteristics of rice production environment and the role of various stakeholders is important to identify factors to be considered in strategies to improve production. Key among these factors include the importance of rice among other crops in the area, rice production constraints, causes of low adoption of improved varieties, causes of abandonment of the adopted improved varieties, and farmer's preferences. Rice in this area is essentially cultivated under irrigation. Irrigation schemes, especially under arid and semiarid environments, are experiencing increasing levels of salt-affected soil because of mismanagement of the soils, irrigation and drainage principles, poorly designed and managed irrigation infrastructures, excessive and irrational use of irrigation water and global climatic change (Kashenge-Killenga, 2010). It is important to know the characteristics of rice production environment, to identify

farmers' needs and preferences and to establish the impact of salinity among other production constraints in rice production area of Niger.

The specific objectives of the study were to:

- ⇨ identify farmer's rice production constraints.
- ⇨ assess the current status of salinity problems.
- ⇨ determine farmers coping strategies with salinity problems.
- ⇨ identify farmer's preferences and selection criteria for rice varieties.
- ⇨ determine the extent of salinity in farmers' fields' soils.
- ⇨ assess the contribution of irrigation to field's salinity.

3.2. Materials and Methods

3.2.1. Study area

Participatory rural appraisal (PRA) was conducted in three regions of western Niger (Figure 3.2), located in the semi-arid zone where rice is cultivated (FAO, 2007). These regions were:

- Gaya: With average maximal annual temperatures of 36°C and annual rainfall of 883 mm. Its geographical coordinates are 11° 53' 16" North, 3° 26' 48" East, with total population of 917, 005 in 2010.
- Niamey: With average maximal annual temperatures of 36.8°C and annual rainfall of 505 mm. The geographical coordinates are 13° 40' 0" North, 1° 47' 0" East and population of 1, 222, 066 in 2010.

- Tillabery: With average maximal annual temperatures of 37.6°C and annual rainfall of 417 mm. Its geographical coordinates are 14° 12' 42" North, 1° 27' 11" East and with population of 2, 500, 454 in 2010 (INS, 2010).

About 97% of national rice production comes from these regions. The PRA was carried out during March 2012. This is a peak period of dry season rice production activities.

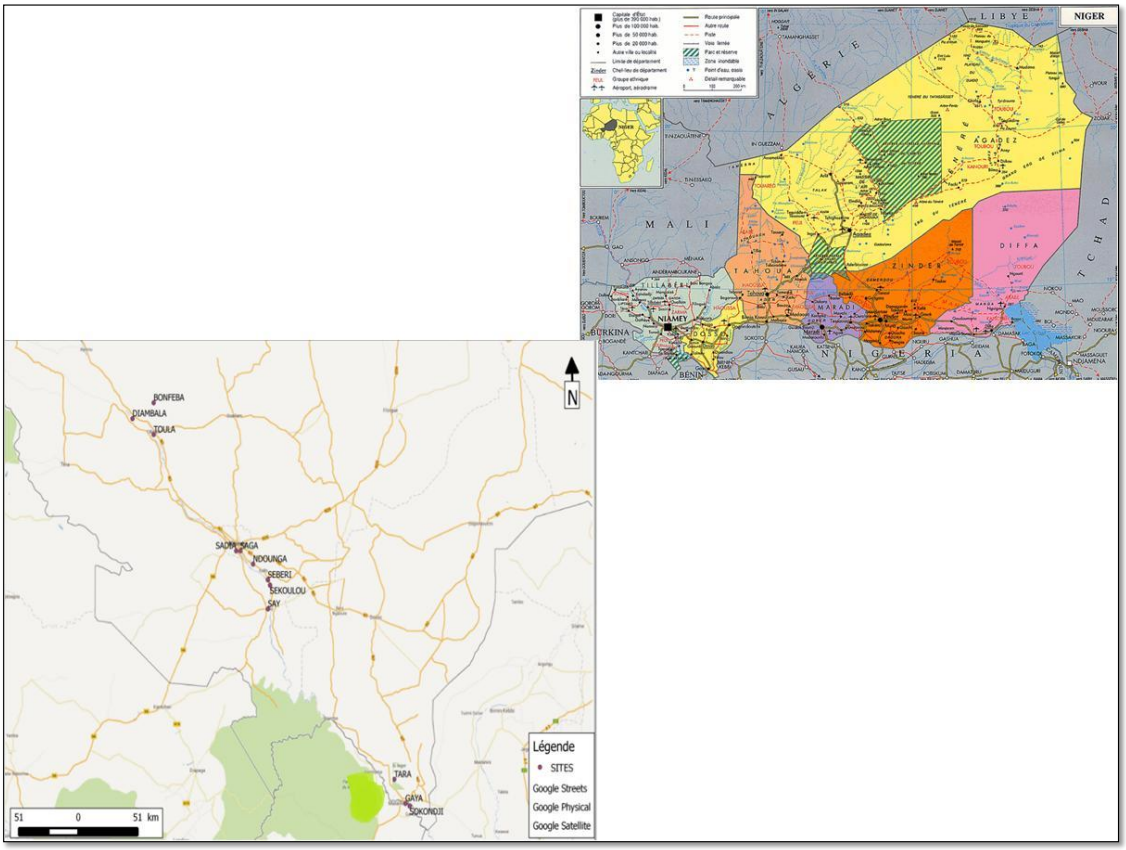

Figure 3.2: Villages used for the PRA.

3.2.2. Sampling procedures

3.2.2.1. Irrigated schemes sampling

A total of 31 irrigated schemes of different ages are present in Niger, some involve several villages. A stratified random sampling was used for this study. The irrigated schemes were

divided into two subgroups composed of 23 and 8 irrigated schemes for the first and second stratum respectively.

- The first subgroup was where irrigation has been practiced equal or more than 30 years. This is equivalent to at least sixty seasons under irrigation (2 seasons per year).
- The second stratum was where the irrigation has been practiced less than 30 years.

Random samples of 30% of the farmers were taken in each of the two strata. This led to a sample size of 11 irrigated schemes (Table 3.1). This includes 3 irrigated schemes less than 30 years and 8 more than (or equal to) 30 years old. The sample size of each stratum in was proportion to the population size of the stratum when viewed against the entire population

Table 3.1: The selected villages for the PRA

Irrigated scheme	Area (Ha)	Number of Farmers	Number of years of growing rice	Number of irrigated seasons
Sokondji Birni	25	100	17	34
Bonféba	327.11	641	20	40
Gaya Amont	187	748	22	44
Djambala	621	1522	30	60
Sébéri	381	1098	32	64
Toula	243	632	37	74
Tara	124	496	37	74
N'Dounga 2	286	1179	39	78
Saadia amont	111	362	39	78
Saga	390.25	1112	46	92
Say I	250	438	67	134

3.2.2.2. Farmer sampling

The head of the irrigated scheme was informed in advance before visit. He was to inform farmers about the meeting's purpose, and to ask them to participate. The work was done with all the participants together in one group, if the number was less than 15, but if the participants' number was beyond this, they were split into two or more groups of eight to twelve farmers. The number of farmers in each village is presented in Table 3.2.

Table 3.2: Number of selected farmers per village

Village	Bonféba	Djambala	Toula	Gaya Amont	Tara	Sokondji Birni	Sébéri	Saga	N'Dounga 2	Sadia Amont	Say1
Number of selected farmers	17	21	19	15	18	15	20	19	17	16	20

3.2.2.3. Soil sampling

Ten farms, each from a different irrigation scheme along the bank of the higher river and whose owners had been used for the PRA were selected for the study. Composite soil samples from the plough depth (20 cm) were taken from each site. Composite soil samples were also taken from similar soils adjacent the sites which have had no history of irrigation. These latter samples served as control. After sampling, the soil samples were air dried ground and passed through a 2 mm sieve to obtain the fine earth fraction. The fine earth fraction was then stored in plastic bags for laboratory analyses.

3.2.2.4. Water sampling

The water from the rivers used for irrigation was sampled by taking into account the two different cropping seasons (dry season and rainy season). The river water is used to irrigate during both seasons and may change in aspect and hence property during the two seasons. Five (5) water samples were taken in dry season: Three (3) from the Tillabery area, and two (2) from Gaya. Two (2) other samples in addition to pipe borne water were collected in Niamey area in the rainy season. Thus, a total of eight (8) samples were taken for laboratory analyses.

The soil and water samples were analyzed in the soil laboratory of INRAN (National Institute of Agronomic Research, Niger).

3.2.3. Data collection and analysis

3.2.3.1. Group Discussions

Group discussions were held to obtain community level information in an informal way at each village. The group of participants was guided by a moderator who introduced topics for discussion and helped the group to participate. The discussions were guided by a semi-structured questionnaire to get information on areas of interest (Table 3.3). Questions were asked when the interviewer felt it was appropriate to ask them. These were prepared questions or those arising during the interview. Farmers were able to talk about topics in detail and depth. The meanings behind actions were revealed as the interviewees were able to speak for themselves with little direction from interviewer.

Preference ranking was used for the determination of the preferences of individual farmers. Farmers were asked to list some of the important traits of the rice varieties they were sowing or

had grown before. Then they were asked to rank these traits and varieties. The preference ranking was used to know the preferred varieties and traits.

In pair wise ranking, each item on a list was compared in a systematic way with each other item (Table 3.4). To construct this table, each problem or trait was compared in turn with each of the other problems or trait. Thus, problem or trait 1 was compared first with problem or trait 2. For instance, if the community saw that one was more important than two, then 1 was placed in the first cell. This was then repeated with all the other traits or problems. The pair-wise method was used to identify the preferred crop and the most important constraint of rice production in the area. The estimation yield loss due to salinity was done by comparing the current production to what they used to produce before the problem. Farmers were asked if this difference can attain less a quarter, a quarter, half or more than half of initial situation.

Individual interviews were made to supplement the findings from the focus group discussion and for in-depth verification of key issues discovered. These were a follow-up to confirm and give more credence to the group findings. They were done with heads of local irrigated schemes. These are men with extensive and useful knowledge in rice production. Other farmers were also interviewed individually to enable them to express their own opinions without any influence from the community.

Table 3.3:Semi-structured interview questionnaire

Information	Techniques used
Information crop production	
Cultivated crops and their importance	Interrogation and iteration
Preferred crops	Pair wise ranking
Reasons of preference	Interrogation and iteration
Information on rice production	
Rice history	Iteration, probing , and cross-check
Period of irrigated scheme rice cultivation	
Evolution of rice yield over years	
Crop management practices	
Information on rice production constraints	
Biotic constraints	Pair wise ranking
Abiotic constraints	
Information on salinity problem	
Awareness about salt problems	Interrogation and iteration
Area of salt affected soils	Interrogation and iteration
Affect of salt on rice plants	Interrogation and iteration
Affect of salt on rice production	Interrogation and iteration
How they overcome salinity problem	Interrogation and iteration
Information on rice varieties	
Varieties that were cultivated	Interrogation and iteration
Varieties that were abandoned	Interrogation and iteration
Origin of the varieties	Interrogation and iteration
Varieties that are being cultivated now	Iteration and field visit
Most preferred varieties	Preference ranking
Information on farmers' preferred traits	
Rice traits	Interrogation and iteration
Preferred traits	Pair wise ranking

Table 3.4: pair-wise ranking techniques

Problem or trait	Problem or trait number						Score	Rank
	1	2	3	4	5	6		
1		1	1	1	5	1	3	2
2			2	2	5	2	4	1
3				3	5	6	1	4
4					5	4	1	4
5						6	4	1
6							2	3

To verify information from farmers' field visits were conducted on farm. These involved three representative fields with salinity and without salinity problem. The Figure 3.3 showed farmer's fields visited after interview.

Figure 3.3: A healthy rice field (left) and a field affected by salinity (right) at Bonféba.

3.2.3.2. Laboratory analyses

The under listed soil and water properties with their respective methods of analysis (Table 3.5) were measured.

Table 3.5: Parameters and methods of analysis

Soil property	Method of analysis
Soil reaction, pH (H_2O)	electrometric, soil: water (1:1)
Electrical conductivity	Electrometric, soil: water (1:1)
Organic carbon	Walkey-Black (1934)
Total Nitrogen (%)	Macro Kjeldhal digestion
Available P	Olsen's method
Exchangeable Bases	Ammonium acetate
Cation exchange capacity	1M ammonium acetate
Calcium carbonate	Bernard (1972) Method
Texture	Hydrometer or Pipette Method
Total dissolved ions (TDS)	Electrometric

Table 3.6: Ions in a sample of water to estimate the TDS

Ion	Symbol	mol/L
Sodium	Na^+	
Calcium	Ca^{++}	
Magnesium	Mg^{++}	
Potassium	K^+	
Chloride	Cl^-	
Sulfate	$SO4^{- -}$	
Bicarbonate	$HCO3^-$	

The water total dissolved salt was estimated using the ions content (Table3.6). The total salt deposited per hectare of irrigated field was obtained by multiplying the salt per liter content (mg/L) by the estimated water volume per ha for each cultivation cycle.

The quantity of water (Qw) per season per hectare was calculated by using the formula:

$$Qw = \frac{(\text{Volume of water pumped} * \text{flow per hour} * \text{Number of hour per day} * \text{irrigation days per season}) - \text{wasted water}}{\text{Area of irrigated scheme}}$$

The wasted water was estimated to be 20% of the total (ONAHA non published doc).

SPSS 16.0 software was used for socio-economic data analyses. GenStat 9.2 and Minitab14 software were also used for statistical analysis. T-test and mean difference confidence interval approach were used for data comparison between irrigated and non-irrigated fields. Excel 2007 was used descriptive analysis and chart drawing.

3.3. Results

3.3.1. PRA results

3.3.1.1. Crops in the study area

Millet, sorghum, rice, cowpea, onion, okra, marrow and tomato were the most cultivated crops in the study area (Table 3.7). Among these crops, rice appeared to be the most preferred followed by millet, sorghum and cowpea. Rice was scored 7 and ranked first when millet and sorghum were scored 6 and 5. These 2 crops occupied the second (for millet) and third places. Cowpea was the fourth crop and was scored 4. Okra, Marrow and tomato were the less preferred crops.

Table 3.7: Ranking of crops cultivated in the area

	Millet	Rice	Sorghum	Cowpea	Okra	Marrow	Onion	Tomato	SCORE	RANK
Millet		Rice	Millet	Millet	Millet	Millet	Millet	Millet	6	2nd
Rice			Rice	Rice	Rice	Rice	Rice	Rice	7	1st
Sorghum				Sorghum	Sorghum	Sorghum	Sorghum	Sorghum	5	3rd
Cowpea					Cowpea	Cowpea	Cowpea	Cowpea	4	4th
Okra						Okra	Onion	Tomato	1	6th
Marrow							Onion	Marrow	1	6th
Onion								Tomato	2	5th
Tomato									1	6th

3.3.1.2. Farmers 'perceptions of rice production constraints

All the farmers recognized that rice yield was decreasing over the years but diverged about the causes and extents. Forty-eight (48) percent of the farmers said that yield was reduced about 25% compared to 20 years ago. For twenty three (23) percent the yield reduction was about 50%. Eight (8) percent of the farmers said that it was more than 50% and twenty one (21) percent said that it was less than 25% (Figure 3.4).

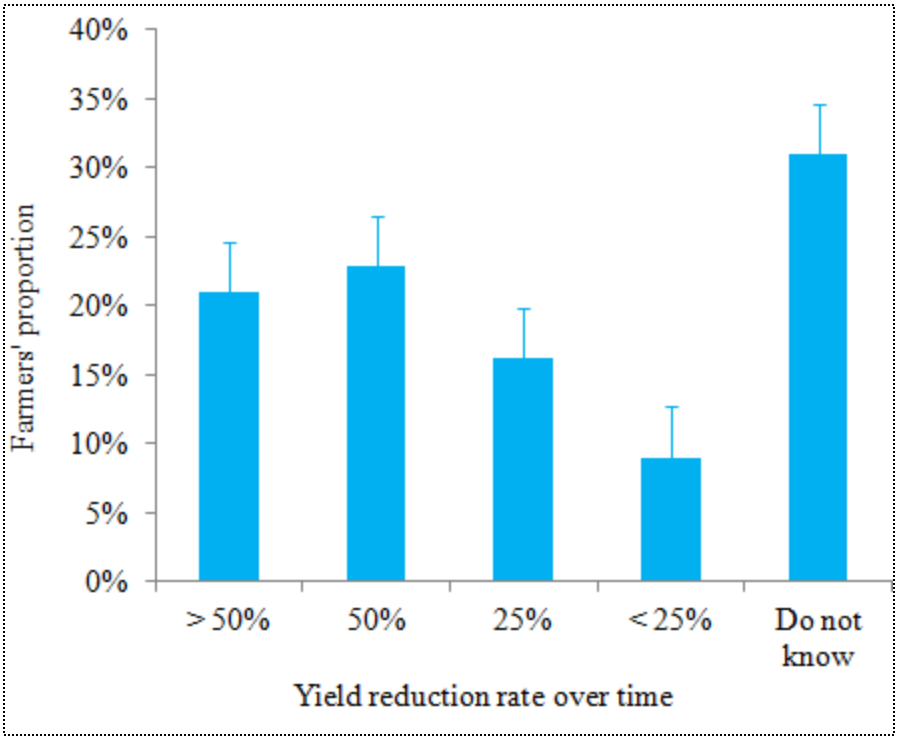

Figure 3.4: Farmer's estimation about yield reduction over time.

Table 3.8: Ranking of rice production constraints

	Lack of fertilizer	lack of good varieties	problem of salinity	Diseases	Birds	Flooding	Score	Rank
Lack of fertilizer		Lack of fertilizer	Salinity	Lack of fertilizer	Lack of fertilizer	Lack of fertilizer	4	1st
lack of good varieties			Salinity	diseases	lack of good varieties	lack of good varieties	2	4th
Salinity				diseases	Birds	Salinity	3	3rd
Diseases					diseases	diseases	4	1st
Birds						Birds	2	4th
Flooding							0	6th

Rice production was constrained by several problems (Table 3.8) such as lack of fertilizers, lack of good varieties, salinity, diseases, birds attack and flooding. Salinity was scored 3 and ranked third. Thus, it appeared the third most serious problem impeding rice production after diseases

and the lack of fertilizers. The lack of fertilizer and diseases were the two most serious problems and they were scored 4 and ranked first. Varieties problem and birds attack were scored 2 and were ranked fourth. Flooding notified in only one village was the less serious problem in the area.

3.3.1.3. Farmers 'perceptions of salinity problem and effects

Ninety percent (90%) of the farmers in the area were aware of salt problem while the others were not aware. Salinity was recognized by farmers through its appearance at the soil level (40% of the farmers) and the symptoms on rice plants (50% of the farmers) (Figure 3.5). Farmers also suspected salinity in cases of yield reduction despite applying recommended levels of mineral fertilizer. According to the farmers that experienced salt problems on their fields, salinity symptoms on leaves of rice plants disappeared after the application of manure. Twenty-seven (27) percent though that salinity came from soil degradation under irrigation and 32% thought it was inherited (Figure 3.6). For the latter some soils were naturally salty under irrigation or not.

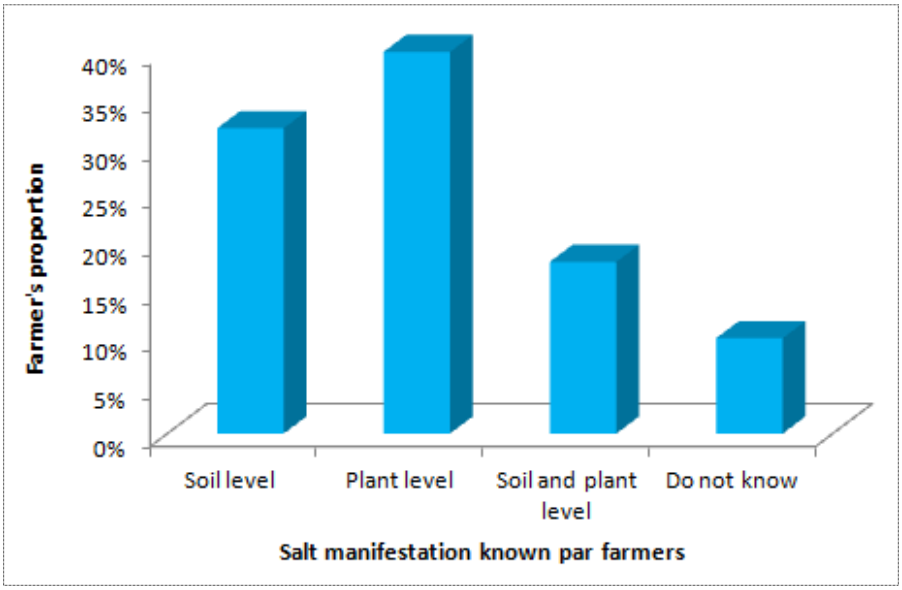

Figure 3.5: Farmer's methods to identify salt problems

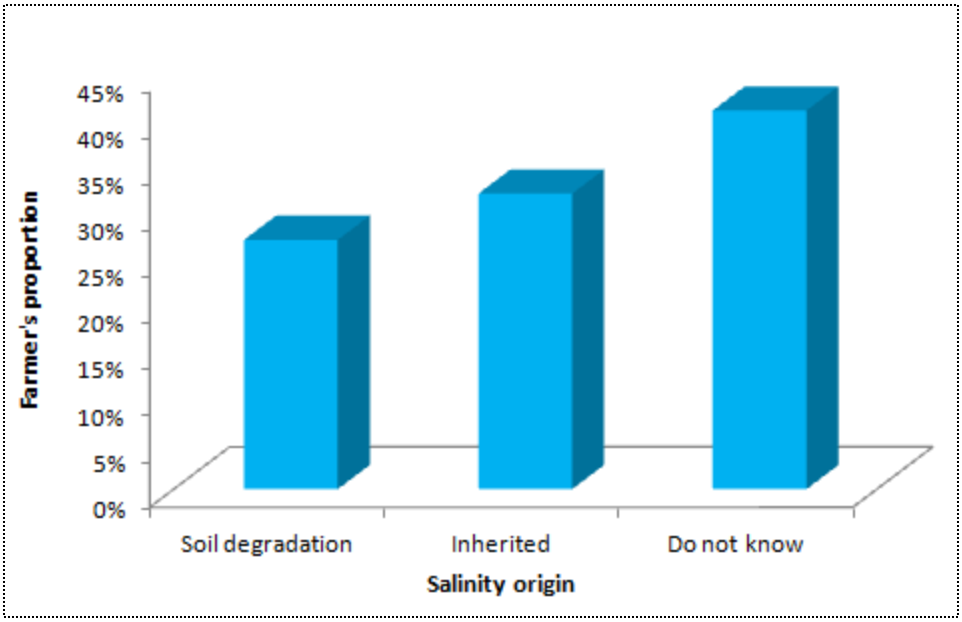

Figure 3.6: Farmer's explanation about salinity origin.

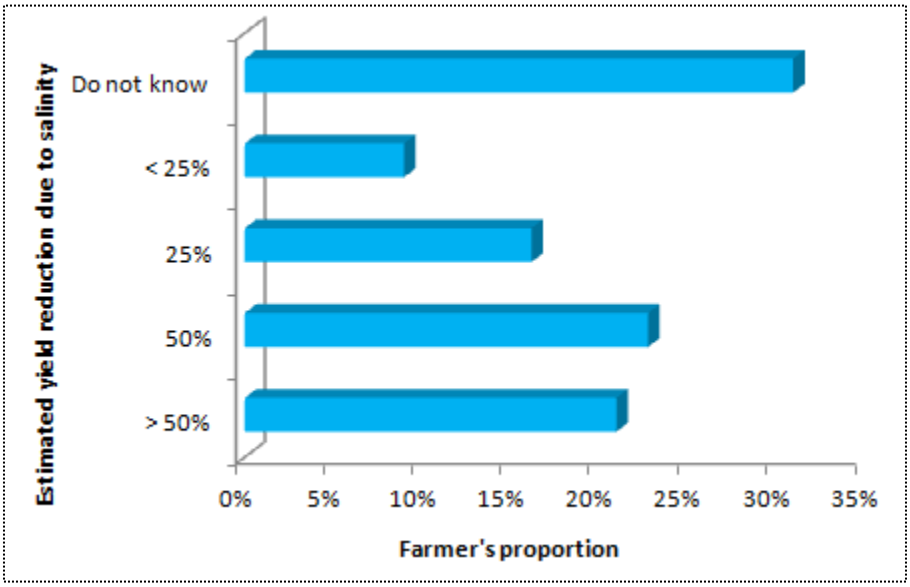

Figure 3.7: Estimated yield loss due to salinity in salt prone area

The yield reduction due to salinity ranged less than 25% at Sebery to 100% at some abandoned fields of Saga (Figure 3.7).

3.3.1.4. Farmer's coping strategies

To improve productivity under salinity various strategies were employed by farmers. These were: the avoidance of urea application (4%), Application of manure (11%) or a combination of manure and ashes (6%) and Application of straw (9%). However, seventy percent (70%) of the farmers use no method to overcome salinity problem (Figure 3.8).

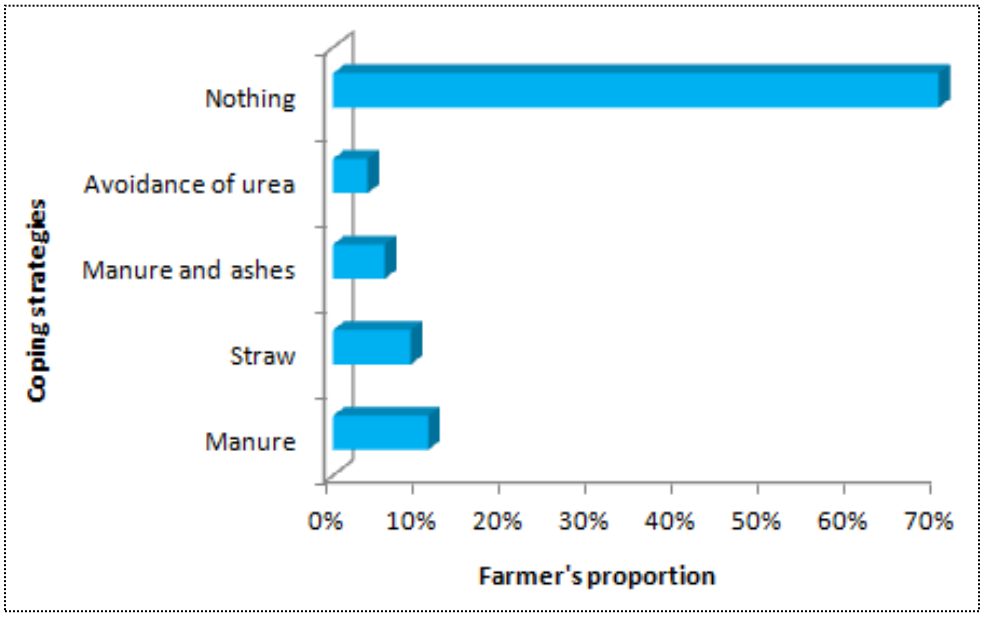

Figure 3.8: Farmers coping strategies

3.3.1.5. Farmers' preferred rice cultivars

A number of improved varieties have been released for irrigated rice cultivation (Table 3.9):

The improved variety IR1529 was used about 20 years ago because of its relatively high yield and good taste. It was abandoned because the decreasing yields due to susceptibility to diseases and the high demand for fertilizer. Other varieties such as WITA 8 and WITA 9 were cultivated because of good yield and ability to grow under low nitrogen conditions. They were abandoned because of their sensitivity to drought, lodging, salinity and birds. BG, NERICA, and Gambiaka, were other improved varieties in use.

Locale varieties (unknown origin), Kassimo (early maturing, good yield, less bird problems), Kardjikoyo (good yield and good taste), are now cultivated. These cultivars developed by a farmer named Kassim.

Table 3.9: Rice cultivars that have been used in the area

Variety Name	Released period	Growth duration (days)	Raisons of adoption	Raisons of abandonment
IR 1529-680-3	1975-1999	130-135	• Good taste	• Sensibility to diseases • Yield decreasing • Demanding of lot fertilizers
BG 90 2	1995-1999	130-135		• Yield decreasing
WITA 8	1997			• Low yielding • Lodging
WITA 9	1997			• Low yielding • Lodging
KASSIMO	Not Known		Yield	
KARDJIKOYO	Not Known		Yield and taste	
NERICA L49	Not Known		Yield	
GAMBIAKA	Not Known		Yield taste and grain quality	

Preference ranking of these varieties showed that the most preferred variety in the area of Tillabery and Niamey was Kassimo followed by Kardjikoyo. In the sector of Gaya the preferred varieties were: Gambiaka (first), followed by NERICA-L-49.

The foremost trait preferred by farmers was yield (score 7) followed by resistance to diseases. The tolerance to salinity was the third preferred trait. High tillering ability was scored 4 and was ranked fourth (4). Other farmers preferred traits such as height, earliness and low nitrogen efficiency that were respectively classified fifth, sixth and seventh. Grain quality such as good

taste was the last trait preferred by rice growers (Table 3.10). However, this was the most preferred traits at Gaya.

Table 3.10: Farmers preferred traits

	Yield	Resistant to diseases	Tolerant to salinity	Low nitrogen efficiency	Earliness	Taste	Tillering	Height	Score	Rank
Yield		Yield	Yield	Yield	Yield	Yield	Yield	Yield	7	1st
Resistant to diseases			Resistant to diseases	Resistant to diseases	Resistant to diseases	Resistant to diseases	Resistant to diseases	Resistant to diseases	6	2nd
Tolerant to salinity				Tolerant to salinity	Tolerant to salinity	Tolerant to salinity	Tolerant to salinity	Tolerant to salinity	5	3rd
Low nitrogen efficiency					Earliness	4	Tillering	Height	1	7th
Earliness						Earliness	Tillering	Height	2	6th
Taste							Tillering	Height	0	8th
Tillering								Tillering	4	4th
Height									3	5th

3.3.2. Soils results

3.3.2.1. Soil Texture

A huge variability exists within irrigated field (IF) soils and among irrigated and non irrigated soils texture (Table 3.11). This variability existed also within non irrigated fields. Irrigated soils of Gaya Amont, Gaya Aval, Sokondji, Ndounga Sebery and Ndounga were predominantly composed of clay and silt. At Saga, Toula and Bonfeba, sand was the main component of the texture. Most of none irrigated fields (NIF) were sandy soils. Irrigated fields soils were

significantly different from non-irrigated fields' soils (at 0.001 probability level) in terms of clay content. Significant differences also existed among the two types of fields in terms of sand content (at 0.05 probability level). However, in terms of silt content the irrigated fields were not different from non-irrigated fields.

Table 3.11: Soil texture of irrigated fields and none irrigated fields

	Irrigated fields			Non-Irrigated fields		
	Clay (%)	Silt (%)	Sand (%)	Clay (%)	Silt (%)	Sand (%)
Gaya Aval	44.6	37.2	18.2	28.3	41.5	30.2
Gaya Amont	48.1	41.2	10.7	11.3	29.7	70.2
Diambala	32.2	52	15.7	21.2	46.8	32
Sakondji Birni	48.6	20.7	31.7	45.2	41.1	13.8
Saguia	41.8	43.9	14.2	4.3	20.5	75.2
Saga	11.6	13.1	75.3	7	14.1	79.8
Bonfeba	12.5	19.7	67.7	7.6	10.1	82.4
Sebery	48.4	26.6	25	6.3	8.8	84.9
Toula	11.8	14.7	73.5	14.1	18.7	67.2
Ndounga	51.4	25.8	22.8	4.5	8.5	87.2

3.3.2.2. Soil pH

The pH of both irrigated and non-irrigated soils in the communities selected for the study are shown in Figure 3.9. From the Figure, apart from Bonfeba which soil was neutral in pH all the soils were acidic (pH < 7). The lowest pH in KCl of 3.5 was recorded at Gaya. The pH in KCl at Saguia and Sakondji Birni were strongly acid with pH in below 5. There was no significant difference in pH between irrigated and non irrigated plots within each selected field.

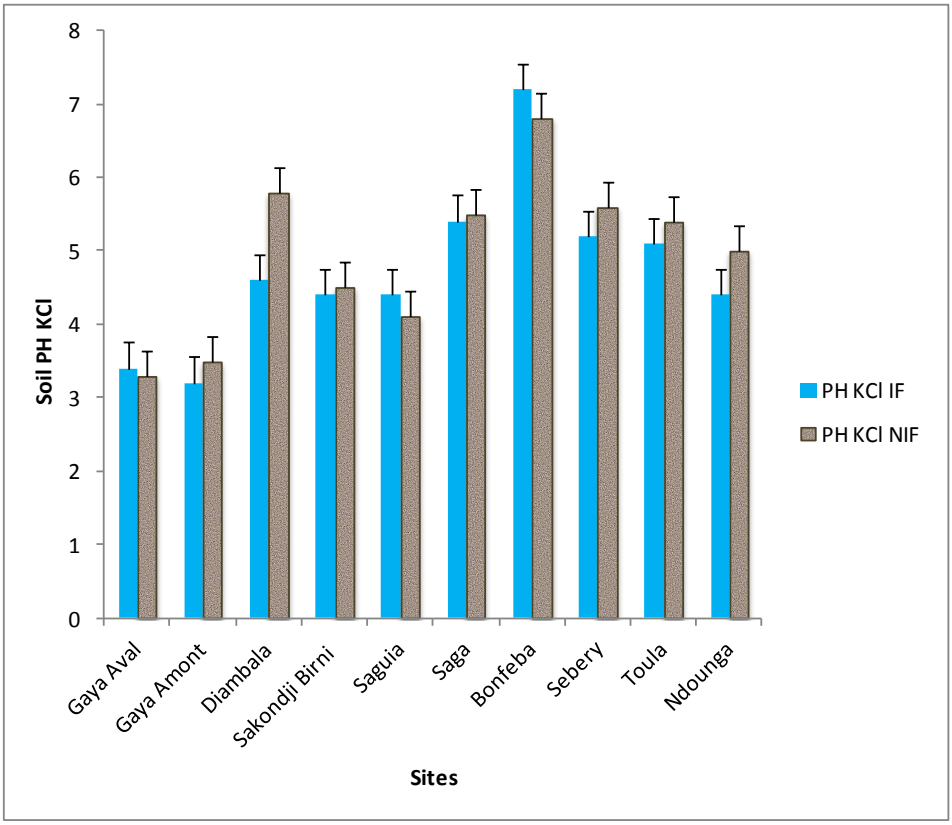

Figure 3.9: pH in KCl of irrigated Fields (IF) and non-irrigated fields (NIF)

3.3.2.3. Soil Sodium content and sodium adsorption ration

For all the sites sodium adsorption ratio of irrigated fields (SAR IF) was significantly higher than sodium adsorption ratio of non-irrigated fields (SAR NIF) (Figure 3.10). The greatest level of SAR was observed at Saga irrigated fields and the lowest at Saguia. Sodium content in irrigated fields was also significantly higher compared to non-irrigated fields (Figure 3.11). The exchangeable sodium percentage was also higher in irrigated scheme than non-irrigated fields (Figure 3.12). The SAR and sodium contents were particularly very high at Saga.

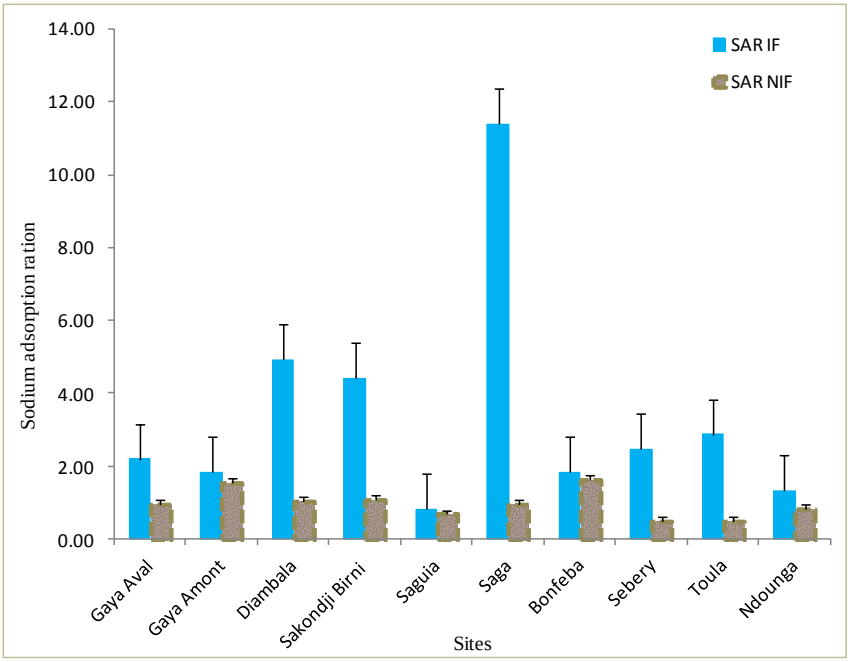

Figure 3.10: Sodium adsorption ration of irrigated fields (SAR IF) and none irrigated fields (SAR NIF).

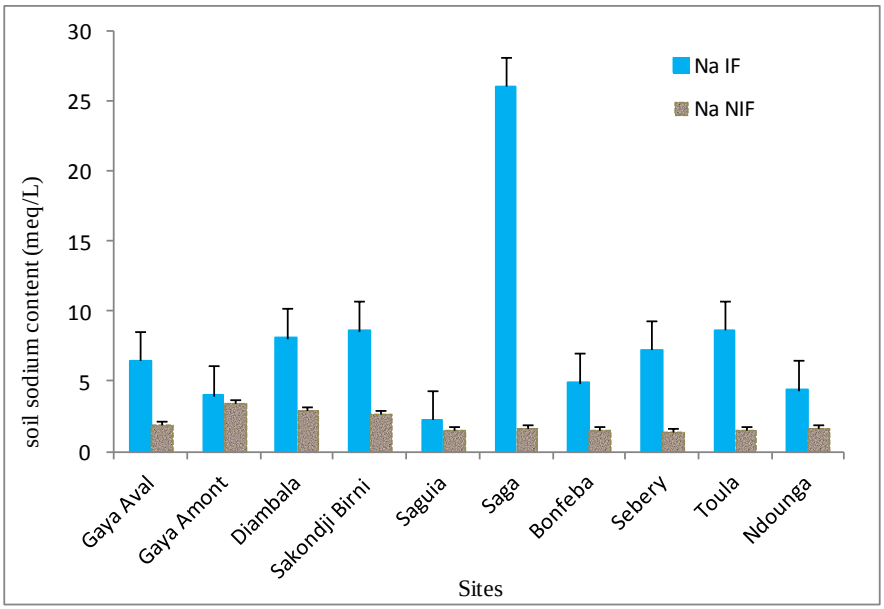

Figure 3.11: Sodium content in the study area

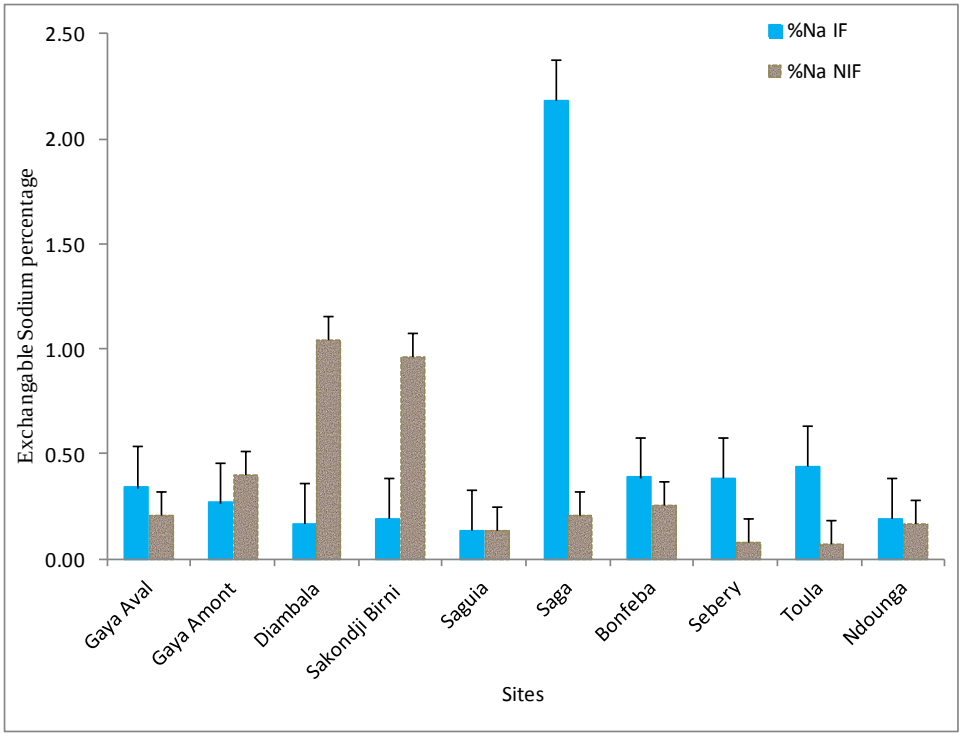

Figure 3.12: Exchangeable sodium percentage in the study area

3.3.2.4. Sodium and potassium ratio and PAR (potassium adsorption ratio)

The ratio Na/K ranged from 1 to 12 in the irrigated field and from 0.3 to 7.8 in non irrigated field (Figure 3.13). The Na/K was significantly higher in irrigated fields than non-irrigated fields. The PAR was higher in non-irrigated fields than irrigated ones (Figure 3.14). However, the differences were not significant.

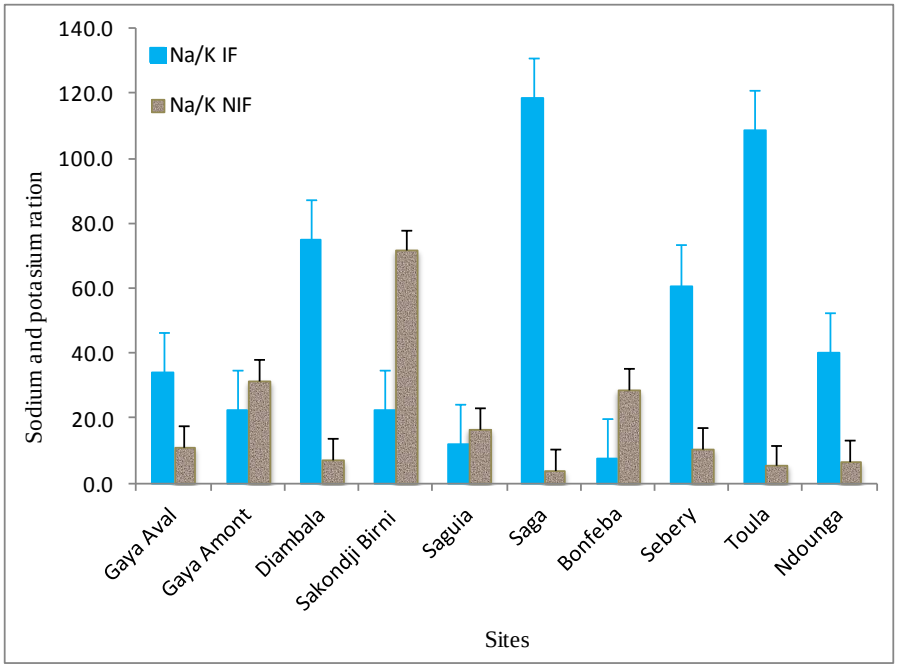

Figure 3.13: Sodium and potassium ratio of the study area

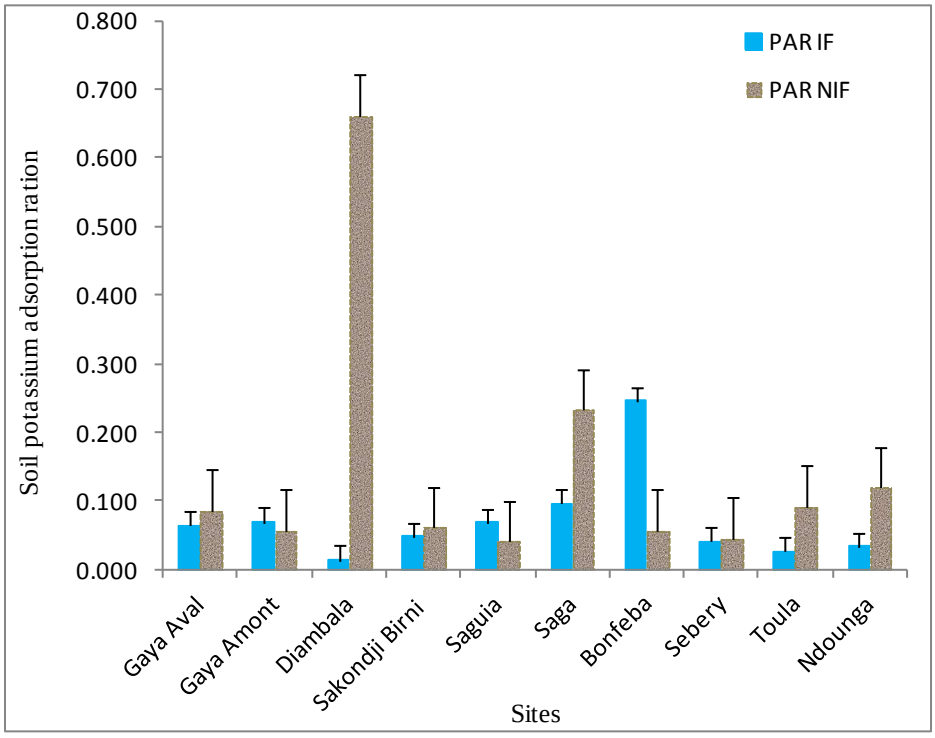

Figure 3.14: Potassium adsorption ratio (PAR)

3.3.2.5. Total dissolved solids (TDS) and cation exchange capacity (CEC)

The TDS and CEC were significantly higher in irrigated schemes compared to non-irrigated ones (Figures 3.15 and 3.16). However, at Bonfeba, the irrigated fields had lower values of TDS and CEC.

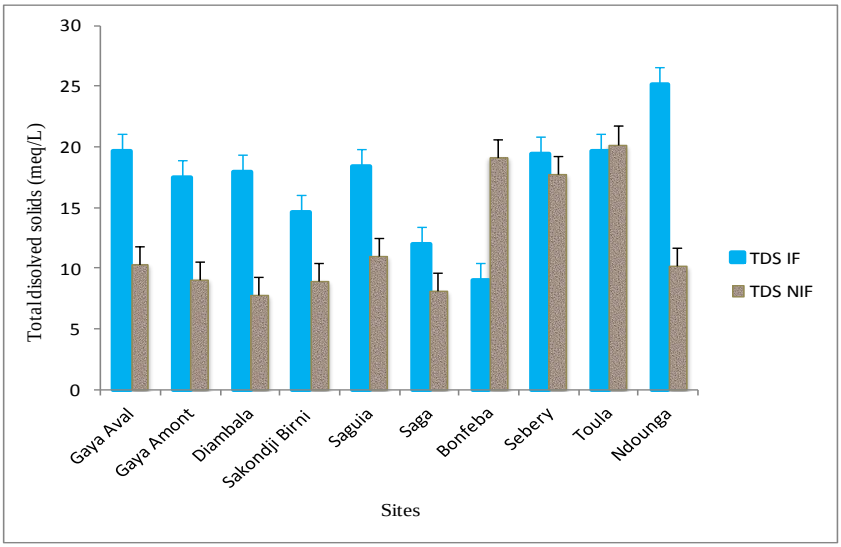

Figure 3.15: Total dissolved solids of the study area

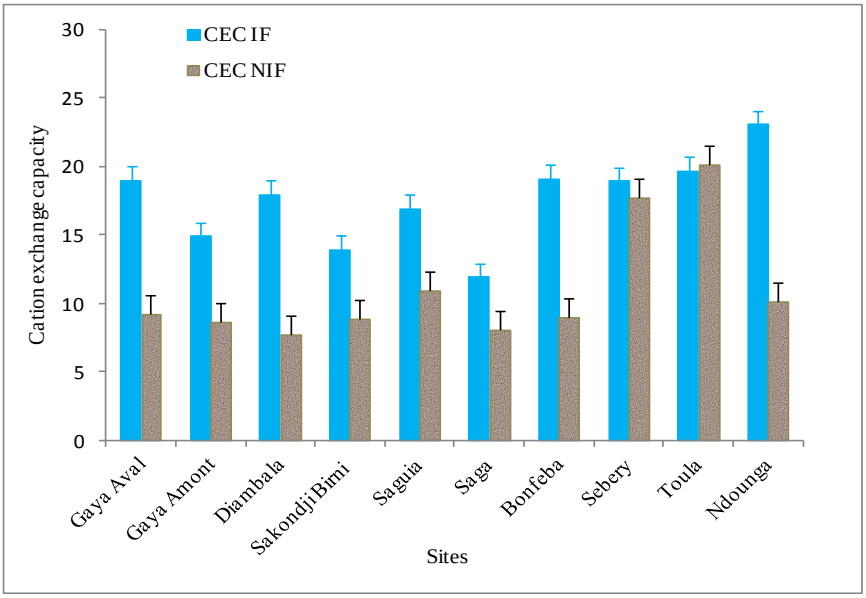

Figure 3.16: Cation exchange capacity of the area

3.3.2.6. Soil electrical conductivity (EC)

The electrical conductivity of irrigated fields (EC IF) is significantly higher than non-irrigated fields one (Figure 3.17). Rice fields EC ranged from 5.2dScm^{-1} at Sakondji Birni to 16.5 dScm^{-1} at Bonféba with an average of 7.7 dScm^{-1}. The non-irrigated fields EC varied from 2.2 dScm^{-1} (Saga) to 8.5 dScm^{-1} at Bonféba with an average of 5 dScm^{-1}.

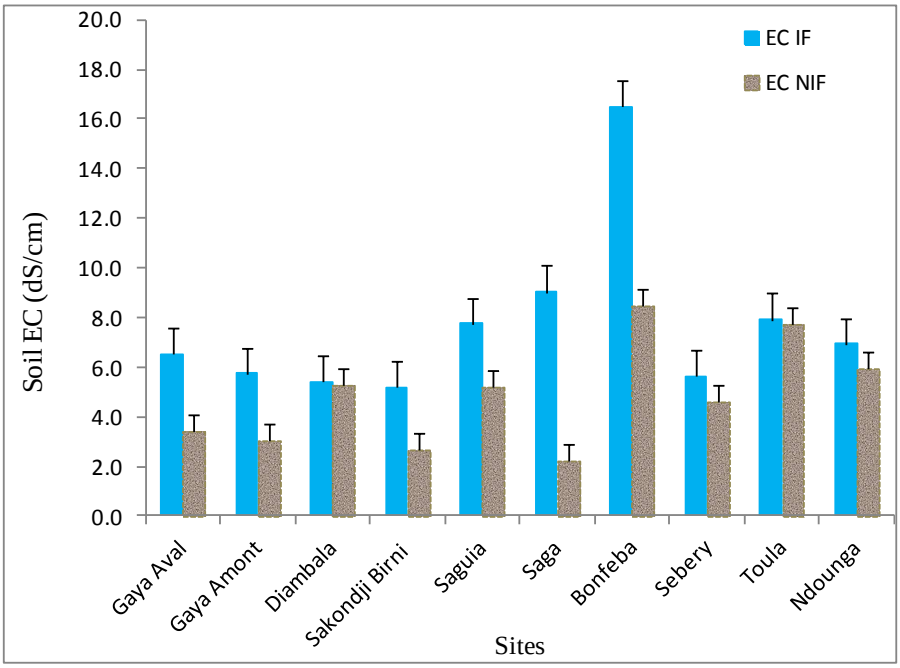

Figure 3.17: Soil electrical conductivity (EC)

3.3.2. Water results

3.3.2.1. SAR, PAR and TDS of irrigation water

The water SAR (Figure 3.18) ranged from 0.07 at Sebery to 0.24 at Diambala. The PAR (Figure 3.19) varied from 0.001 to 0.1. The highest TDS (387 mg/l) was observed in tap water of Niamey and the lowest (251 mg/l) at Diambala (Figure 3.20).

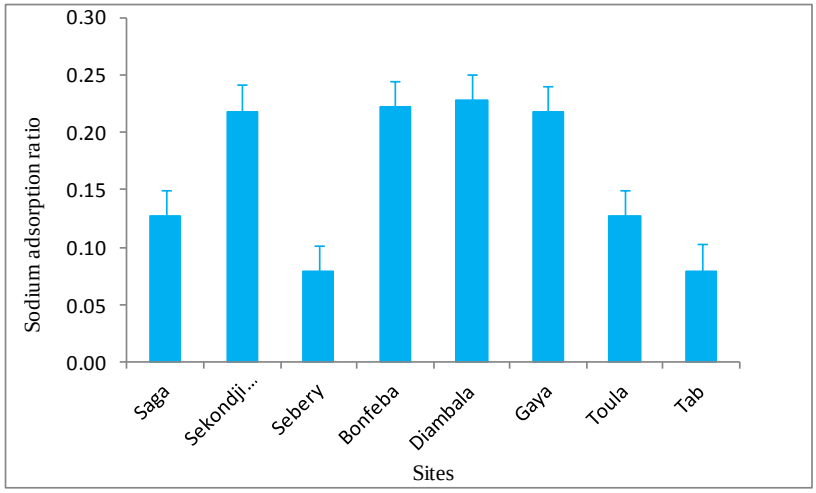

Figure 3.18: Irrigation water sodium adsorption ration

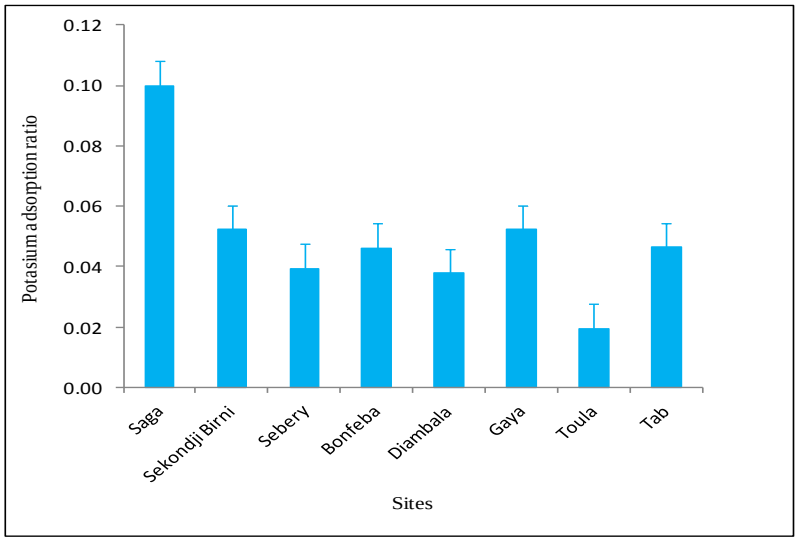

Figure 3.19: Irrigation water potassium adsorption ration

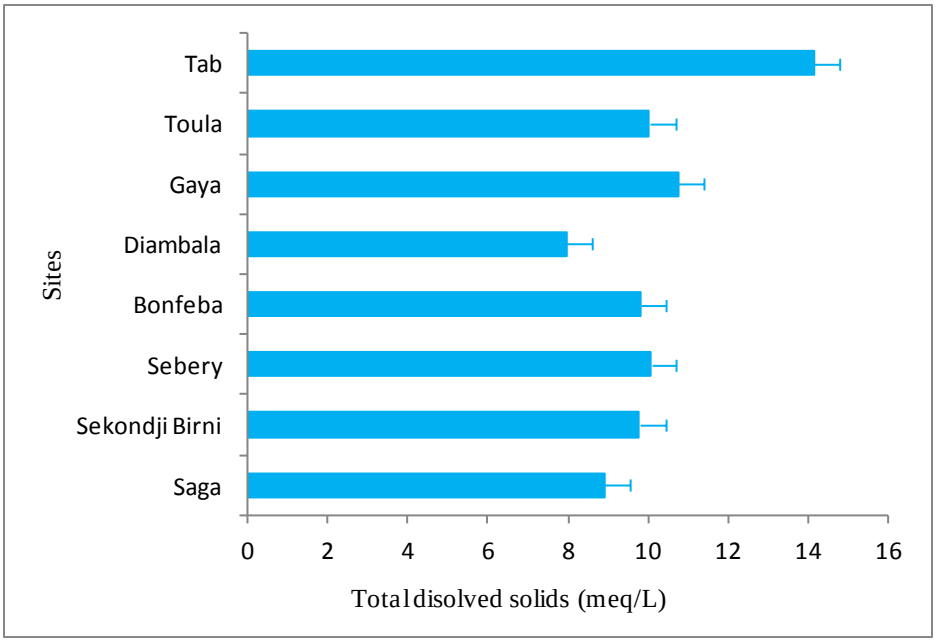

Figure 3.20: Irrigation water TDS

3.3.2.2. Sodium quantity deposited by irrigation water

Niger River contains sodium (Na) like other salts. The Na content ranged from 2.3 mg/l at Sebery to 5.75 mg/l at Gaya with an average of 4.23 mg/l (Figure 3.21). The total sodium quantity estimated to be deposited per hectare per year varied from 87 kg/ha/year to 218 kg/ha/year (Figure 3.22).

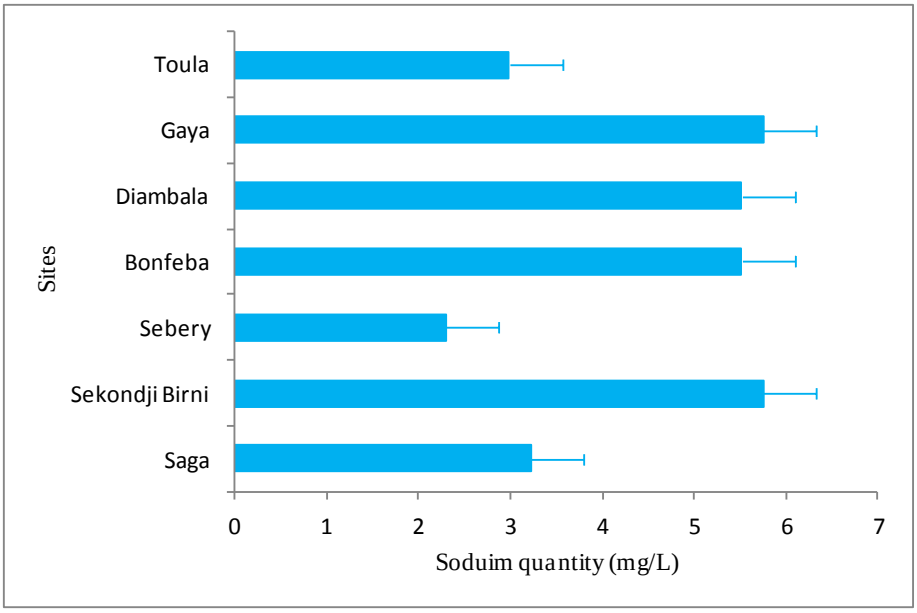

Figure 3.21: Estimated sodium content of the irrigation water (mg/L)

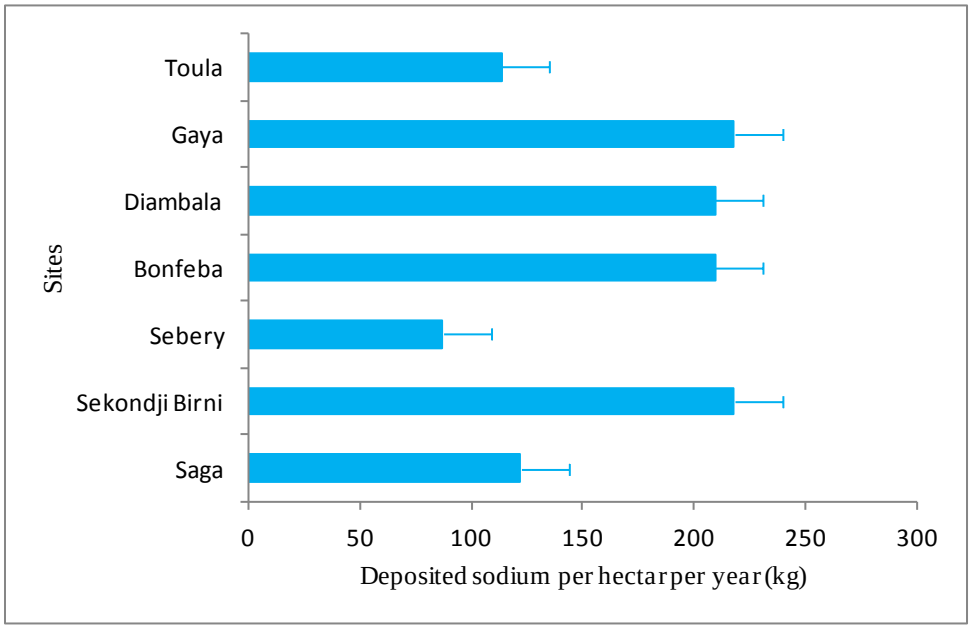

Figure 3.22: Estimated deposited sodium due to irrigation water

3.4. Discussion

3.4.1. Importance of rice and framers' perception of its production constraints

Rice was the most preferred crop in the area because of three main raisons. First, rainfed crops production is constrained by several factors such as drought, pests and diseases and the use inherently low-yielding varieties of crops. Secondly, rice, more than all the others cereals, serves as food and also helps to solve economic problems. Depending on the level of mechanization, net income/farm/season varies between 100 000 and 400 000 FCFA. The contribution of the irrigated schemes to the family basic food coverage varies from 30 to 190 percent (IPTRID, 2004). Thirdly, rice is cultivated two times a year giving it an advantage over other crops.

Others crops were still cultivated in the study area because of food culture and social reasons. This is different from the situation of the rest of the country, where pearl millet and sorghum were the main rain-fed subsistence crops.

One the most serious problems rice growers were facing was the lack of fertilizers which were provided either insufficiently or too late. The first application that should be done just after transplantation was sometimes delayed for two (2) to four (4) weeks. This delay affects rice growth, development and yield. The fertilizer problem can be explained by poor organization at the cooperative level that is incapable to make fertilizer available at the right time. But some time farmers instead of applying fertilizer to the rice plant sold it or applied it on other crops such as vegetables.

Diseases were also highly ranked constraints. Farmers experienced huge yield losses due to these disorders. RYMV, bacterial blast, rice stem borer were cited to be the most destructive.

Salinity was the third most serious constraint after diseases and the lack of fertilizers. Farmers considered salt problem as a phenomenon that was imposed by nature and that occurrence can neither be predicted nor prevented. They thought that salt problem unlike the two first is a daily routine constraint that once appeared will remain for no limited time. So they have to be used of it and applied some measure that will allow them to harvest some paddy rice.

Farmers also lacked good varieties. Many varieties were grown on one irrigated scheme, or in one village and sometimes on the same field. Some were improved (NERICA-L-49, IR1529, etc…) developed by research centres, others were selected by farmers (Kassimo, Weyhidjo, Arhidjo…), but all had lost their high yielding ability because of farmer's practices. Hence they often grew several varieties on one field and seed was often a mixture of cultivars.

Birds attack and flooding were others problems that were not common among all the irrigated schemes. Flooding was a serious problem at Diambala, whilst bird' attack was mentioned at Toula, Bonféba and Sebery.

3.4.2. Farmers 'perceptions of the salinity problem, coping strategies and expectations

Ninety percent of the farmers were aware of the salt problem. In all the villages but "Gaya Amont" this problem was mentioned. Yield loss can range from 10 to 100% depending on the salinity level and the cultural practices. Thirty-two (32) percent of farmers recognized salt problem at the soil level, but the early signs of soil salinity are often difficult to identify visually and a soil test is required to determine the degree of salinity (Australia, 2005). Symptoms like leaf tip burning, yellowing and stunting are due to salt problem can be corrected with manure application. Salinity origin was not well known by farmers but 27% claimed that it was due to intensive irrigation that leads to soil degradation, while others thought that some soils are

naturally saline. According to Jacques *et al.* (1994) human activity, particularly superfluous irrigation, constitutes the most pertinent explanation of the salinization processes. The major source of salinity problems is usually irrigation water (Provin and Pitt, 2008) because, in every river basin, prior to the introduction of irrigation, there exists water balance between the rainfalls on the one hand and stream flow, groundwater level and evaporation and transpiration on the other. This balance is disturbed when large additional quantities of water are artificially spread on the land for agriculture (Gardner and Fireman, 1958; Sharma and Prihar, 1973).

Because of soil degradation, farmers realized that their field cannot produce using only mineral fertilizer. According to them, application of 500kg of fertilizer can have the same result as application of 100kg (50kg urea and 50 kg N 15-15-15) supplemented by manure. In some cases usage of urea was completely avoided by farmers because it was said to burn the rice plants. Straw, manure and ashes were also used in the area to deal with salt problems. Rice glumes and ashes were mostly used in Gaya, while manure was applied in Tillabery. Applying residue or mulch to the soil can help lower evaporation rates and improve rice development. These coping strategies aim to avoid effects of salt on soil structure by providing organic matter. The effects of exchangeable sodium on plants are either direct on plant nutrition or indirect through deterioration of the soil structure, leading to decreased hydraulic conductivity and aeration (Mahmood *et al.*, 2004). Organic matter application would enhance soil structure and reduce evaporation.

In this area with poor drainage, tilling may help the water move downward through the soil. While deep tillage will help temporarily, the parts of the soil not permanently broken up may reseal. Tilling was not performed against salinity in the areas studied.

The most preferred rice characteristics were: yield, resistance to diseases, tolerance to salinity high tillering ability and height. Farmers were interested in yield because after harvesting they usually divide the paddy into three parts. One part served to reimburse credits to the cooperatives; this is compulsory for each farmer in order to pay fees for irrigation and fertilizers that were provided by the cooperative. The second part was to be sold and the last part to be consumed by family members. In this system anything that will contribute to reduce yield (diseases and salinity) must be avoided. All the improved varieties introduced in the region have been adopted because of their relative high yield, and rejected some years later because of their low yielding due to deterioration. The preference of disease and salinity tolerance is justified by the desire to gain high yield. WITA 8 and WITA 9 were cultivated because of the good yield potential, and efficient growth under low nitrogen. They were abandoned because of their sensitivity to stress (bird's attacks, salinity, and diseases) that reduced their productivity. The reasons for high preference for tall plants were that the majority of the farmers harvested rice panicles using knives while standing. Tall plants lessened the burden of bending to harvest (Efisue, 2007). Apart from this, tall plants had long rice straws and more biomass. Farmers used straw to feed their animals. Other preferred rice characteristics were earliness, low nitrogen efficiency, and taste. Short duration rice allow farmers to grow other crops or to consecrate their time to others activities. Because of the lack of fertilizer provision, farmers like varieties that can produce more with little amounts of fertilizer.

3.4.3. Rice production environment

Seventy percent (70%) of non-irrigated fields were sandy soils, while eighty percent (80%) of irrigated field soils were clay and silt. The higher percentage of the irrigated fields being silty and clay was due to the fact that these irrigation schemes were located in the low lying areas of

the Niger River. These soils have capacity to store and supply water and air for plant growth (Adamu and Aliyu, 2012). Clay soils have a greater water holding capacity than sandy soils. In addition, well drained soils typically have good soil aeration meaning that the soil contains air which is conducive to healthy root growth, and thus, a healthy crop (Berry *et al.*, 2007). Results of non-irrigated fields confirmed FAO (1994) report which said that sahelian soils are generally sandy with sand percentage varying between 71 to 99% at the surface layer. This is because most of the sahelian soils devoted to agriculture are upland sandy soils. They are mostly cultivated by millet and cowpea. About 80-90% of them are upland sandy soils. The lowlands soils of the inland valleys Niger River valley represent less than 15% of cultivated soils in a country like Niger.

The results showed that the pH of all fields was lower than 5.6 except for Bonfeba. This was too low for optimum development of rice. Most agronomic crops require soil water pH values between 5.7 and 7 for optimum plant growth (Kidder *et al.*, 1988; Kalra, 1995; Miller and Kissel, 2010). Soil pH is among the important environmental factors which can influence plant growth. Initially, each type of soil has a certain level of acidity and pH depending upon its composition, native vegetation, and rainfall amounts; however, various factors over time cause changes in soil pH (Reeuwijk, 2002). Soils formed under low rainfall conditions tend to be basic with soil pH readings around 7.0 However, intensive farming over a number of years with nitrogen fertilizers or manures can result in soil acidification (Cliff, 2005). This may be the reason why the irrigated soils pH was more acidic than non-irrigated soil.

Irrigated Fields EC was too high (16.5dS/cm at Bonfeba and 9dS/cm at Saga) to induce severe yield reduction of rice. In these two areas fields are completely abandoned. This great EC level may partly be due to irrigation because irrigated fields had greater value of electrical

conductivity compare to non-irrigated one. However, some fields that have never been irrigated had also high EC. This was due to inherent soil salts composition.

The sodium adsorption ratio (SAR), as well as exchangeable sodium percentage (ESP) was significantly higher in irrigated fields than non-irrigated one. This may be due to irrigation water which contained a SAR that can affect soils over years and can cause severe permeability problems when applied to fine-textured soils (Foundation, 2007). The SAR and ESP are two different criteria currently recognized in the scientific literature as indices of salinity. However, the value we got were below the reported threshold of 12 (cmol kgG) for SAR (Mohsen *et al.*, 2009). Munshower (1994) reported that SAR along with pH, characterize salt-affected soils. When the SAR rises above 12 to 15, serious physical soil problems arise and plants have difficulty absorbing water. The greatest level of SAR was observed at Saga irrigated fields. These fields were abandoned because of salt problem. So the great salt content may explain this high SAR.

Sodium to potassium ratio was significantly higher in irrigated schemes than non-irrigated schemes despite application of K fertilizer application in the irrigated rice fields and this could be attributed to the high addition of Na from the irrigation water.

The higher PAR in non-irrigated fields was due to relative high potassium content in these soils.

The CEC of irrigated field was significantly higher than for non irrigated fields likely due to their higher clay and organic matter contents. Low CEC soils were more likely to develop potassium and magnesium deficiencies, while high CEC soils were less susceptible to leaching losses of these cations. The lower the CEC, the faster the soil pH will decrease with time.

ESP was lower in irrigated field of Gaya Diambala and Sakondji than non-irrigated fields, because of high TDS.

The irrigated soils were characterized by a pH less than 7, an EC (Electrical conductivity) above 4 a SAR (Sodium adsorption Ratio) below 13 an ESP (Exchangeable sodium percentage) below 15 and flocculated structure. These are the typical characteristics of saline soil (Horneck *et al.*, 2007).

Irrigation water included some amount of dissolved substances, collectively called salts. These salts include dissolved solids derived from the weathering of rocks and soil by water and salts dissolved from soil the water previously passed through (Ayers and Westcot, 1976; Miller and Donahue, 1995). Salts are added to the soil with each irrigation and a huge quantity of sodium from irrigation water is deposited in rice fields. About 200kg of sodium were estimated to be deposited each year per hectare. But these results did not reflect the soil actual salinity level. The salinity level is lower than it could have been expected. This is because of leaching fraction that was not known so was not taken into consideration in our calculation. The drainage even though was poor could also have effect on the expected salt level. The water SAR and PAR vary from site to site because of irrigation canal that are defective. This made water to pass through the soil, so water ions can either get into soil or vice versa.

Salt accumulates and concentrates in soil when water evaporates from the soil surface, when plants use water, when leaching is not adequate to leach salts beyond the root zone, and/or when precipitation does not wash salts off the land surface (Schafer, 1983; Rhoads and Akandiah, 1992).

Another important point regarding the relationship between irrigation water and soil salinity is that the salinity of soil water would be equal to approximately three times the salinity of irrigation water (Ayers and Westcot, 1976; Nikos *et al.*, 2002). In conditions of relatively high

leaching fractions, the soil water solution and drainage water will have a salinity level slightly greater than the irrigation water. When considering salinity effects of the irrigation water, the plants and soil actually are subject to the salinity of the resultant soil solution, which is a function of the salinity of the applied water (Barbour *et al.*, 1998; Usda, 2002).

3.5. Conclusion

This study, focused on only irrigated rice, has identified high yield, tolerance to salinity and diseases, high tillering ability and tall plants as the preferred traits. Early maturity was also an important trait. Through this study more was known about the attitudes of farmers, and the reason for adoption of improved rice varieties at the farm level. Such information could potentially help in improving the efficiency of the breeding programs through the application of selection criteria that will increase the probability of varieties being adopted by large numbers of rice farmers in the region.

Soils and irrigation water contain various proportions of cations (Na+, Mg++, K+, and Ca++) and anions (Cl−, SO4 −−, CO3 −− and HCO3 −) that eventually appear in soil in form of salt crust deposit, or solution. The soil content of these ions is a function of the inherent properties and the irrigation water it receives. The soil electrical conductivity (EC), the sodium adsorption ratio (SAR), the exchangeable sodium percentage (ESP) and the cation exchange capacity (CEC) were also significantly higher in irrigated fields compared to non-irrigated ones. Most of the farmers' rice fields were saline soils. The study showed that the soil ions content may have been brought about partly by irrigation water. A huge amount of salt can be deposited in soil trough irrigation. This imperatively led to Salinization that severely affects soil productivity.

CHAPTER FOUR

4.0. PHENOTYPIC VARIABILITY OF RICE GENOTYPES UNDER DIFFERENT LEVELS OF SALT STRESS

4.1. Introduction

Salinity is a worldwide problem of serious nature in arid and semi-arid regions where most of the developing countries happen to fall.

Plant growth on these soils is adversely affected because of reduced water uptake, salt toxicity, and nutrient imbalances (Abdelbagi *et al.*, 2007). Degree of salt stress can affect the different crops differently. For rice, soil salinity beyond ECe ~ 4 dSm^{-1} is considered moderate salinity while more than 8 dSm^{-1} becomes high (Singh, 2009). Soil solutions high in sodium chloride with electrical conductivity values of 6–10 dSm^{-1} are associated with 50% decrease in yield (Frans and Nico Van, 1978) because of both osmotic and ionic stresses (Siringam *et al.*, 2009).

Plant scientists have adopted various strategies to overcome the salinity. One of them is to exploit the genetic variability of the available germplasm to identify a tolerant genotype (Ashraf *et al.*, 2006). Existence of appropriate genetic variation is a prerequisite for the improvement of any character, through selection and breeding (Mahmood *et al.*, 2009). Fortunately, diversity in salt tolerance at the intra specific level has been found in a considerable number of rice varieties (Bari and Hamid, 1988; Mishra *et al.*, 2000; Zeng *et al.*, 2002). The screening of rice genotypes is necessary to identify the salt tolerant germplasm for breeding programs (Muhammad *et al.*, 2010). Study on the response of rice to salinity stress may be helpful in breeding salt tolerant cultivars.

The specific objectives of this study were to:

- determine the variability of rice genotypes in response to salinity tolerance.
- identify and select promising salt tolerant genotypes as parents for a development of new varieties with salinity tolerance

4.2.Materials and methods

A greenhouse pot experiment was conducted at the Regional Agronomic Research Centre (CERRA) of Kollo in 2011 wet season to determine the response of rice varieties to different levels of saline water at different growth stages. Kollo is geographically located in latitude 13º 19´ 43´´ N, longitude 2º 19´ 16´´E and 250 m altitude.

4.2.1. Properties of soil used for the experiment

The soil transported from the rice salt free field was clay with dark color. The soil was clayey and was composed of 52.8% clay, 30.4% silt and 16.7% sand.

The experimental soil was acidic (pH=3.9), the sodium adsorption ratio (SAR) and potassium adsorption ratio (PAR) were equal and very low. The exchangeable sodium percentage was about 6%. The electrical conductivity (0.12dS/cm), organic matter percentage and the cation exchange capacity were also low (Table 4.1).

Table 4.1: Chemical characteristics of the experimental soil

Soil properties	pH	SAR	PAR	CEC	ESP (%)	EC	MO percentage (%)
Value	3.9	0.06	0.06	10.16	6	0.12	2.26

4.2.2. Properties of irrigation water used for the study

The water used for irrigation (table 4.2) had a sodium percentage (1%) and electrical conductivity that was also low (1.42). The total dissolved solids were estimated to be 14.15 meq/l. The SAR was too low and slightly higher than the PAR.

Table 4.2: Characteristics of the irrigation water used in this study

Characteristic	TDS (meq/L)	Ecw (dS/m)	SAR	PAR	SPAR	%Na	Total cation (meq/L)	Total anion (meq/L)
Value	14.15	1.42	0.013	0.008	0.021	1%	9.26	4.89

TDS= total dissolved solids, ECw= Water electrical conductivity, SAR = sodium adsorption ration, PAR =Potassium adsorption ration, SPAR= sodium potassium adsorption ratio.

4.2.3. Soil preparation for experiment

The soil was exposed to ambient temperature temperature 45^{o}C to 48^{o}C for two (2) weeks. The aim of this exposition was to remove the moisture in order to know the real dry soil weight that each pot would contain. Two hundred (200) round pots of 20 cm diameter, with a capacity of 4 kg soil were used for the experiment. Pots were then filled up to desired level and weighed on an electronic scale. Each pot was filled with 4 kg of dried soil. The watering was done using tap water. An average of one liter was needed for each pot to be fully moistened. The pots were maintained irrigated when pre-germination of seeds was started.

4.2.4. Sowing in nursery

Seeds of 20 entries were placed in Petri dishes with moistened filter paper for pre-germination. Four pre-germinated seeds of test entries were sown per pot. Twenty days after seeding (DAS), seedlings were thinned to two per pot and the water level was raised to about 1 cm above soil.

The water level was maintained daily and the plants were protected from any pests and diseases by spraying insecticide and fungicide. The first fertilization was done at 20 DAS (after the thinning). The fertilizer used was NPK 15-15-15 at a rate of 2 grams/pot. The second fertilization was done at 58 DAS with urea at the rate of 2 grams/pot.

4.2.5. Salinization

Salinization was done by adding to the soil a salinized solution, which was obtained by adding table salt to water up to desired electrical conductivity (EC) which equals to 10 dSm^{-1} and 12 dSm^{-1}. One liter of salinized water was added to each pot and used till termination of experiment. An EC meter (Atago Digital EC Meter (DEC-2) was used to calibrate the desired level of electrical conductivity. For the preparation of salinized water solution the following methods were used:

- Salinization was made by adding NaCl while stirring up to the desired EC (5 and 6 g NaCl/L gives an EC of 10 and 12 dSm^{-1} respectively) (Gregorio *et al.*, 1997). This means that 500 mg/L= $1dSm^{-1}$.
- Approximately 640 mg/L of NaCl is equivalent to $1dSm^{-1}$ (Shannon *et al.*, 1998).

For each method 40 L of tap water were used. Two hundred (200 g) and 240 g of NaCl (table salt) were dissolved in 40 L, to obtain respectively 10 DSm^{-1} and 12 DSm^{-1} with Gregorio method. Two hundred fifty six grams (256 g) and 307.6 g of NaCl (table salt) were dissolved in 40 L, to obtain respectively 10 DSm^{-1} and 12 DSm^{-1} with Shannon method (Table 4.3).

When the seedlings were 28 days old, all the water in the pots was drained out from pots. Then the pots were filled up with salinized water solution. This was the stage of ideal transplanting moment in farmers 'fields.

No leaching from the pots was possible any water loss was due to evapo-transpiration, and the only factor that could change salt content was the root uptake. The water level was maintained daily (5 cm above soil surface) by adding ordinary water. Untreated soil served as the check.

Table 4.3: Salinization process

Method	EC (ds/m)	Salt quantity/L (g)	Total Salt quantity (g)	Volume of salted water/pot (L)	Volume of salted water/method (L)
Gregorio 10	10	5	200	1	40
Gregorio 12	12	6	240	1	40
Shanon10	10	6.4	256	1	40
Shanon12	12	7.68	307.2	1	40

4.2.6. Genetic material

Fifteen (15) genotypes known as salt tolerant from IRRI and five (5) farmer's varieties from INRAN (National Institute for Agronomic Research of Niger) were used. Table 4.4 shows all the 20 genotypes and their origins.

Table 4.4: Germplasm used in the study their source and origin

Entry	Stock ID	Name	Source	Origin
1	IRGC 30	ENGKATEK	IRRI	Malaysia
2	IRGC 249	C10022	IRRI	India
3	IRGC 15963	BOULOUF	IRRI	Senegal
4	IRGC 17038	DAMODAR	IRRI	India
5	IRGC 17041	GETU	IRRI	India
6	IRGC 17229	BANIH KUNING	IRRI	Indonesia
7	IRGC 26612	DUSHOR	IRRI	Bangladesh
8	IRGC 37084	GIRMI	IRRI	Bangladesh
9	IRGC 37179	KULPOD	IRRI	Bangladesh
10	IRGC 108921	POKKALI	IRRI	India
11	IRTP 22027	NSIC RC 106	IRRI	Unknown
12	IRTP 22024	IRRI 126	IRRI	Unknown
13	IRTP 22023	IRRI 113	IRRI	Unknown
14	IRTP 22030	IRRI 124	IRRI	Unknown
15	IRGC 116937	CSR 36	IRRI	INDIA
16	Kogoni 91-1	GAMBIAKA	INRAN/Niger	
17		IR1529-680	INRAN/Niger	
18		NERICAL49	INRAN/Niger	WARDA
19		GIZA 175	INRAN/Niger	Egypt
20		BG 90-2	INRAN/Niger	

4.2.7. Experimental design

The experiment design was a standard split–split plot with two replicates. The treatments were five salt levels: 10 dSm^{-1} (Shannon), 10 dSm^{-1} (Gregorio), 12 dS m^{-1} (Shannon), 12 dSm^{-1} (Gregorio) and control, and 20 rice genotypes. The salt levels were assigned as the main plot factor and the treatments of genotypes were assigned as sub-plot factors. All factors were considered as fixed.

4.2.8. Data collection

Data were recorded at both vegetative stage and maturity.

4.2.8.1.Scoring

The symptoms of salt stress for each plant were recorded 42 days after salt treatment according to the standard evaluation system (Table 4.5) used at International Rice Research Institute (Gregorio and Senadhira, 1995). Five evaluation grades were used: 1. Normal growth, no leaf symptoms; 3. Nearly normal growth, but leaf tips or few leaves whitish and rolled; 5. Growth severely retarded; most leaves rolled; only a few are elongating; 7. Complete cessation of growth; most leaves dry; some plants dying; 9. Almost all plants dead or dying. The Figure 4.1 shows examples of plants states and the given score during our evaluation.

Table 4.5: IRRI standard evaluation system for salt symptoms scoring

Score	Observation	Tolerance
1	Normal growth, 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

Figure 4.1: Scoring of rice germplasm for tolerance to salt stress

4.2.8.2. Data collected

Salinity induces among other things white leaf tip followed by tip burning and leaves death. The older leaves dying first. So the number of green leaves of each genotype on the main Culm was scored before senescence. Tillering ability is also a good characteristic of tolerant genotypes as salinity induces low tillering. The number of tillers per genotype was counted at maximum tillering stage. Panicle number per plant, the panicle weight, flag leaf weight, and dry weight were also recorded at maturity stage.

4.2.8.3.Selection indices

Weights (Wi) for the selection index were allocated based on the relative importance of each measured trait as an indicator of salinity tolerance (Efisue, 2007) (Table 4.6). The results from the participatory rural appraisal on preferred traits rice farmers were also considered in assigning weight on each trait. The selection index (SI) of each genotype was calculated as:

SI = PiWi+ PjWj+ ---------- + PnWn (Efisue, 2007)

Where Pi = standardized phenotypic value of the trait observed; Wi = is the assigned weight value to the trait in the selection index.

Table 4.6: Sign and weight based on the relative importance of traits in the selection index

Traits	Sign	Weight (Wi)	Importance
Salinity Score	-	5	Little or no leaf symptoms
Tillers Number	+	3	High tillering ability
Panicle number	+	4	High panicle number
Green leaves number	+	2	More green leaves
Panicle weight	+	4	Heavy panicle
Dry weight	+	1	More biomass

NB: negative sign (-) = not desired; Positive sign (+) = desired

4.2.9. Data analysis

SAS 9.2 software was used for statistical analysis. Proc univariate procedure was used for descriptive statistics. The CORR Procedure was used for association analysis and GLM Procedure for analysis of variance. The LSD method was used for means separation. Excel 2007 was used for plots drawing.

4.3.Results

4.3.1. Visual scoring for salt tolerance

The average mean score ranged from 4 (tolerant) to 9 highly susceptible (Table 4.7). The local genotypes were all susceptible. Some IRRI genotypes known as salt tolerant also showed some susceptibility.

Table 4.7: Genotypes behavior under salt stress based on visual scoring

Genotypes	Scoring	Tolerance
Pokkali	4	Tolerant
IRRI 126	4.5	Tolerant
IRRI 113	4.8	Tolerant
IRGC 17229	5.5	Moderately tolerant
NSIC RC 106	5.5	Moderately tolerant
GIZA 175	6	Moderately tolerant
IRGC 17041	6.1	Moderately tolerant
IRRI 124	6.8	Moderately tolerant
CSR 36	6.8	Moderately tolerant
GAMBIAKA	7	Susceptible
NERICA L49	7	Susceptible
BG 90-2	7.3	Susceptible
IRGC 17038	7.5	Susceptible
IRGC 30	7.8	Susceptible
IRGC 249	8	Susceptible
IRGC 37179	8	Highly susceptible
IRGC 37084	8.5	Highly susceptible
IRGC 15963	8.7	Highly susceptible
IRGC 26612	9	Highly susceptible
IR1529-680	9	Highly susceptible

4.3.2. Traits analysis

4.3.2.1. Analysis of variance of traits

Table 4.8: Traits ANOVA table

Source	DF	TN	GLN	PN	PW	DW
Replication	1	364.59	15.68	450	415.64	4433.05
Treatment	4	7093.36***	680.4 ***	1728.35**	3000.44**	15276.94**
Replication*Treatment	4	54.05 ns	51.22**	170.85 ns	191 ns	1452.59ns
Genotype	19	1039.0 ***	261.88**	4682.70**	6350.37**	36080.92**
Treatment*Genotype	76	1453.31ns	236.32ns	5685.45*	7667.09*	46630.85ns
Error	94	1691.31	220.10	3894.15	5391.37	32169.40

*, **, and *** significant at 0.05 , 0.01 and 0.001 probability level respectively. TN= Tillers number, GLN = green leaves number, PN= panicle number, DW = dry weight.

The number of tiller significantly differed among genotypes. Differences also existed among treatments across genotypes (Table 4.8). Significant differences existed among treatments and also among genotypes in terms of green leaves number. The later trait was significantly influenced by the interaction between replication (block) and treatment. Genotypes were significantly and differently affected by salt treatments as far as panicle number is concerned. The panicle number was significantly affected by genotype treatment interaction. In terms of panicle weight some evidences showed that there was significant variability among genotypes as well as among treatments. An interaction also existed between salt level and genotypes for this trait. The analysis of variance also showed that biomass produced greatly varied among genotypes and also among treatments.

4.3.2.2.Traits statistics

The traits measurement across genotypes and treatments showed a maximum of 44 tillers per plant and a minimum of 0. The panicle number ranged from 0 to 36. The panicle weight varied from 0 to 44g. For all the traits it was observed a high standard deviation and a minimum of zero (Table 4.9).

Table 4.9: Means and standard errors for agronomic traits across genotypes and salt levels

Variable	Mean	Std Dev	Range	Probability
GL	2.96	2.71	0-9	**
TN	5.35	7.68	0-44	**
PN	5.95	9.13	0-36	**
PW	6.41	10.75	0-43.77	**
FlW	0.89	1.28	0-5.8	*
DW	17.97	26.27	0-91.23	***

*, **, and *** significant at 0.05 , 0.01 and 0.001 probability level respectively. TN= Tillers Number, PN= panicle number, GL= green leaves number, PW= panicle weight, FlW= flag leaf weight, DW=dry weight.

4.3.2.3.Traits association

The tiller number (TN) and green leave number (GL) had strong positive association (Table 4.10). Genotypes that had good tillering ability had also the ability to keep longer their leaves green. But both had weak positive association with all the other traits. A strong significant positive association also existed between Panicle number, panicle weight, and flag leaf weight. Traits such as panicle number, panicle weight, and flag leaf weight significantly co-vary. Thus, the higher panicle number was associated with heavier panicle and heavier flag leaf. Genotypes that produced lesser panicles under salt stress bore lighter panicles and lighter flag leaf.

Table 4.10: Correlation coefficients among studied traits of 20 rice genotypes

Traits	GL	TN	PN	PW	FlW
TN	0.70 **				
PN	0.29 *	0.29*			
PW	0.32 *	0.30*	0.90***		
FlW	0.35*	0.28*	0.70**	0.70**	
DW	0.29 *	0.23*	0.88 ***	0.86**	0.75**

*, **, and *** significant at 0.05, 0.01 and 0.001 probability level respectively. TN= Tillers Number, PN= panicle number, GL= green leaves number, PW= panicle weight, FlW= flag leaf weight, DW=dry weight.

4.3.2.4.Tiller number

All the genotypes had their tiller number reduced, the tolerant having more tillers than susceptible except for Pokkali that did not produce many tillers even in salt free condition. The highly susceptible genotypes did not have tillering ability under salt condition. Thus, salt level induced significant reduction of tillers compared to the control (Figure 4.2).

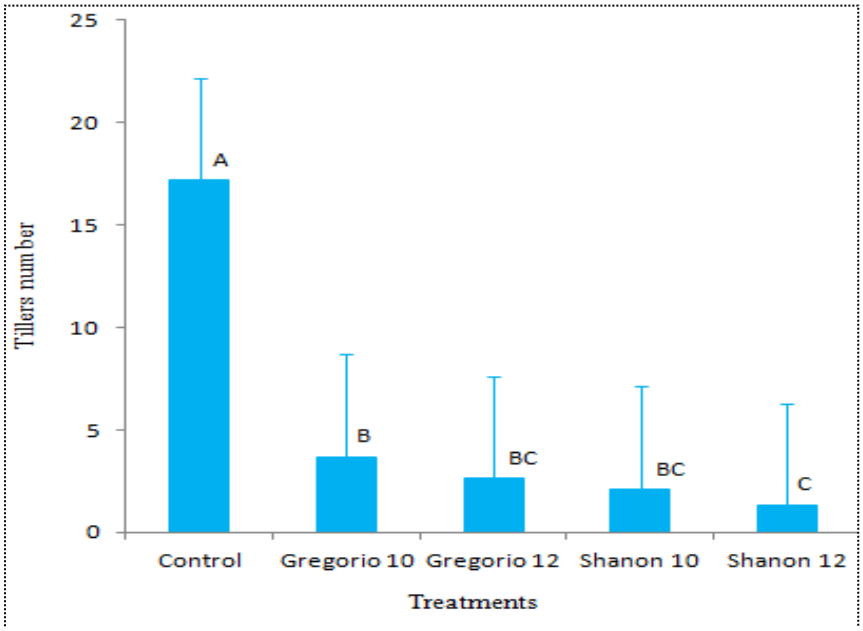

Figure 4.2: Tiller number means across treatments.

NB: Means with the same letter are not significantly different

A reduction of tiller number was observed for all the genotypes in salt stress condition compared to the control. Differences were observed among salt treatments Shanon12 being the most severe in terms of tiller reduction. Treatments means comparison displayed 3 groups: The control was the group1. Shannon 10, Gregorio 10 and Gregorio 12 formed group2. Shannon10, Shannon12 and Gregorio 12 composed the group3. Thus, the control mean was significantly different from the others. Shannon 12 mean was significantly different from Gregorio10. But the experiment failed to display significant differences between Shannon10, Shannon12 and Gregorio12.

Genotypes means comparison (Figure 4.3) displayed four groups the most performing being IRGC 17229, IRRI 126 and IRRI113. The second group was composed of IRRI 126, IRRI113, IRGC30, and Pokkali.

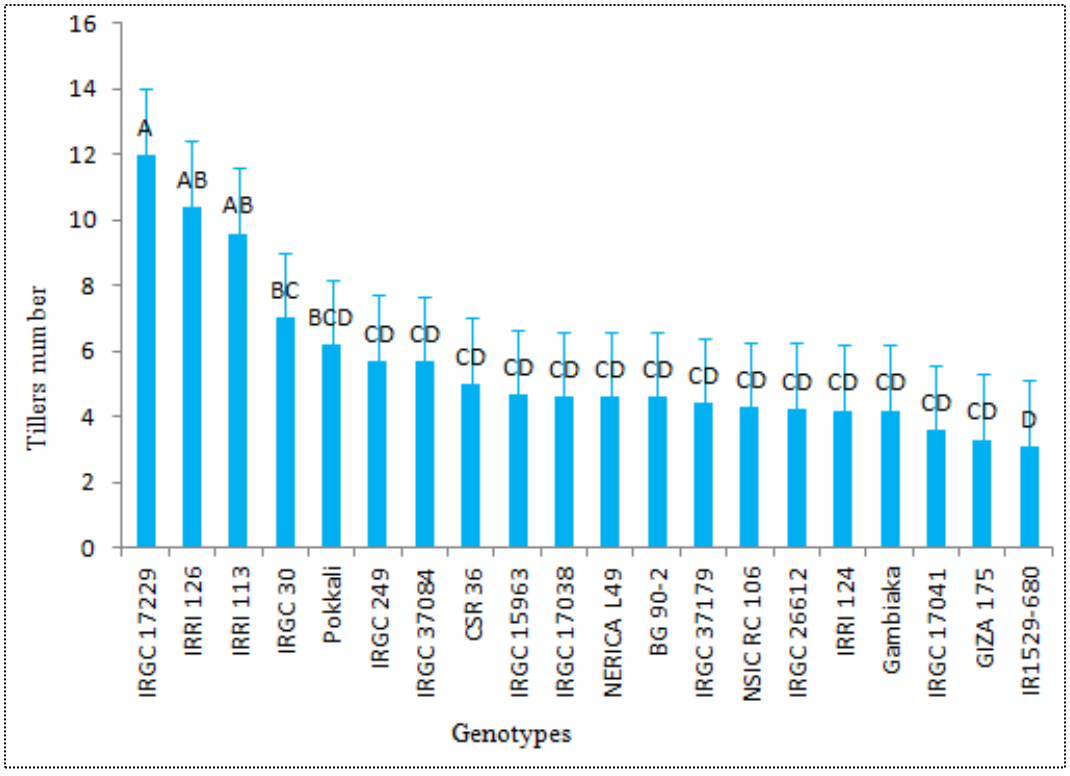

Figure 4.3: Genotypes tillers number means comparison

NB: Means with the same letter are not significantly different

4.3.2.5.Green leaves

Leaves number was significantly affected by salt level compared to the control (Figure 4.4). The extent of leaves loss depended on the genotypes and the salt concentration. Higher concentration occasioning more leaves losses. Treatment means separation showed that Shannon 12 affected more significantly the green leaves number than Gregorio 10 treatment. But the difference among Shannon 10 and 12 and Gregorio 12 was not significant.

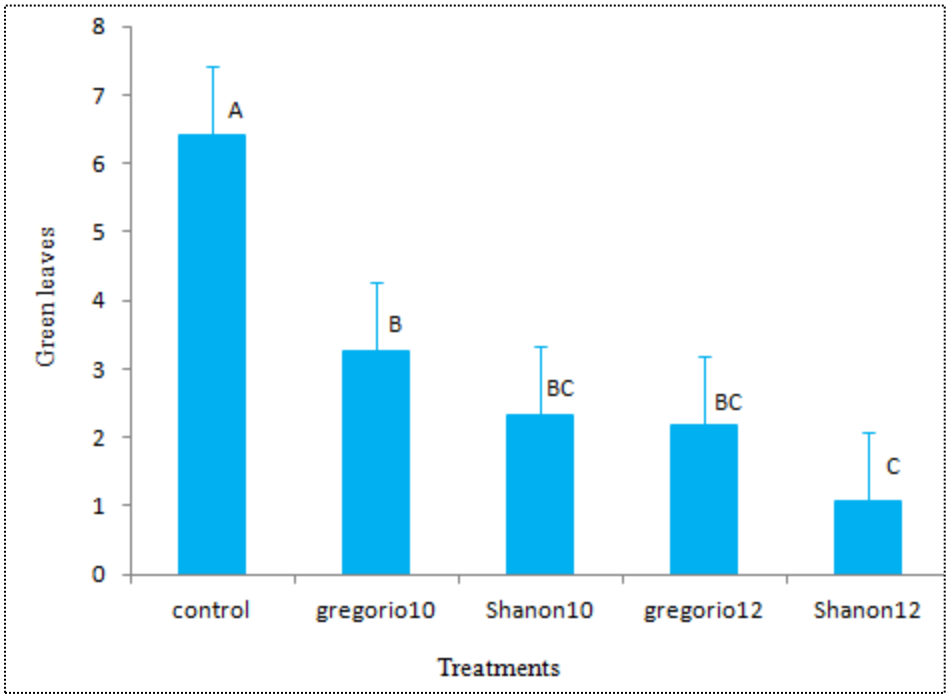

Figure 4.4: Green leaves means comparison across treatments.

NB: Means with the same letter are not significantly different

The means comparison across genotypes (Table 4.11) showed that Pokkali, IRRI126, IRGC 17229, IRRI113 and NSIC RC 106 had significant ability to keep more green leaves in salt condition. IR1529 and Gambiaka had poor performance of bearing functional leaves in salt condition. Significant diversity existed among genotypes concerning this trait.

Table 4.11: Means comparison of green leaves number

Genotypes	Green leaves number	Group
Pokkali	5.1	A
IRRI 126	4.6	AB
IRGC 17229	4.3	AB
IRRI 113	4.1	ABC
NSIC RC 106	4.1	ABC
GIZA 175	4.0	ABC
IRGC 17041	3.5	ABCD
IRGC 17038	3.4	ABCD
CSR 36	3.3	ABCDE
NERICA L49	3.2	BCDE
IRRI 124	3.1	BCDE
IRGC 30	2.6	CDEF
BG 90-2	2.6	CDEF
IRGC 37084	2.5	CDEF
IRGC 249	2.4	CDEF
IRGC 37179	2.4	CDEF
IRGC 15963	1.9	DEF
IRGC 26612	1.5	EF
Gambiaka	1.5	EF
IR1529-680	1.0	F
$LSD_{0.01}$=1.8		

NB: Genotypes followed by the same letter are not significantly different

4.3.2.6.Panicle number

Number of panicle differed significantly among genotypes within treatment and among salt levels. Effects of salt treatments were significantly different from the control. Salt levels had also significant different effect on panicle number (Figure 4.5).

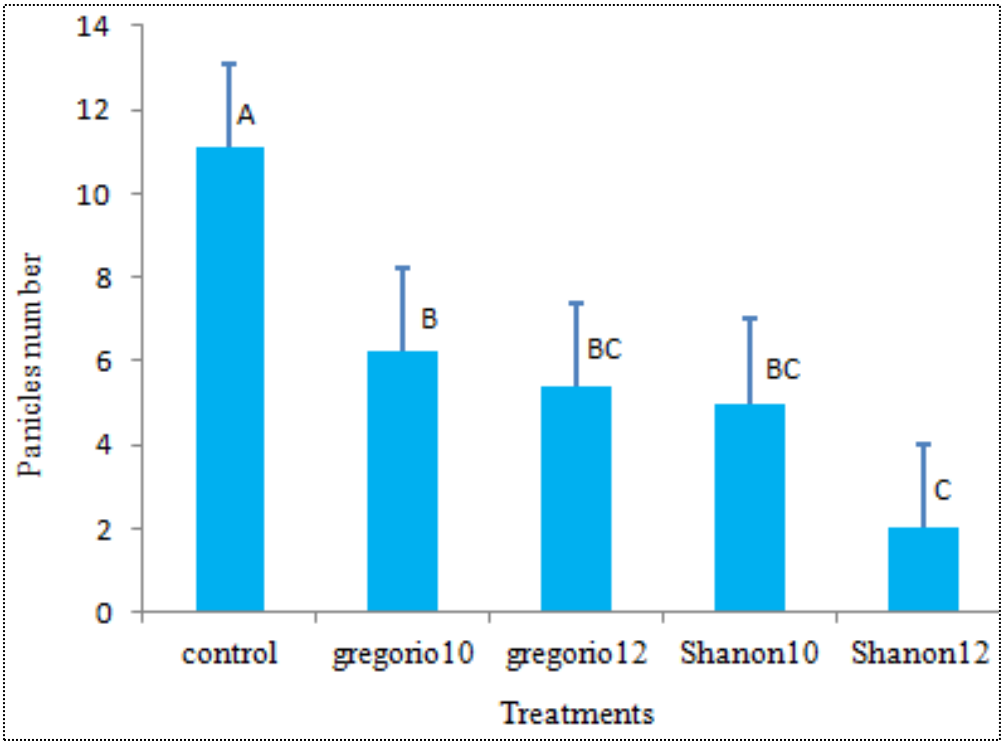

Figure 4.5: Means comparison panicle number across treatments.

NB: Means with the same letter are not significantly different

Means comparison (Table 4.12) showed that panicle number differed significantly among genotypes across treatments. There was clear evidence that the best genotypes concerning panicle number were IRRI113, IRRI126, IRRI124, NSIC RC 106.

Table 4.12: Means comparison of panicles number

Genotypes	Panicles number	Groups
IRRI 113	14.7	A
IRRI126	12	AB
IRRI 124	11.4	BC
NSIC RC 106	7.7	BCD
IRGC 30	7	BCD
GIZA 175	6.7	BCD
Pokkali	6.3	BCD
CSR 36	6.2	CD
NERICA L49	6	CDE
IRGC 17041	4.8	DE
IR1529-680	4.3	DE
GAMBIAKA	3.6	DE
IRGC 17038	3.5	DE
IRGC 37084	3.2	DE
IRGC 15963	1.1	E
IRGC 26612	1.1	E
BG 90-2	1	E
IRGC 17229	1	E
IRGC 249	0.5	E
IRGC 37179	0.5	E
$LSD_{0.01}$= 5.684		

NB: Genotypes followed by the same letter are not significantly different

4.3.2.7.Panicle weight

Significant weighty panicles were obtained in salt free conditions (control) compared to the treatments (Figure 4.6). Salt treatments also led to significant different result in terms of panicle weight. Two groups were clearly distinguished, Gregorio 10 and 12 and Shannon 10 in one side and Shannon 10 and 12 and Gregorio 12 in the other side. This means that only Shannon 12 and Gregorio 10 gave significantly different impact on panicle weight.

Genotypes means comparison (Table 4.13) showed that there was strong evidence that IRRI113, IRRI126, IRRI124 and NSIC RC 106, were genotypes that bore the heaviest panicles. However, they shared the same group with CSR 36, Pokkali and Giza 175.

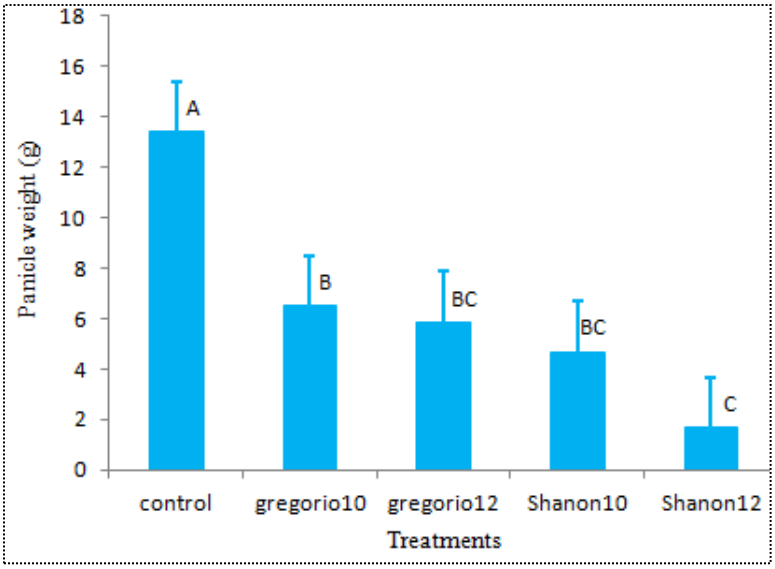

Figure 4.6: Panicle weight means comparison across treatments.

NB: Means with the same letter are not significantly different

Table 4.13: Genotypes panicle weight means comparison.

Genotypes	Panicle weight (g)	Groups
IRRI 113	19.50	A
IRRI 124	17.39	AB
IRRI 126	12.97	ABC
NSIC RC 106	12.66	BC
IRGC 30	8.79	CD
CSR 36	8.64	CD
Pokkali	8.06	CD
GIZA 175	6.68	CD
IR1529-680	5.36	D
IRGC 17229	4.91	D
GAMBIAKA	4.87	D
BG 90-2	4.25	D
IRGC 249	4.25	D
NERICA L49	3.71	D
IRGC 17041	3.12	D
IRGC 37179	1.75	D
IRGC 37084	1.47	D
IRGC 17038	0.77	D
IRGC 26612	0.18	D
IRGC 15963	0.15	D
LSD 0.05 = 6.7		

Genotypes with the same letter are not significantly different

4.3.2.8.Dry weight

Clear evidence of superior biomass of the control was shown compared to salt treatments. Hence, genotypes produced more biomass in salt free conditions (Figure 4.7). Differences were also observed among salt treatments. Thus, Gregorio10 and Shannon 12 had significantly different effect on dry weight. However, Gregorio 12 and Shannon 10 showed similar biomass reduction like Gregorio 10 and Shannon 10.

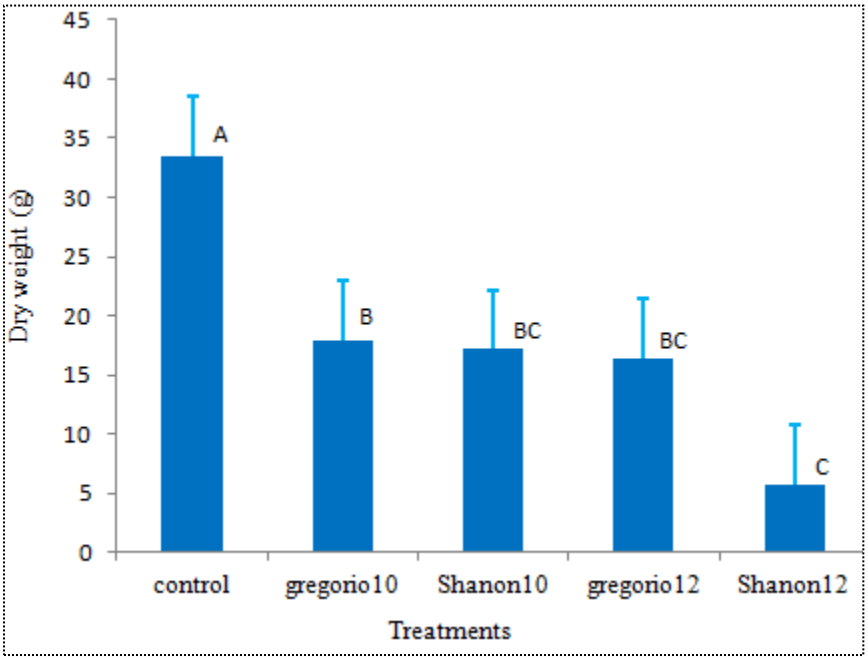

Figure 4.7: Dry weight means comparison across treatments.

NB: Means with the same letter are not significantly different

The top group of genotypes in terms of biomass included IRRI126, Pokkali, IRRI113 and CSR36 (Table 4.14). Genotypes that produced lesser dry weight were IRGC 249, IRGC 37179, IRGC 17229, IRGC 26612 and IRGC 15963.

Table 4.14: Genotypes dry weight means comparison.

Genotypes	Dry weight (g)	Groups
IRRI 126	46.313	A
Pokkali	38.1625	AB
IRRI 113	37.716	ABC
CSR 36	30.327	ABCD
IRGC 30	29.391	BCDE
IRRI 124	25.553	BCDEF
GIZA 175	22.561	BCDEFG
NSIC RC 106	22.435	BCDEFG
IRGC 17041	21.551	CDEFG
NERICA L49	17.465	DEFGH
GAMBIAKA	17.372	DEFGH
IR1529-680	16.492	DEFGHI
IRGC 17038	13.118	EFGHI
BG 90-2	9.269	FGHI
IRGC 37084	7.566	GHI
IRGC 15963	3.637	HI
IRGC 26612	3.446	HI
IRGC 17229	1.188	HI
IRGC 249	0.25	I
IRGC 37179	0.225	I
$LSD_{0.05}$ =16.43		

NB: Genotypes with the same letter are not significantly different

4.3.3. Selection index

The selection index value ranged from -20.68 to 187.5 (Figure 4.8). Based on selection index the best five (5) genotypes were IRRI113, IRRI126, IRRI124, Pokkali and NSIC RC106 with respectively a value of 187.5, 164, 125.5, 104.4 and 97.5.

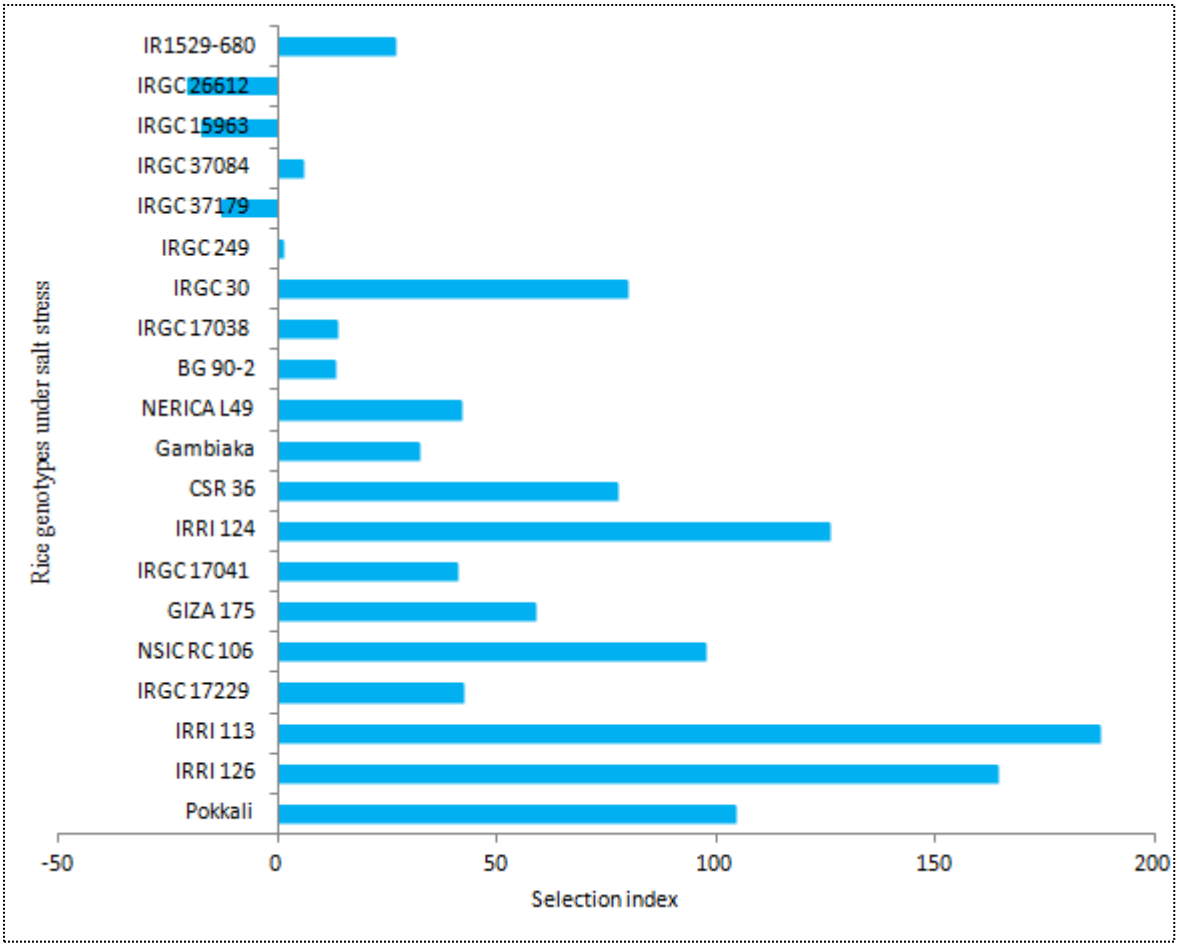

Figure 4.8: Selection index of 20 rice genotypes under salt stress

4.3.4. Principal component analysis

The first two principal components (PC1 and PC2) explained 67% and 17% of the total variation, respectively. The main traits that had the highest loading scores (and hence contributed most for the differences) were the scoring index, panicle number, panicle weight and dry weight for component 1, tiller number and green leaves for component2. The Figure 4.9 showed the two grouping patterns.

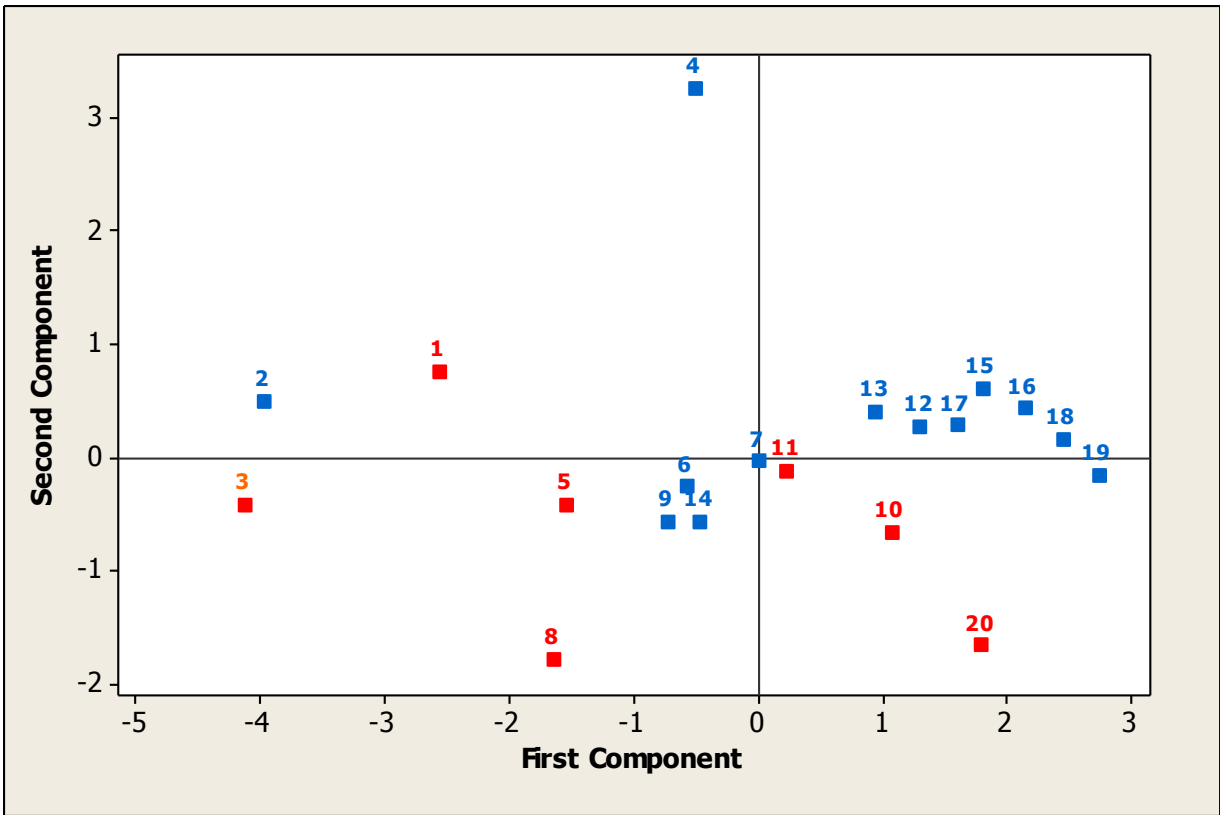

Figure 4.9: Principal component analysis of 20 parents:

NB: Numbers in the figure indicate the 20 genotypes as in table 4.4

These components were used to draw a dendrogram with single linkage and Euclidean Distance (Figure 4.10). At a distance of 3.96 thirteen clusters were distinguished. The local genotypes (Gambiaka, IR1529 and NERICAL49) were in the same cluster. At a distance of 7.92 five groups were separated. Pokkali, IRRI126, IRRI113 were each and alone in separate group.

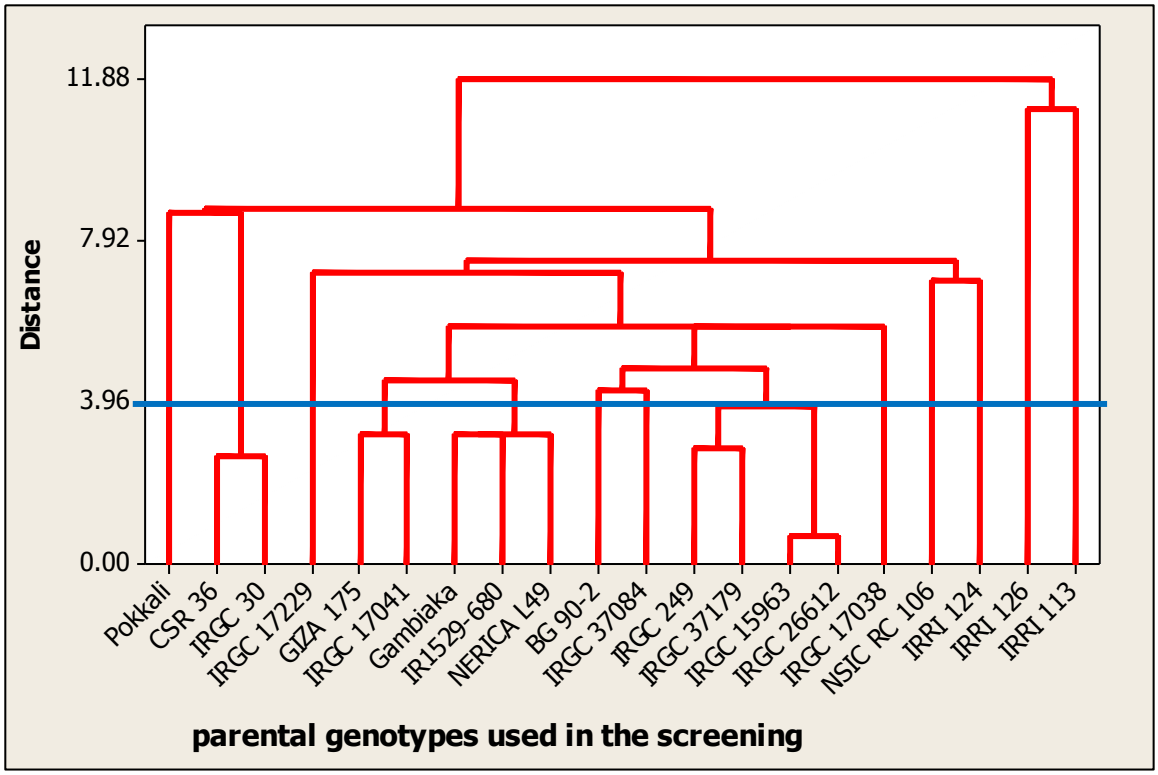

Figure 4.10: Dendrogram of 20 parental genotypes

4.4.Discussion

4.4.1. Genetic variability and performance of genotypes under salt stress

All the tolerant genotypes observed came from IRRI. These included Pokkali known, to be highly salt tolerant (Xie *et al.*, 2001; Gregorio *et al.*, 2002; Ismail *et al.*, 2007). However, some IRRI genotypes, known as salt tolerant showed susceptibility. This may be due to confined experimental conditions that may not be conducive to root growth. Gregorio and Senadhira (1993) reported the clear relation of low Na-K ratio to salinity tolerance. Na-K ratio in shoot is also the best indicator of grain yield (Gill and Singh, 1995). Moreover, Na-K ratio is related to visual score (Lee and Senadhira, 1996). The score based on visual symptoms related well to grain yield and yield reduction due to salt stress (Saharay and Amirul Islam, 2008).

Results indicated salinity had a profound influence on plant tillers number. Effects of salinity on the number of tillers were reported by researchers (Aschl *et al.*, 2000; Beatriz and Bernstein, 2001) found a reduction of tiller number under salt stress. This is due salt stress because high salinity interferes with plant growth and development and can also lead to physiological drought conditions and ion toxicity. Therefore, high salinity affects mostly all aspects of plant physiology and metabolism (Zhu, 2002) including germination, vegetative growth and reproductive development (Munns, 2002). These results showed that rice is also salt sensitive at the vegetative stage; this is different from many studies which have shown that rice is salt sensitive plant during the early seedling stage and gains resistance during vegetative growth (Kaddah *et al.*, 1973; Bhattacharya, 1981; Abdullah and Khan, 1997). However, no significant difference existed among the three levels of salt treatments effect (Gregorio 10 and 12 and Shannon 10) because they were too close (10dS/m and 12 dS/m). Significant differences existed among genotypes under salt conditions. This may be due to genotypic variability among genotypes.

More green leaves indicate more photosynthesis and more production of organic matter. This means more accumulation in stem and sheet for translocation at the grain filling stage. Genotypes with more green leaves may have more tissue tolerance to salt toxic affect. This may also be a result of salt exclusion at root or cellular level. Genotypes under salt conditions bore fewer green leaves than controls. This was due to salt affect because salinity induces leaf senescence (Yeo and Flowers, 1983; Leidi *et al.*, 1991; Yeo *et al.*, 1991). If excessive amounts of salt enter the plant, the concentration of salt will eventually rise to a toxic level in older transpiring leaves causing premature senescence and reduced photosynthetic leaf area of a plant to a level that cannot sustain growth (Munns, 2002; Hassan *et al.*, 2012). A statistically significant difference was recorded among genotypes and the green leaves number was always higher in salt-tolerant genotypes (Pokkali, IRRI126, IRRI113, NSIC RC106, IRRI124 etc...), whatever the NaCl dose. In rice, concentration of salt in leaves is found to cause different degrees of toxicity in different varieties, which is termed tissue tolerance (Yeo and Flowers, 1983; Ismail *et al.*, 2007). According to Al-Karaki (2000) 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 $Ca2^+$ depletion.

Panicle weight was decreased by salinity compared to the control, because, according to Aschl and Marco (2001), salinity in reproductive stage, decreases the number of filled panicles, fertile panicle, weight of 100 grains and percentage of fertile grains and increases fertile tillers. Tiller number per plant and spikelet number per panicle contributed the most variation in grain weight per plant under salinity. Reductions in tiller number per plant, and spikelet number per panicle were the major causes of yield loss (Zeng and Shannon, 2000b). Again there was genotypic variation in the response of these genotypes to salinity.

All genotypes that had no tillering ability, little or no green leaves, and lacked panicles were also scored high: these were the susceptible genotypes. Some have more tillers, more green leaves and less panicle weight. This is the result of tolerance at vegetative stage and susceptibility at reproductive. This may be due either to tiller non productivity (tillers did not flower) or florets abortion. The tolerant genotypes at vegetative stage had highest dry matter quantity. Tolerant genotypes had high positive selection index and susceptible genotypes had low or negative selection index.

4.4.2. Selection of salt tolerant genotypes

Based on the selection index the best salt tolerant parents were IRRI113, IRRI126, IRRI124, Pokkali, and NSIC RC106 respectively first, second, third, fourth, and fifth. In terms of selection index the best genotype known as salt tolerant (Pokkali) was the fourth. This is because Pokkali despite its ability to withstand high level of salt remains poorer in terms of agronomic performances: poor tillering ability, few panicle and poor yield. IRRI113 and IRRI126 respectively first and second in terms of selection index had less scores under salt stress compared to Pokkali but they had better agronomic performances such as high tillering ability, high panicle number and better yield. Besides all the dendrogram showed that at a distance of 11.88 the two genotypes were in the same group when all the others appeared in one other group. These two genotypes seem to be related. This is a valuable reason to select only one of them along with the 3 follower in terms of selection index. These 4 genotypes and the 3 farmers preferred varieties were selected to serve as parents in our breeding program.

4.5.Conclusion

Evaluation of twenty genotypes in pots showed different responses under salt stress for all the traits measured. The scoring under different levels of salt stress showed that the genotypes ranged from tolerant (low score) to highly susceptible (high score). Salt affects significantly all the genotypes growth development and performances compared to salt free condition. However, significant differences existed among genotypes within salt conditions as well as within genotype across salt conditions. For the same traits in the same salt level genotypes were significantly different. For the same genotype significant different responses were observed with different salt condition. Analysis of the data suggests that genotypic variation exists in the extent to which salinity affects aspects of the plants vegetative and reproductive physiology and development. Selection index combined overall performance of the genotypes for all the traits across treatments. The selection index ranged from negative index for the highly susceptible to a high positive index for the tolerant. The best performing varieties were IRRI113, IRRI126, IRRI124, Pokkali and NSIC RC 106. This huge variation will be used in attempts to enhance the tolerance of rice to salinity. For this purpose the best salt tolerant genotypes (IRRI113, IRRI124, Pokkali and NSIC RC 106) and local ones (Gambiaka, IR1529, NERICA L49) will be used in hybridization for further studies.

CHAPTER FIVE

5.0. GENETIC MECHANISMS CONTROLLING SALT TOLERANCE IN F3 POPULATIONS OF RICE

5.1. Introduction

One of the major inputs for increasing crop productivity is irrigation. But irrigation over a long period of time exacerbates soil salinity problems (Singh and Singh, 2000). Genetic manipulation of crop plants to develop genotypes tolerant to saline conditions is, one of the most cost effective means of maintaining crop production in areas with salinity problem (Blum, 1988; Singh and Singh, 2000). Successfully changing the characters of the population using hybridization is predictable only from knowledge of the degree of correspondence between phenotypic values and breeding values. An important function of the heritability parameter is expression of the degree of correspondence between the phenotypic and genotypic values (Munshower, 1994). Narrow sense heritability, expresses the extent to which phenotypes are determined by the genes transmitted from the parents and the proportion of the total variance that can be attributed to the average gene effects (Falconer, 1989; Falconer and Mackay, 1997; Fahliani *et al.*, 2010). Genotypic expression is, however, influenced by environmental factors (Falconer, 1989).

The release of rice cultivars with improved performance in saline environments is reliant on an understanding of genetic control of traits of plant exposed to salinity. This study focused on studying genetic control of salt tolerance in F3 populations

The specific objectives were to:

- ⇨ assess the degree of inheritance of salt tolerance in rice.

- ⇨ determine the gene action involved in salt tolerance of rice
- ⇨ assess the genotype by environment interaction.

5.2.Materials and Methods

5.2.1. Diallel (7*7) Crossing blocks

Planting was staggered for flower synchronization at interval of a week. Seven crossing blocks were established (Figure 5.1) and for each block one entry of the seven was considered as male (i) Pokkali (ii) IRRI113 (iii) IRRI124; (iv) NSICRC 106; (v) NERICA L49; (vi) Gambiaka, and (vii) IR1529.

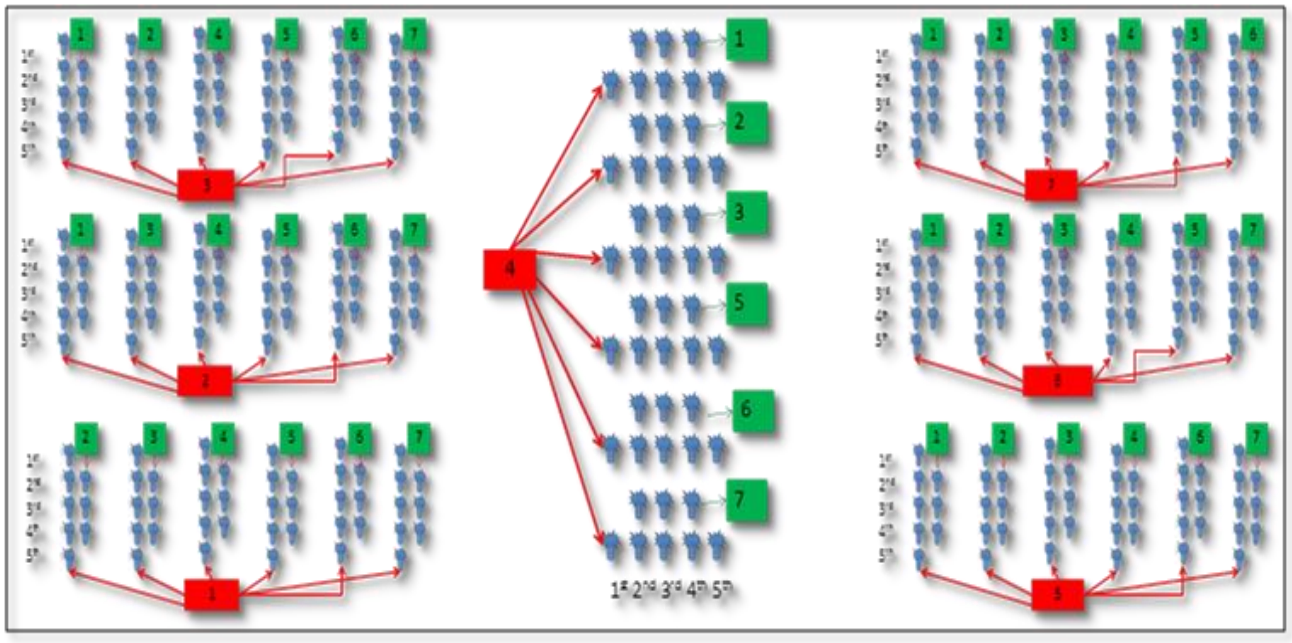

Figure 5.1: crossing design

NB: 1= Pokkali, 2=IRRI 113, 3= IRRI 124, 4= NSIC RC 106, 5=NERICA L49, 6=Gambiaka, 7= IR 1529. Number in red color = male; Number in green color = female

5.2.2. Crossing

When the panicles were 50-60% emerged, they were selected and separated from surrounding ones to make it easy to work on. The upper and lower florets were removed with scissors. All florets from the top that had undergone anthesis (extrusion of anthers) were cut away as well as the young florets from the bottom of the panicle. The top of each spikelet in a panicle was cut off at a slight angle using scissors and the six anthers were removed with forceps for emasculation. When every spikelet of the panicle was emasculated the emasculated parent was placed in proximity of pollen parent. When on the pollen parent anthers were beginning to be extruded so their pollen could be shed, the two panicles were covered with pollination bag. The bag was then closed with paperclip (Figure 5.2) and was regularly shaken during midday of 3 days after crossing.

5.2.3. First and second generations handling

After harvesting F_1 seeds were exposed to ambient temperature and stored for 45 days in oder to break seed dormancy. Then F_1 seeds and parents were sown in pots under green house during the dry season of 2013 (Figure 5.3). Each pot was a round plastic pot of 20 cm diameter (314 cm^2). Soil was collected from rice fields not affected by salinity. The first fertilization was done when plantlets were at 5 leaves stage. The fertilizer used was NPK 15-15-15 at a rate of 2 grams/pot. The second fertilization was done at booting stage with urea at the rate of 2 grams/pot. Irrigation was done once a day at the early vegetative stage and twice at maximum tillering to maturity. The F_2 seeds were harvested at 95% maturity. F_2 seeds and parents were sown at the experimental station of Sebery during the wet season of 2013. The transplantation was done at 4

leaves stage. The NPK fertilizer was applied just after the transplantation and urea at panicle initiation. Weeding was done by using herbicide (Londax) and manually.

5.2.4. Sites characteristics

The study was carried out on two sites namely, Saga with a latitude of 13°28'N and a longitude of 2°08' and Sekoukou latitude of 13°15'N and a longitude of 2°22'. The F_3 evaluation was conducted on farmer's rice fields known to be affected by salt problem. The soil properties of these sites were reported in chapter 4.

The average annual rainfall from 1950 to 2010, ranged from minimum of 321mm to a maximum of 796 mm (Figure 5.2). The average minimum temperature from 1950 to 2010 varied from 21 to 24°C and the average maximum varied from 35 to 39°C.

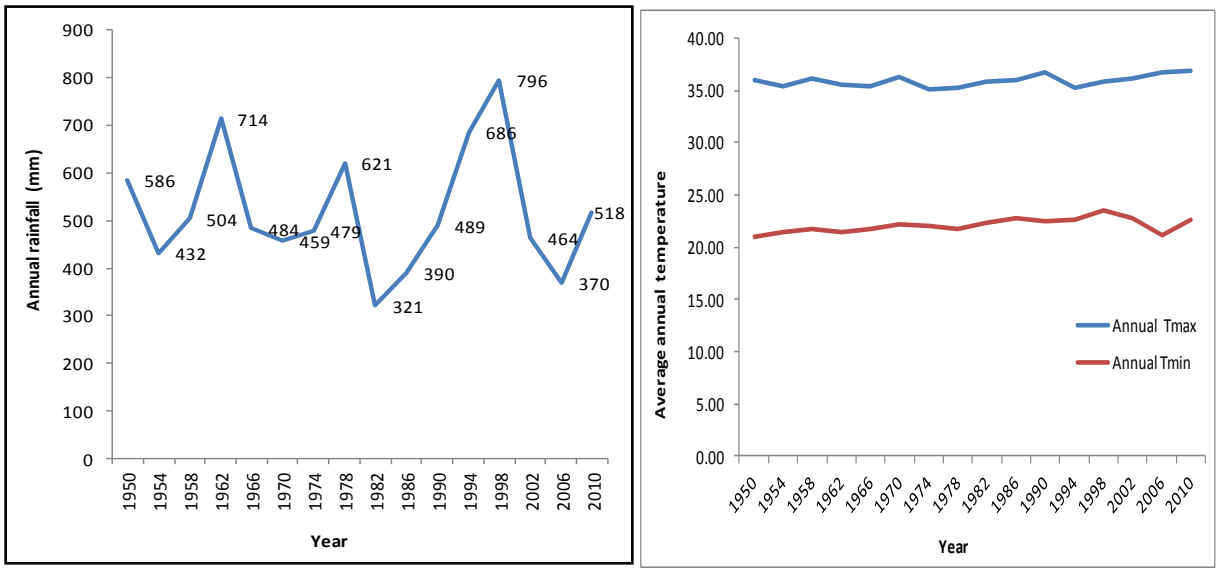

Figure 5.2: annual rain and temperature from 1950 to 2010.

5.2.5. Plant material

Because of problem of F_1 seeds germination; seedling death and abortion of F_1 plants flowers that were experienced in some crosses. Only one hundred twenty (120) F_3 families derived from the cross in diallel design of four parents (IRRI113, NSIC RC 106, Gambiaka, and IR1529), and the parents were tested in farmer's field conditions.

5.2.6. Experimental Design

The experimental design was Alpha lattice (25*5) with five blocks and three repetitions. Each incomplete block contained 25 lines with 10 plants on lines and each line was constituted of one F_3 family. The inter-plant space (on line) was 0.2 m and the between line space was 0.5 m. RCBD design with 3 replications, was used for the F_2 evaluation respecting the same spacing as for F_3.

5.2.7. Data scoring

At maturity plant height, total tillers number per plant, reproductive tillers number per plant, panicle number, panicle weight, and paddy yield were scored. The time to 50% flowering, time to 85% maturity were also recorded.

5.2.8. Data analysis

Data were analyzed using SAS software version 9.2 and GenStat version 9.2.

A general ANOVA was performed using SAS Glm procedure with random effect Model. The linear model used was: $y_{ijg} = \mu + \tau_i + \beta_j + e_{ijg}$.

Where: μ = *mean*

τ_i = ith treatment effect where i=1,t

β_j = jth block effect where j=1,b, where blocks were random

g = n_{ij} where n_{ij} =0 if the ith treatment doesn't appear in the jth block

n_{ij} =1 if the ith treatment does appear in the jth block

$e_{ijg} \sim (0,\sigma^2)$ = error.

The intra-class correlation (ICC) was calculated as follow: ICC= $\delta^2_A/ (\delta^2A + \delta^2)$ (Dowdy *et al.*, 2004). Where δ^2_A = among group variance = (MSa-MSe)/n (MSa = means square among, MSe = means square error, n = number of individual). $\delta^{2\,=}$ Within Group variance: $\delta^{2\,=}$ MSe.

An estimator for broad sense heritability for the family means is:

$$\hat{h}_f^2 = \frac{\hat{\sigma}_F^2}{\hat{\sigma}_P^2}$$

Where σ^2_F is the family variance component in the reference population and σ^2_P is the phenotypic variance of family mean deviation.

The heritability was estimated using REML Method. Proc mixed asycov, and proc iml were used. The Rep, block (rep) and entries were considered as random. Mid parent offspring regression was also used for heritability estimation.

The Hayman diallel model (Hayman, 1954) was used for gene action study. The model used is shown below.

Y = U + rep + a + b + c + d + a*rep + b*rep+ c*rep+ d*rep, where

- U = grand mean
- rep= replication effects

- a = additive effects

b = dominance effects

Partitioning of dominance effects (b)

b = is partitioned into:

- b1, indicates direction of dominance (unidirectional if significant; equiv. to Parent vs. crosses contrast)
- b2, tests asymmetry of alleles
- b3, shows that some dominance is peculiar to some crosses

Y = U+rep + a+b+c+d+a*rep+b*rep+ c*rep+ d*rep

  - b*rep partitioned into: rep*b1, rep*b2 and rep*b3

- c = additive maternal effects
- d = maternal interaction effects

Interactions of reps with genetic effects

- a*rep + b*rep+ c*rep+ d*rep = interaction of the reps with the model components

5.3.Results

5.3.1. Traits analysis of variance (ANOVA) across families

ANOVA showed that significant differences existed among F3 families for all the traits across the two environments (Table 5.1).

Table 5.1: ANOVA table of nine traits across families.

Source of variation	DF	Yield	Pwt	Tpwt	Gwt	Pnum	Tnum	Height	Mat	Flw
Saga										
Block(Rep)	14	76.89***	25.99***	13423***	7689.33***	1608***	2024.8***	2752.8***	362.3***	362***
Family	124	49.36***	11.38***	6760.***	4936.4***	1599***	1254***	856.7***	1244.7***	1244***
Sekoukou										
Block(Rep)	14	105***	4.4***	10783.2***	10519.1***	4834.4***	6460.48	3275.8***	1763.4***	1763.4***
Family	124	9.8***	6.2***	1257.2***	985.3***	3427.9***	4076.54	366.8***	469***	468.9***

***= significant at 0.001 probability level. Pwt= panicle weight, Tpwt = total panicle weight, Gwt = grain weight, Pnum = panicle number, Tnum= tiller number, Mat = time to maturity and Flw= time to flowering.

5.3.2. Intra-class correlation (ICC)

The intra-class correlation varied among traits and among sites for the same trait (Table 5.2). Thus, of the total variance in yield 81% and 15% were due to the differences among the families respectively at Saga and Sekoukou. From the total variance of the traits the amount that was explained by families ranged from 57% for the duration (time to flowering) to 82% for total panicles weight at Saga. At Sekoukou, the part of variance that can be attributed to family differences varies from 11% for panicle weight to 74% for the duration. The percentage of the explainable sums of squares among the total individuals in the study is given by the R-square.

Table 5.2: Intra-class correlation of traits variances across sites

Traits	Yield	Pwt	Tpwt	Gwt	Pnum	Tnum	Height	Mat	Flw
Sekoukou									
ICC	15%	11%	23%	15%	44%	43%	46%	74%	74%
R-square	14%	8%	24%	14%	22%	22%	37%	50%	50%
Saga									
ICC	81%	63%	82%	81%	70%	61%	82%	57%	57%
R-square	47%	35%	57%	47%	38%	31%	58%	21%	21%

Pwt= panicle weight, Tpwt = total panicle weight, Gwt = grain weight, Pnum = panicle number, Tnum= tiller number, Mat = time to maturity and Flw= time to flowering.

5.3.3. Heritability

5.3.3.1. Broad sense heritability

The proportion of phenotypic variance of family means that was due to family genetic effects ranged from 66% for the duration to 0% for panicle weight at Sekoukou (Table 5.3). The most inherited traits were duration (66%), the number of panicle per plant (47%), tillering ability (41%) and yield (27%). The heritability varied at Saga from 81% for total panicle weight to 31% for the duration. In general, the populations expressed higher genotypic effects for all the traits except duration (38%) at Saga. However, the pooled heritability which was the heritability across environments was high for the duration (61%). The tillers number, panicle weight and height were poorly heritable across environments with a respective heritability of 2%, 14% and 0%.

Table 5.3: Traits broad sense heritability (h^2_f)

		Flw	Mat	Height	Tnum	Pnum	Tpwt	Pwt	Gwt	Yield
Sekoukou	h^2_f	66	65	24	41	47	28	0	27	27
	SE	0.040	0.040	0.07	0.06	0.055	0.07	0.070	0.057	0.057
Saga	h^2_f	38	38	76	53	61	81	69	77	77
	SE	0.052	0.052	0.028	0.05	0.043	0.024	0.035	0.026	0.026
Pooled	h^2_f	61	61	0	2	23	43	14	30	30
	SE	0.07	0.07	0	0.28	0.20	0.14	0.2	0.17	0.17

Pwt= panicle weight, Tpwt = total panicle weight, Gwt = grain weight, Pnum = panicle number, Tnum= tiller number, Mat = time to maturity and Flw= time to flowering.

5.3.3.2. Narrow sense heritability

A very low heritability was observed in terms of time to flowering and time to maturity when IRRI113 was used as female parent (6% at Sekoukou and 17.88% at Saga). For the same traits the heritability was much higher when IRRI113 was the male parent: 40% and 58% respectively at Sekoukou and Saga (Table 5.4). The total panicle weight, panicle weight, grain weight and yield had higher heritability when IRRI113 was the female parent.

Table 5.4: IRRI113 offspring heritability

	FLW	MAT	Height	Tnum	Pnum	TPwt	Pwt	Gwt	Yield
Sekoukou									
Half sib (IRRI113 fem)	5.9	5.9	40.58	63	58	75.60	41.29	77.25	77.25
Half sib (IRRI113 male)	40	40	65.58	71	40	25.64	24.69	12.86	12.86
Saga									
Half sib (IRRI113 fem)	17.88	17.88	100	23.89	5.32	16.01	15.69	16.81	16.81
Half sib (IRRI113 male)	58.04	58.04	6.1	26.92	3.87	5.43	9.38	2.8	2.8

Pwt= panicle weight, Tpwt = total panicle weight, Gwt = grain weight, Pnum = panicle number, Tnum= tiller number, Mat = time to maturity and Flw= time to flowering.

The most highly heritable traits are total panicle weight (70 to 81%) panicle weight (7.22 to 76.3%) grain weight and yield (66.8 to 98.94%). For these traits the narrow sense heritability was high either NSIC RC 106 was male or female parent (Table 5.5). The tillering ability and the panicle number were lowly heritable at Sekoukou but medium at Saga in all cross directions. For the duration high heritability was observed only when NSIC RC 106 was used as female parent.

All the traits were poorly heritable when Gambiaka was used as female except the panicle weight that showed high heritability at Saga (table5.6). On the two sites only two traits such as tillering ability, and total panicle weight were highly heritable when Gambiaka was used as male.

Table 5.5: NSIC RC 106 offspring heritability

	Flw	Mat	Height	Tnum	Pnum	TPwt	Pwt	Gwt	Yield
Sekoukou									
Half sib (NSIC RC106 fem)	58	58	3.41	15.68	14.28	78.13	7.22	66.8	66.8
Half sib (NSIC RC106 male)	15	15	0.7	13.38	24.92	81.42	13.28	98.94	98.94
Saga									
Half sib (NSIC RC106 fem)	68	68	72.4	62	58	70.1	66.3	85.2	85.2
Half sib (NSIC RC106 male)	17.58	17.58	51.45	52.90	58	73.01	76.3	78.2	78.2

Pwt= panicle weight, Tpwt = total panicle weight, Gwt = grain weight, Pnum = panicle number, Tnum= tiller number, Mat = time to maturity and Flw= time to flowering.

Table 5.6: Gambiaka offspring heritability

	FLW	MAT	height	Tnum	Pnum	TPwt	Pwt	Gwt	Yield
Sekoukou									
Half sib (Gambiaka fem)	33.54	33.55	51.9	23.61	36.31	17.77	44.78	29.24	29.24
Half sib (Gambiaka male)	32	32	23.44	74.37	99.71	72.81	21.26	56.11	56.11
Saga									
Half sib (Gambiaka fem)	19.92	19.92	44.94	41.18	8.2	32.06	74.30	4.48	4.48
Half sib (Gambiaka male)	19.01	19.00	20.30	89.71	22.52	91.36	35.28	8.60	8.60

Pwt= panicle weight, Tpwt = total panicle weight, Gwt = grain weight, Pnum = panicle number, Tnum= tiller number, Mat = time to maturity and Flw= time to flowering.

IR1529 offspring's revealed high heritability of total panicle weight (68%), panicle weight (74.35%), grain weight (67.23%) and yield (67.23%) when their common parent was male at Sekoukou, whereas only the height (76.21%), tillering ability (75.39%), and panicle weight (81.34%) were highly heritable at Saga. When the common parent (IR1529) was the female only trait (panicle weight) showed high heritability at Saga (Table 5.7).

Table 5.7: IR1529 offspring heritability

	FLW	MAT	height	Tnum	Pnum	TPwt	Pwt	Gwt	Yield
Sekoukou									
Half sib (IR1529 fem)	62.46	62.46	16.80	54.8	70.10	25.72	28.68	2	2
Half sib (IR1529 Male)	12.35	12.35	42.45	12.32	13.62	67.88	74.35	67.23	67.23
Saga									
Half sib (IR1529 fem)	4.34	4.32	30.16	31.08	6.04	50.34	64.25	14.22	14.22
Half sib (IR1529 Male)	30.27	30.27	76.21	6.99	75.39	34.68	81.34	31.88	31.88

Pwt= panicle weight, Tpwt = total panicle weight, Gwt = grain weight, Pnum = panicle number, Tnum= tiller number, Mat = time to maturity and Flw= time to flowering.

5.3.4. Genes action

A highly significant additive gene action was notified in terms of tiller number (Table 5.8). However, the dominance effect and maternal effect were not significant for this trait. The time to flowering (duration) was significantly influenced by dominance effect and maternal additive effect. For the height only maternal additive effect had significant influence. The grain weight and panicle weight were significantly impacted respectively by dominance effect and additive effect.

Table 5.8: genes action on traits

Gene action	d.f.	Flw	Tnum	Pnum	height	Tpwt	Pwt	Gwt
A	3	95.20ns	157.09**	138.12ns	55.69ns	142.25ns	0.41*	55.82ns
b1	1	17.63ns	0.15ns	0.19ns	23.73ns	400.70ns	0.25ns	228.20ns
b2	3	39.77*	81.68ns	42.09ns	3.87ns	100.33ns	0.07ns	52.70ns
b3	2	7.19ns	22.40ns	20.68ns	0.56ns	20.94ns	0.098ns	14.09*
B	6	25.22ns	48.34ns	27.97ns	6.08ns	123.93ns	0.11ns	69.08ns
C	3	79.49*	17.29ns	49.06ns	7.87*	222.50ns	0.27ns	98.73ns
D	3	7.3ns	12.62ns	10.48ns	16.27ns	10.41ns	0.043ns	2.44ns

** = highly significant, * = significant, ns = non significant. Pwt= panicle weight, Tpwt = total panicle weight, Gwt = grain weight, Pnum = panicle number, Tnum= tiller number, and Flw= time to flowering.

The covariance (Wr) is related to the variance (Vr) by straight regression of a slope less than 1.0 implied the presence of non-allelic interaction and with dependent distribution of genes among the parents for the trait time to flowering (Figure 5.3). The regression line cuts the Wr above the origin (positive intercept) this suggested a partial dominance. The parents with most dominant genes were IRRI113 and IR1529 because they were close to the origin where Gambiaka had equal dominant and recessive genes. NSIC RC106 was the parent with most recessive genes. A slope less than 1.0 and a positive intercept suggested a presence of gene interaction and partial dominance for the trait height (Figure 5.4). The parents with more dominant genes controlling plant height were IRRI113, NSIC RC106 and IR1529. Gambiaka was the parent with most recessive gene for height.

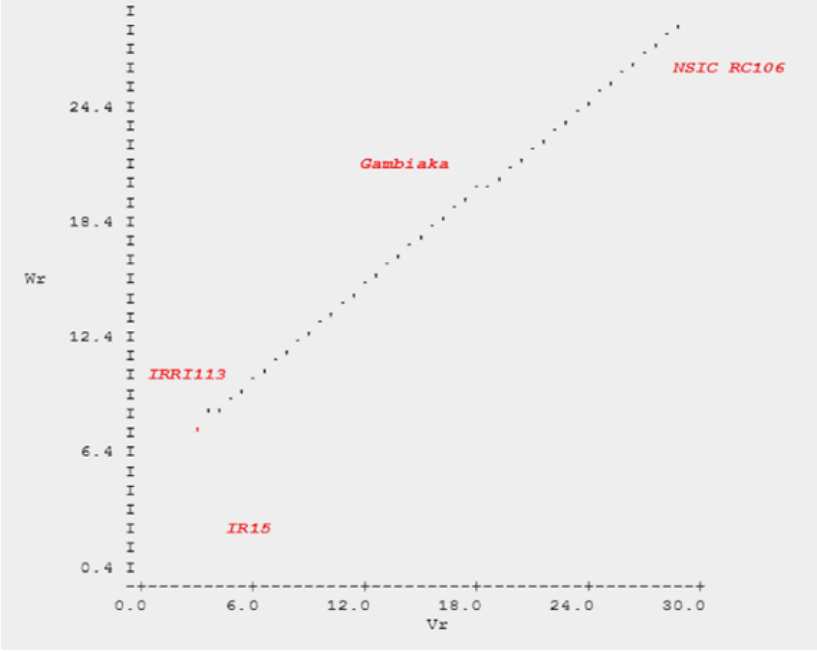

Figure 5.3: Variance and covariance regression for time to flowering

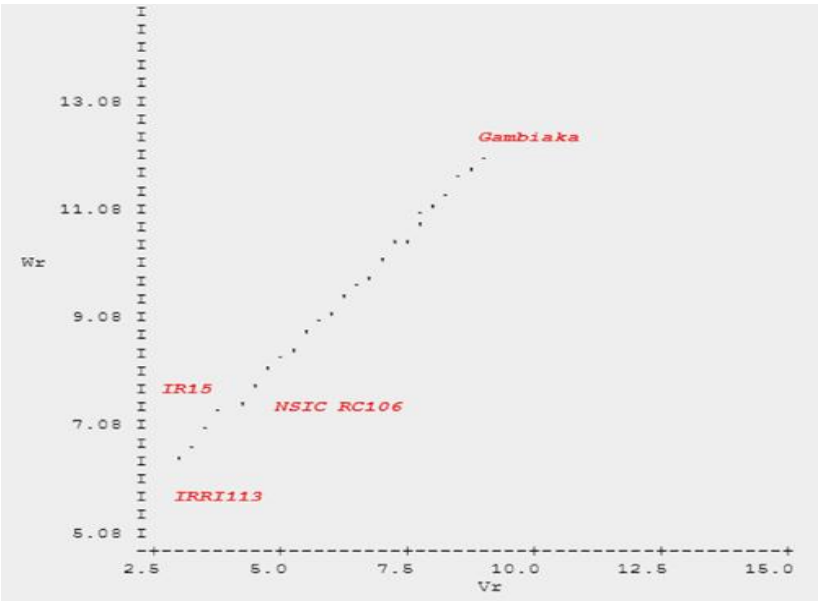

Figure 5.4: Variance and covariance regression for plant height

For tiller number (Figure 5.5) and panicle number (Figure 5.6) an absence of non-allelic interaction with independent distribution of genes among the parents was suggested, this was

explained in the flowing Figures by Wr that is related to Vr by straight regression of unity slope, 1.0.

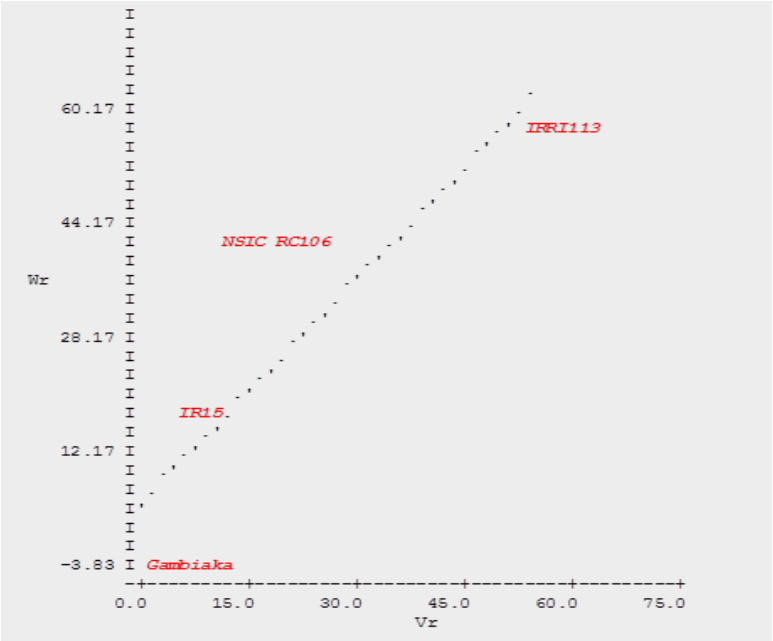

Figure 5.5: Variance and covariance regression for tiller number

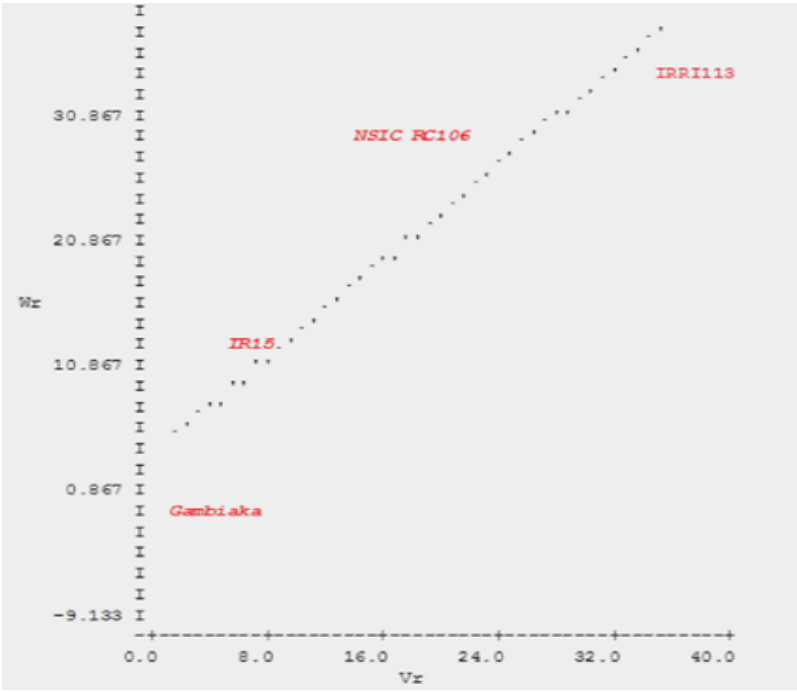

Figure 5.6: Variance and covariance regression for Panicle number

The Figure 5.7 showed partial dominance effect on total panicle weight. This is indicated by a positive intercept of the regression line with the covariance. The parents with most dominance genes were NSIC RC106, Gambiaka and IR1529 were IRRI113 bears most recessive genes.

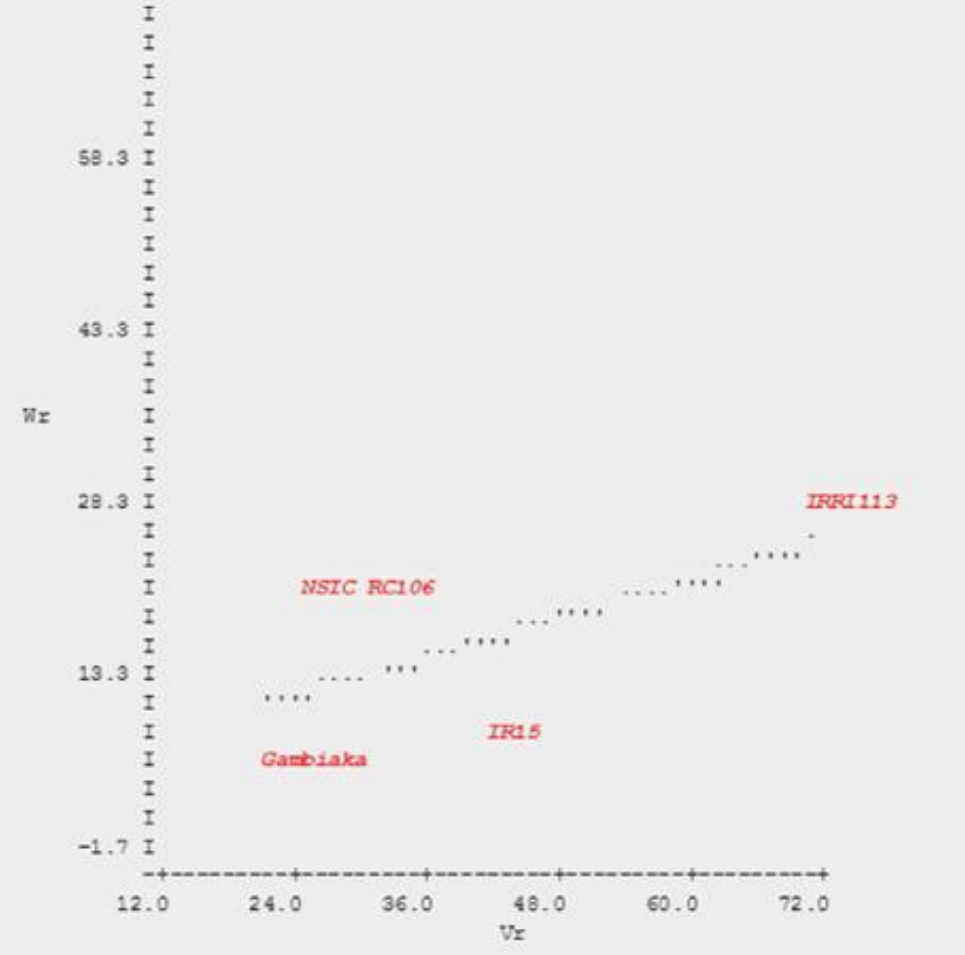

Figure 5.7: Variance and covariance regression for Total panicle weight

Panicle weight variance and covariance regression (Figure 5.8) showed an absence of allelic interaction. However, partial dominance (positive intercept) affected grain weight (Figure 5.9). The parents with most dominant genes (P>75%) were NSIC RC106 and Gambiaka where IR1529 and IRRI113 had most recessive genes (P< 25%).

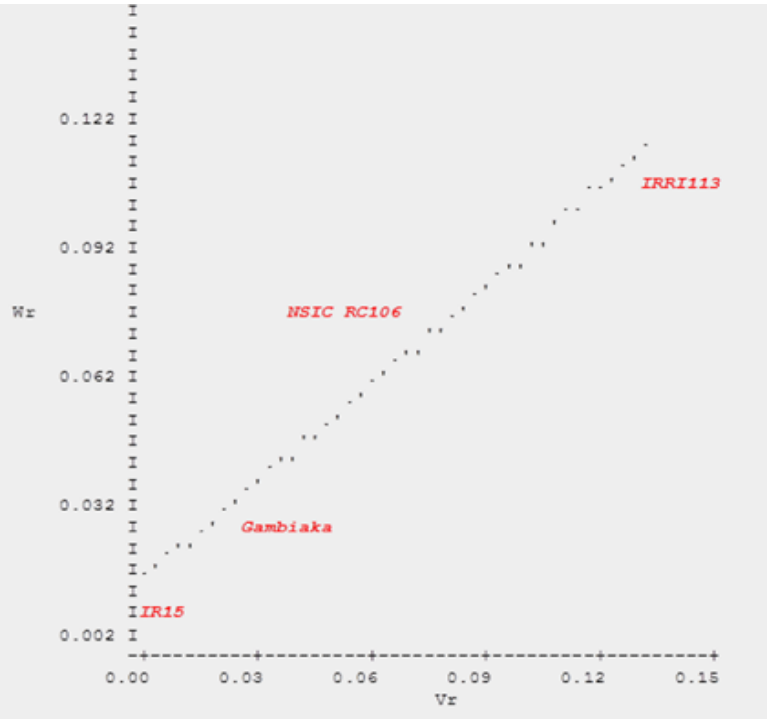

Figure 5.8: Variance and covariance regression for Panicle weight

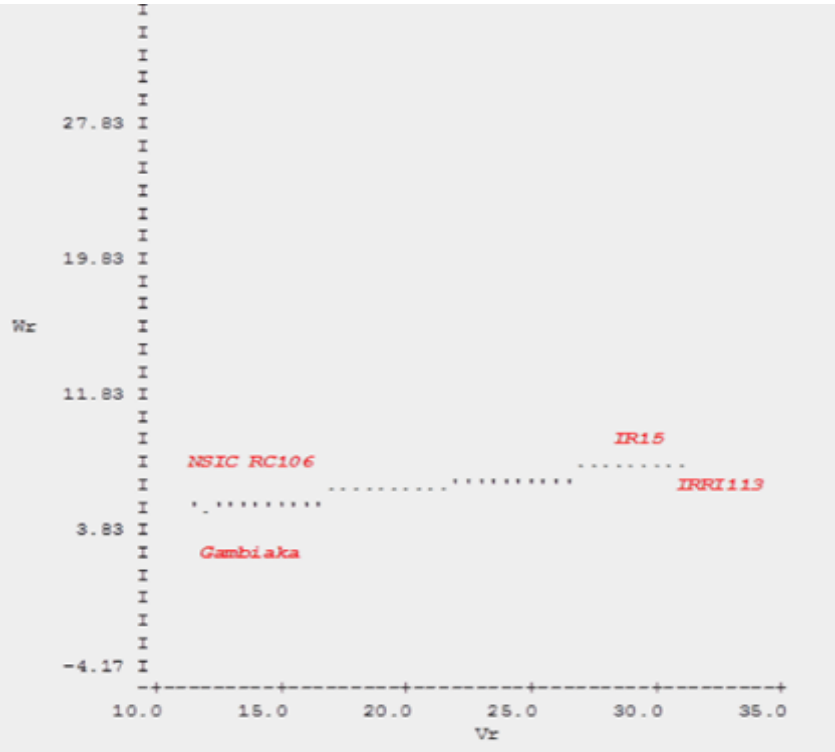

Figure 5.9: Variance and covariance regression for grain weight

5.3.5. Genotypes by environment interaction

5.3.5.1.Families and environment interaction

The environments (sites) were significantly different for all the traits measured (Table 5.9). Highly significant differences also existed among F_3 families. Family performances were highly significantly influenced by environment effects. This was so because families and environment interaction was highly significant for all the traits.

Table 5.9: families and environment interaction

Source	d.f.	Flw	Tnum	Pnum	height	Tpwt	Pwt	Gwt
Env	1	166821.84***	882752.6***	623944.43***	342190.56***	838150.7***	248.28***	342967.16***
Family	124	1098.36***	2812.42**	2742.03**	1098.36**	4321.86**	8.15**	2833.79**
Env*family	124	411.29**	2667.03**	2475.01**	411.29**	4434.37**	8.94**	3650.05**

***= significant .at 0.001 probability level. Pwt= panicle weight, Tpwt = total panicle weight, Gwt = grain weight, Pnum = panicle number, Tnum= tiller number, and Flw= time to50% flowering.

5.3.5.2.Genes and environment interaction

Effect of environment on gene action (additive, dominance, and maternal effect) existed but was not significant for all the traits measured (Figure 5.10). However, for tiller number, significant interaction existed between dominance effect and the environment.

Table 5.10: genes action and environment

Interaction	d.f.	Flw	Tnum	Pnum	height	Tpwt	Pwt	Gwt
Env*a	3	18.76ns	0.95ns	26.43ns	25.22ns	60.86ns	0.03ns	54.44ns
Env*b1	1	25.66ns	50.33ns	45.91ns	1.27ns	166.64ns	0.96ns	76.28ns
Env*b2	3	3.65ns	169.39*	107.97ns	13.06ns	163.89ns	0.61ns	91.47ns
Env*b3	2	3.32ns	4.93ns	4.23ns	6.62ns	6.37ns	0.01ns	0.27ns
Env*b	6	7.21ns	94.73ns	63.05ns	8.95ns	111.84ns	0.47ns	58.54ns
Env*c	3	8.00ns	18.35ns	35.39ns	0.25ns	192.60ns	0.28ns	130.65ns
Env*d	3	2.01ns	18.09ns	23.34ns	10.07ns	57.25ns	0.08ns	22.18ns

*= significant at .0.05 probability level. Pwt= panicle weight, Tpwt = total panicle weight, Gwt = grain weight, Pnum = panicle number, Tnum= tiller number, and Flw= time to50% flowering.

5.4.Discussion

5.4.1. Heritability

The intra-class correlation (ICC) indicated that significant percentage of the variance of all the traits can be attributed to family differences. The causes may be heredity, environment, or both.

Results showed that the heritability on the family means basis was high for almost the traits at Saga. Thus, height, tiller number, panicle number, panicle weight, grain weight and yield were more heritable at Saga than at Sekoukou. It supposes that it is better to breed for these traits under high stress conditions. Sure enough, high heritability is good to achieve meaningful results for that site. Many Reports on diallel analysis have indicated significant additive and dominance genetic effects and a high degree of heritability values in most trait studies (Moeljopawiro and Ikehashi, 1981; Akbar *et al.*, 1985). The duration was more heritable at Sekoukou than Saga. This means that we can easily breed for early, late or medium maturing under less salt conditions than high stress. The duration heritability across environments was high. This means a stability of the F_3 population for this trait. For the same trait the heritability differed according to the site and the parents used in the cross. This conformed with results of Betran *et al* (2009) who found that heritability for the same trait and their magnitude depends on several factors. These factors are the environment, the reference population, the sample of genotypes evaluated, the generation or progenies. Heritability is used to estimate expected response to selection and to choose the best breeding approach to improve the target trait(s). Traits with high heritability can be selected on a single-plant basis, faster, and in a low number of environments. In contrast, traits with low heritability require selection on a family basis and in a greater number of environments to determine breeding values of genotypes (Fehr, 1987; Betrán *et al.*, 2009). For direct selection, the heritability of the traits to be subjected to selection is fundamental.

Traits were more heritable under salt stress when IRRI 113 and NSIC RC106 were used as female parents. The results showed that heritability was high for tiller number (63%), panicle number (58%), total panicle weight (75.6%) and yield (77%) when IRRI113 was the female parent. For the same traits the heritability was low when IRRI113 was the male parent. The duration heritability was high across environment when IRRI113 was the male parent or when NSIC RC106 was the female parent. The yield was highly heritable across sites and crosses when NSIC RC106 was either female or male parent. But when Gambiaka and IR1529 were involved as female parents the traits had low heritability except for the duration that was highly heritable when IR1529 was used as female parent. Thus, panicle number, tillering ability and yield were more heritable across sites when Gambiaka was the male parent. This may be brought about by maternal or cytoplasm effect. The implications of this are that, for offspring that female parents were either IRRI113 or NSIC RC106 we can continue the selection on the plant basis to advance in one environment for traits such as yield, tiller and panicle number and height. But for duration family basis selection on multi-environment will be necessary to advance. For offspring that female parents were either Gambiaka or IR1529 the plant based selection can work only for the duration. So we have to use family means basis selection over several environments to select for the others traits.

5.4.2. Gene action

This study indicated additive effect for tiller number, panicle number, and panicle weight. This means that these traits were not influenced by allelic inter-action and that segregation does not have any effect on them. This confirmed findings of Mishra *et al.* (1998) that indicated the role of few major genes along with numerous minor genes involved for salinity tolerance. However, additive maternal effect was noticed for duration and height this is not conform to the result of

the same authors who inferred that salinity tolerance trait is polygenic in nature and lacks maternal influence. Partial dominance effect was detected in some traits such as height and duration. So breeding for early maturing height can be influenced by segregation. This is because dominance effect is an inter-action of alleles at the same locus. As the study was on segregating populations these traits may change substantially over generations.

5.4.3. Genotype by environment interactions

The genotype by environment interaction (G × E) was highly significant for all the traits. This may be due to the fact that environment stress such as salinity is unpredictable and variable (Grando and Ceccarelli, 2009). However, G × E interaction acts to reduce heritability (Cooper *et al.*, 1999). It can also be seen that increasing replication across sites and years and within year-site combinations can increase heritability. Where G × E interactions exist, a condition for any estimate of heritability to be a reliable indicator of the scope for making progress from selection is that the results must be based on a sample of environments that matches the expected mixture of "types" of environments. Thus, the incidence of genotype-by-environment (G × E) interactions within the target genotype-environment system of the breeding program necessitates the use of multi-environment trials to evaluate genotype adaptation of fixed lines in the future.

Increasing the number of environments affects selection response more than increasing the number of replications. However, the environments have to be representative of the target area because if the environments are very different, δ^2_{GE} can increase substantially and thereby reduce selection gain (Betrán *et al.*, 2004; Cooper *et al.*, 2006).

5.5.Conclusion

The F_3 populations evaluated were composed of families that were significantly different. The heritability was high at Saga, which could lead to higher response to selection. The latter could prelude meaningful results achievement. The F_3 families derived from the cross of either IRRI113 or NSIC RC 106 as female parent had higher heritability. For these families, selection for advancement can be done on the plant basis. For the other families with low heritability, selection can only be done on a family mean basis in multi-environmental trials. Traits such as tiller number, panicle number, and panicle weight were controlled by additive genetic effect. These traits were more stable than those controlled by dominance genetic effect. Multi-environmental trial should be used for the future fixed lines. This is because the genotype by environment interaction was highly significant for all the traits.

CHAPTER SIX

6.0. AGRONOMIC PERFORMANCE OF EARLY SEGREGATING POPULATIONS OF RICE UNDER SALT STRESS

6.1. Introduction

The world population is increasing rapidly and may reach 9.3 billion by the year 2050 (Tuteja, 2007), whereas the crop production is decreasing rapidly because of the negative impact of various environmental stresses. Abiotic stresses are the principal causes of decreasing the average yields of major crops by more than 50%, which causes losses worth hundreds of million dollars each year (Mahajan and Tuteja, 2005) and are becoming the major limiting factors for crop production especially under globally changing climatic conditions (Wang *et al.*, 2012). Among the various factors limiting rice yield, salinity is one of the oldest and most serious environmental problems in the world (Mcwilliam, 1986; Islam *et al.*, 2007). Thus, salinity happens to be a major constraint to the sustainability and expansion of rice cultivation in areas where rice production has not kept up with increasing demand from a growing population (Ma *et al.*, 2007). High salinity stress is the most severe environmental stress, impairing crop production on at least 20% of irrigated land worldwide. In addition, the increased salinity of arable land is expected to have devastating global effects, resulting in up to 50% land loss by the middle of the twenty-first century (Mahajan and Tuteja, 2005). The amount of salt-affected land is a threat to agriculture (Flowers, 2004). Furthermore, there is a deterioration of about 2 million ha of world agricultural lands because of salinity each year. Soil salinity may be a result of poor water management, high evaporation, heavy irrigation, (Ashraf, 1994; Mahajan and Tuteja, 2005; Ashraf, 2009). In addition, a severe shortage of good quality water is also forcing growers to use

poor quality ground water for irrigation, which further aggravates the salinity problem (Ma *et al.*, 2007). Salinity results in reduced growth and development, loss of yield and productivity in crop species (Hasegawa *et al.*, 2000; Singh *et al.*, 2007; Qadir *et al.*, 2008).

Rice germplasm is known to have genetic variability for salt tolerance (Akbar *et al.*, 1985; Gregorio and Senadhira, 1993). However, most of the varieties grown in saline environments are traditional pure line selections that are very well adapted to local conditions. They have a long duration of growth and are tall, photoperiod sensitive and low yield in grain (Xie *et al.*, 2001). Therefore, there is a clear need for breeding high yielding salinity tolerant varieties (Akbar *et al.*, 1985). The purpose of this study was to assess agronomic performance of early rice populations under salt stress in Niger.

The specific objectives were to:

- ⇨ assess yield potential of F_3 families under salt stress.
- ⇨ select the best performing families for advancement.

6.2.Materials and Methods

6.2.1. Plant material

One hundred and twenty F_3 families derived from F_2 individual plants, 4 parents and a check (Table 6.1) were evaluated on farmer's fields at Saga and Sekoukou.

Table 6.1: list of rice families used in the evaluation

Family	Parents		Number	Status	Family	Parents		Num	Status
	Fem	Male				Fem	Male		
Kol2-1	IR15	GAM	1	F_3	Kol14-39	IRRI	IR15	23	F_3
Kol2-2	IR15	GAM	2	F_3	Kol14-41	IRRI	IR15	24	F_3
Kol2-3	IR15	GAM	3	F_3	Kol14-42	IRRI	IR15	25	F_3
Kol2-4	IR15	GAM	4	F_3	Kol14-43	IRRI	IR15	26	F_3
Kol2-5	IR15	GAM	5	F_3	Kol14-45	IRRI	IR15	27	F_3
Kol2-12	IR15	GAM	6	F_3	Kol14-47	IRRI	IR15	28	F_3
Kol2-13	IR15	GAM	7	F_3	Kol14-63	IRRI	IR15	29	F_3
Kol2-14	IR15	GAM	8	F_3	Kol14-78	IRRI	IR15	30	F_3
Kol2-15	IR15	GAM	9	F_3	Kol11-1	NSIC	IRRI	31	F_3
Kol2-17	IR15	GAM	10	F_3	Kol11-5	NSIC	IRRI	32	F_3
Kol15-1	IRRI	GAM	11	F_3	Kol11-6	NSIC	IRRI	33	F_3
Kol15-3	IRRI	GAM	12	F_3	kol11-7	NSIC	IRRI	34	F_3
Kol15-8	IRRI	GAM	13	F_3	Kol11-8	NSIC	IRRI	35	F_3
KOl15-13	IRRI	GAM	14	F_3	Kol11-9	NSIC	IRRI	36	F_3
Kol15-14	IRRI	GAM	15	F_3	Kol11-12	NSIC	IRRI	37	F_3
Kol15-15	IRRI	GAM	16	F_3	Kol11-13	NSIC	IRRI	38	F_3
Kol15-16	IRRI	GAM	17	F_3	Kol1114	NSIC	IRRI	39	F_3
Kol15-27	IRRI	GAM	18	F_3	Kol11-16	NSIC	IRRI	40	F_3
Kol15-30	IRRI	GAM	19	F_3	Kol5-2	NSIC	GAM	41	F_3
Kol15-33	IRRI	GAM	20	F_3	Kol5-6	NSIC	GAM	42	F_3
Kol14-31	IRRI	IR15	21	F_3	Kol5-8	NSIC	GAM	43	F_3
Kol14-37	IRRI	IR15	22	F_3	Kol5-10	NSIC	GAM	44	F_3
Kol5-11	NSIC	GAM	45	F_3	Kol31-27	GAM	IRRI	67	F_3
Kol5-12	NSIC	GAM	46	F_3	Kol31-28	GAM	IRRI	68	F_3
kol5-13	NSIC	GAM	47	F_3	Kol31-29	GAM	IRRI	69	F_3
Kol5-14	NSIC	GAM	48	F_3	Kol31-36	GAM	IRRI	70	F_3
Kol5-15	NSIC	GAM	49	F_3	Kol29-11	GAM	NSIC	71	F_3
Kol5-16	NSIC	GAM	50	F_3	Kol29-12	GAM	NSIC	72	F_3
Kol4-3	NSIC	IR15	51	F_3	Kol29-14	GAM	NSIC	73	F_3
Kol4-5	NSIC	IR15	52	F_3	Kol29-15	GAM	NSIC	74	F_3

Fem = female parent, IRRI = IRRI113, NSIC= NSIC RC106, GAM = GAMBIAKA, IR15=IR1529

Table 6.1: list of rice families used in the evaluation (continued)

Family	Parents		Number	Status	Family	Parents		Number	
	Fem	Male				Fem	Male		
Kol4-6	NSIC	IR15	53	F_3	Kol29-16	GAM	NSIC	75	F_3
Kol4-7	NSIC	IR15	54	F_3	Kol29-19	GAM	NSIC	76	F_3
Kol4-12	NSIC	IR15	55	F_3	Kol29-23	GAM	NSIC	77	F_3
Kol4-13	NSIC	IR15	56	F_3	Kol29-24	GAM	NSIC	78	F_3
Kol4-15	NSIC	IR15	57	F_3	Kol29-25	GAM	NSIC	79	F_3
Kol4-16	NSIC	IR15	58	F_3	Kol29-31	GAM	NSIC	80	F_3
Kol4-17	NSIC	IR15	59	F_3	Kol27-6	GAM	IR15	81	F_3
Kol4-18	NSIC	IR15	60	F_3	Kol27-7	GAM	IR15	82	F_3
Kol31-51	GAM	IRRI	61	F_3	Kol27-9	GAM	IR15	83	F_3
Kol31-21	GAM	IRRI	62	F_3	kol27-15	GAM	IR15	84	F_3
Kol31-22	GAM	IRRI	63	F_3	Kol27-22	GAM	IR15	85	F_3
Kol31-23	GAM	IRRI	64	F_3	Kol27-23	GAM	IR15	86	F_3
Kol31-25	GAM	IRRI	65	F_3	Kol27-25	GAM	IR15	87	F_3
Kol31-26	GAM	IRRI	66	F_3	Kol27-27	GAM	IR15	88	F_3
Kol27-29	GAM	IR15	89	F_3	Kol23-27	IR15	NSIC	108	F_3
Kol27-30	GAM	IRRI	90	F_3	Kol23-28	IR15	NSIC	109	F_3
Kol25-7	IR15	IRRI	91	F_3	Kol23-31	IR15	NSIC	110	F_3
Kol25-8	IR15	IRRI	92	F_3	Kol21-1	IRRI	NSIC	111	F_3
Kol25-11	IR15	IRRI	93	F_3	Kol21-14	IRRI	NSIC	112	F_3
Kol25-16	IR15	IRRI	94	F_3	Kol21-15	IRRI	NSIC	113	F_3
Kol25-21	IR15	IRRI	95	F_3	Kol21-18	IRRI	NSIC	114	F_3
Kol25-22	IR15	IRRI	96	F_3	Kol21-19	IRRI	NSIC	115	F_3
Kol25-27	IR15	IRRI	97	F_3	Kol21-24	IRRI	NSIC	116	F_3
Kol25-28	IR15	IRRI	98	F_3	Kol21-26	IRRI	NSIC	117	F_3
Kol25-29	IR15	IRRI	99	F_3	Kol21-27	IRRI	NSIC	118	F_3
Kol25-30	IR15	IRRI	100	F_3	Kol21-28	IRRI	NSIC	119	F_3
Kol23-9	IR15	NSIC	101	F_3	Kol21-30	IRRI	NSIC	120	F_3
kol23-10	IR15	NSIC	102	F_3	IRRI				
kol23-11	IR15	NSIC	103	F_3	NSIC				
kol23-13	IR15	NSIC	104	F_3	GAM				
Kol23-24	IR15	NSIC	105	F_3	IR15				
Kol23-25	IR15	NSIC	106	F_3	NER				
Kol23-26	IR15	NSIC	107	F_3					

Fem = female parent, IRRI = IRRI113, NSIC= NSIC RC106, GAM = GAMBIAKA, NER= NERICA L49 (local check), IR15=IR1529

6.2.2. Experimental Design

The experimental design was Alpha lattice (25*5) with five blocks and three repetitions. Each incomplete block was constituted with 25 (families). Each family was planted on one line. Ten individual plants per family were evaluated; this means 10 plants on line. The inter-plant space (on line) was 0.2 m and the

between line space was 0.5 m. The distanced between blocks was 1.5m and the distance between repetition was 3m.

6.2.3. Data scoring

Agronomic traits were collected on each individual F_3 plants: These were:

- ⇨ Time to 50% flowering, time to 85% maturity.
- ⇨ Plant height: the height was measured from the soil surface to the tip of the panicle.
- ⇨ Total number of tillers per plant: all the tillers excluding the dried.
- ⇨ Reproductive tillers number per plant: All tillers that bear panicle.
- ⇨ Panicle number: all the panicle fertile and infertile.
- ⇨ Total panicle weight.
- ⇨ Panicle weight: total panicle weight divided by panicle number.
- ⇨ Grain weight.
- ⇨ Paddy yield.

6.2.4. Selection index

Weights (Wi) for the selection index were allocated based on the relative importance of each measured trait as an indicator of salinity tolerance (Efisue, 2007) (Table 6.2). The results from the participatory rural appraisal on preferred traits rice farmers were also considered in assigning weights on each trait.

The selection index (SI) of each genotype was calculated as:

SI = PiWi+ PjWj+ ---------- + PnWn (Efisue, 2007)

Where Pi = standardized phenotypic value of the trait observed; Wi = is the assigned weight value to the trait in the selection index.

Table 6.2: Sign and weight based on the relative importance of traits in the selection index

Traits	Sign	Weight (Wi)	Importance
Time to 50%flowering	-	1	Earliness
Time to 85% maturity	-	1	Earliness
Height	+	2	Good height
Tiller number	+	3	High tillering ability
Panicle number	+	4	High panicle number
Infertile tiller	-	5	Less infertile tiller
Total panicle weight	+	3.5	High productive panicle
Panicle weight	+	4	Heavy panicle
Grain weight	+	5	High yield

6.2.5. Data analysis

Data were analyzed using SAS software version 9.2. The flowing Table (6.3) indicates the SAS procedures and the type of analysis performed.

Table 6.3: SAS procedures used for data analysis

SAS procedure	Type of analysis
Proc GLM	Analysis of variance
Proc reg	Regression analysis
Proc corr	Correlation analysis
Proc PRINCOMP	Principal component analysis
Proc univariate	Statistics

6.3.Results

6.3.1. Traits Analysis of variance across families and sites

The families and sites interaction was highly significant (Table 6.4). There were significant ($p < 0.001$) differences among genotypes at the two sites for all the traits. The families also differed highly significantly for all the traits. The families and sites interaction was very highly significant.

Table 6.4: ANOVA table across sites

Source	DF	Flw	Mat	Height	Tnum	Pnum	Tpwt	PWT	Gwt
Site	1	166821.8***	342190.56***	35289.21***	882752.6***	623944.43***	838150.70***	248.28***	342967.16***
Block(Rep)	14	827***	827***	2467***	5819***	4384***	12402***	14***	7125***
Family	124	1098**	1098***	735***	2812***	2742***	4321***	8***	2833***
Site*family	124	411***	411***	644***	2667***	2475***	4434***	9***	3650***

*, **, ***= significant at 0.001 probability level. Pwt= panicle weight, Tpwt = total panicle weight, Gwt = grain weight, Pnum = panicle number, Tnum= tiller number, Mat = time to maturity and Flw= time to flowering.

6.3.2. Yield performance

The families yield mean ranged from 1.2 t/ha to 4.3 t/ha (Appendix 2). The average yield of the top performing 20 families at Sekoukou (Table 6.5) ranged from 3.31 to 4.31T/ha under salt conditions. The within family variation was very high with high CVs. However, a maximum of 15.97 t/ha was obtained in the family Kol29-24 with a huge intra-family variation (CV of 112%). The minimum yield in that family was 0. The lowest within family variation was observed in Kol15-16 where the mean yield was about 4 tonnes per ha, the maximum was 8.31 t/ha and the minimum was 0. All the parents gave poorer yield performance than these top 20 families.

Table 6.5: The top 20 high yielding F_3 families at Sekoukou

	family	Mean	S E	C V	Range
Kol25-22	96	4.31	0.45	57.48	0-11.6
Kol14-37	22	4.19	0.5	65.78	0-10.57
Kol29-25	79	3.94	0.39	54.79	0.56-8
KOl15-13	14	3.91	0.57	80.54	0-9.2
Kol15-16	17	3.86	0.35	50.08	0-8.31
Kol21-18	114	3.81	0.63	91.05	0-14.88
Kol2-17	10	3.68	0.51	75.5	0-9.77
Kol31-25	65	3.68	0.45	66.9	0-6.87
Kol29-24	78	3.65	0.75	112.08	0-15.97
kol23-10	102	3.54	0.44	66.46	0-9.77
Kol11-1	31	3.47	0.42	65.53	0-6.83
Kol15-30	19	3.46	0.45	70.48	0-8.58
Kol2-5	5	3.42	0.52	82.89	0-9.63
Kol29-12	72	3.42	0.62	99.67	0-10.02
Kol29-14	73	3.37	0.42	68.27	0-9.57
Kol31-28	68	3.34	0.43	70.22	0-8.26
Kol29-16	75	3.34	0.53	87.34	0-9.89
Kol25-30	100	3.33	0.57	93.53	0-10.67
Kol31-27	67	3.32	0.52	86.24	0-7.73
Kol11-5	32	3.31	0.54	88.68	0-10.34
Kol27-29	89	3.31	0.51	85.12	0-9.39

The families yield performances ranged from 0 to 4.5 t/ha at Saga (Appendix 1). The average yields of the top 20 families varied from 2.53 t/ha to 4.52 t/ha (Table 6.6). The family Kol21-18 had the greatest yield with a high CV (78.15%) and a maximum of 9 t/ha. The highest yielding individual was observed in the family Kol5-14 with about 10 t/ha. However, the CV was large (53%) and the family yield mean was 4 t/ha. Within each family we have maximum yield superior to 6 t/ha and the minimum was 0. In general the CV was high for all the families, the minimum CV being 40.22%.

Table 6.6: The top 20 high yielding F_3 families and parents at Saga

	Family	Mean	SE	CV	Range
Kol21-18	114	4.52	0.64	78.15	0-9
Kol23-9	101	4.32	0.36	46.14	0-9.7
Kol25-21	95	4.3	0.37	47.42	0-7.31
Kol25-28	98	4.19	0.43	56.26	0-7.54
kol23-11	103	4.15	0.34	44.44	0-7.35
Kol5-14	48	4.08	0.4	53.01	0-10.33
Kol21-1	111	4.04	0.3	40.22	0-6.33
Kol4-13	56	3.91	0.43	60.36	0-7.45
Kol21-14	112	3.9	0.38	53.38	0-6.81
Kol25-8	92	3.64	0.45	67.57	0-9.93
Kol25-16	94	3.62	0.44	66.16	0-6.71
Kol21-19	115	3.36	0.47	76.81	0-9.8
Kol4-17	59	3.34	0.49	79.99	0-9.74
Kol23-25	106	3.34	0.3	49.88	0-6.75
Kol21-26	117	3.31	0.36	59.17	0-5.73
Kol21-30	120	3.03	0.48	86.39	0-9.03
Kol21-24	116	2.85	0.52	99.65	0-8.29
Kol23-26	107	2.6	0.54	114.55	0-9.07
Kol21-27	118	2.53	0.44	95.13	0-7.75

The yield across sites varied 0.7 t/ha to 4.17 t/ha for all the families (Appendix 3). The family average performance in terms of yield across the two sites ranged from 2.52 t/ha to 4.17 t/ha for the top performed 20 under saline conditions (Table 6.7). In the highest yielding family a cross sites (Kol21-18) a maximum of about 14.88 t/ha was obtained. However, the high yielding individual was in the family Kol25-28 which was in terms of average yield the fourth of 20 top performers. The yield CV across sites was also high and ranged from 59.12% to 113.42%. For all the families, the minimum was 0T/ha.

Table 6.7: The top 20 high yielding F_3 families across the two sites

	Family	Mean	SE	CV	Range
Kol21-18	114	4.17	0.45	83.78	0-14.88
Kol21-1	111	3.66	0.31	65.26	0-11.29
kol23-11	103	3.64	0.28	59.12	0-7.77
Kol25-28	98	3.39	0.36	83.33	0-15.2
Kol5-14	48	3.32	0.29	66.92	0-10.33
Kol21-14	112	3.28	0.29	68.58	0-6.81
Kol23-9	101	3.27	0.32	75.8	0-9.7
Kol4-13	56	3.2	0.28	67.72	0-7.45
Kol25-16	94	3.19	0.31	75.13	0-7.51
Kol25-21	95	3.08	0.32	79.52	0-7.31
Kol21-19	115	3.07	0.38	94.97	0-10.09
Kol23-25	106	2.95	0.29	76.99	0-8.71
Kol25-30	100	2.83	0.38	105.26	0-10.67
kol23-13	104	2.83	0.35	96.43	0-9.11
Kol25-8	92	2.78	0.3	82.88	0-9.93
Kol21-26	117	2.68	0.27	76.65	0-6.83
Kol5-16	50	2.63	0.23	67.17	0-7.56
Kol25-22	96	2.63	0.34	99.63	0-11.6
kol23-10	102	2.63	0.29	85.63	0-9.77
Kol14-37	22	2.52	0.37	113.42	0-10.57

6.3.3. Selection index

The family selection indices were higher at Sekoukou than Saga. The selection indices varied from -5 to 701 at Saga. At Sekoukou the minimum selection index was 440 and the maximum 1047. Across sites the family selection indices ranged from 239 to 874 (Appendix 4). The selection indices for the top performing 20 families ranged from 721 to 1048 at Sekoukou and from 404 to 701 at Saga (Table 6.8). Thus, the higher selection indices were obtained at Sekoukou. The most performed family in the two sites and across sites was Kol21-18, it ranked first on each site. The second family across sites (Kol23-11) was the sixth at Saga and the nineteenth at Sekoukou. The third family across site (kol23-13) was the fifth at Sekoukou and also the fifth at Saga. The fourth family across sites was the seventeenth at Saga and the eighth at Sekoukou.

Table 6.8: Top 20 family's selection indices on each site and across sites

Sekoukou			Saga			Pooled		
Family	SI	Rank	family	SI	Rank	family	SI	Rank
Kol21-18	1047.9	1	Kol21-18	701.89	1	Kol21-18	874.91	1
kol23-10	921.43	2	Kol25-28	604.93	2	kol23-11	636.48	2
Kol14-41	906.69	3	Kol23-9	561.89	3	kol23-13	624.69	3
Kol2-5	865.84	4	Kol25-21	557.67	4	Kol14-45	619.03	4
kol23-13	863.64	5	KOl4-13	546.34	5	kol23-10	597.78	5
Kol23-31	824.91	6	kol23-11	544.47	6	Kol25-28	593.45	6
Kol29-15	819.3	7	Kol4-17	510.62	7	Kol25-30	589.9	7
Kol14-45	816.83	8	Kol25-30	502.50	8	Kol25-8	586.30	8
Kol11-12	813.01	9	Kol25-16	501.98	9	Kol23-9	583.65	9
Kol31-26	790.07	10	Kol5-14	499.65	10	Kol14-41	579.16	10
Kol11-5	770.76	11	Kol25-8	488.2	11	Kol21-14	562.85	11
Kol2-12	762.95	12	Kol21-1	475.52	12	Kol2-5	557.34	12
Kol27-30	760.99	13	Kol21-14	471.87	13	Kol21-19	555.88	13
Kol23-27	747.92	14	Kol21-24	464.85	14	Kol4-17	554.37	14
Kol14-31	745.21	15	Kol21-19	461.03	15	Kol29-15	549.87	15
Kol5-8	738.59	16	Kol15-3	429.59	16	Kol4-13	549.32	16
Kol15-13	735.43	17	Kol14-45	421.23	17	Kol23-31	548.91	17
Kol31-28	732.99	18	Kol31-51	411.58	18	Kol11-12	547.41	18
kol23-11	728.5	19	Kol21-26	407.35	19	Kol25-21	535.36	19
Kol29-25	721.54	20	Kol23-26	403.95	20	Kol21-30	526.63	20

6.3.4. Traits statistics

At Sekoukou, the time to 85% maturity across families ranged from 95 days to 145 days with a mean of 120 days and a CV of 6% (Table 6.9). The time to flowering varied from 75 to 125 days with an average of 100 days. The average panicle number and tiller number across families were respectively 49 and 60 with a high CV (59% and 53% respectively). The panicle weight was the trait that was the most variable with a CV of about 166% followed by grain weight and yield (CV =93%).

Table 6.9: Mean range and STD Dev of the studied traits at Sekoukou

Variable	Mean	Std Dev	Range	Probability
Flw	99.84	7.67	75-125	***
Mat	119.84	7.67	95-145	***
Height	86.31	10.28	46-135	***
Tnum	60.11	32.04	1-240	**
Pnum	49.19	29.06	1-226	**
Tpwt	42.28	24.94	0.30-188	**
Pwt	1.20	1.99	0-67.6	*
Gwt	25.93	24.16	0-159.7	*

*, **, ***= significant respectively at 0.05, 0.01 and 0.001 probability level. Pwt= panicle weight, Tpwt = total panicle weight, Gwt = grain weight, Pnum = panicle number, Tnum= tiller number, Mat = time to maturity and Flw= time to flowering.

At Saga the duration was much longer than at Sekoukou. The average duration was 136 days for time to 85% maturity (Table 6.10). The minimum was 112 days and the maximum 176. For all the other traits families performed poorer at Saga than at Sekoukou. Thus, families had less tillering ability, less panicle number, light total panicle weight and grain weight. The average height across genotypes ranged from 20 to 129 cm. The means of tillers number, panicles number and total panicle weight were respectively 36, 28 and 19.

Table 6.10: Mean Max min and STD Dev of the studied traits at Saga

Variable	Mean	Std Dev	Range	Probability
Flw	110.87	14.14	87-151	***
Mat	135.87	14.15	112-176	***
Height	81.61	9.09	20-129	***
Tnum	34.57	13.97	0-125	**
Pnum	28.04	13.98	0-110	**
Tpwt	18.66	24.94	0-124.8	**
Pwt	0.82	1.33	0-25.77	*
Gwt	12.38	19.84	0-106	*

*, **, ***= significant respectively at 0.05, 0.01 and 0.001 probability level. Pwt= panicle weight, Tpwt = total panicle weight, Gwt = grain weight, Pnum = panicle number, Tnum= tiller number, Mat = time to maturity and Flw= time to flowering.

Across sites and families, the average tillering ability was 47 tillers per plant (Table 6.11). From these tillers about 38 had ability to bear panicle, the rest being infertile. At maturity the total panicles average weight was 30 g, when an individual panicle had an average weight of 1g. The trait variability remained however, high (high CV) across sites.

Table 6.11: Mean Max min and STD Dev of the studied traits across sites

Variable	Mean	Std Dev	Range	Probability
Flw	106.46	13.14	75-151	***
Mat	129.46	14.33	95-176	***
Height	83.88	9.96	20-135	***
Tnum	47.00	27.62	0-240	**
Pnum	38.31	24.95	0-226	**
Tpwt	30.00	27.59	0-187.9	**
Pwt	1.01	1.70	0-67.64	*
Gwt	19.15	23.12	0-159.7	*

*, **, ***= significant respectively at 0.05, 0.01 and 0.001 probability level. Pwt= panicle weight, Tpwt = total panicle weight, Gwt = grain weight, Pnum = panicle number, Tnum= tiller number, Mat = time to maturity and Flw= time to flowering.

6.3.5. Traits association

The correlation coefficients at Sekoukou are presented in Table 6.12. A strong significant positive association existed between time to maturity and time to flowering. The two traits had weak negative association with tillering ability, total panicle weight, panicle weight, grain weight, infertile tillers and yield. The height had significant positive association with tiller number, panicle number, total panicle weight, grain weight and yield, but was significantly and negatively associated with infertile tillers. The yield was significantly and positively associated with height, total panicle weight, panicle weight and grain weight.

Table 6.12: Trait correlation coefficient at Sekoukou

	Flw	Mat	Height	Tnum	Pnum	InfTi	Tpwt	Pwt	Gwt	Yield
Flw		1***	0.028^{ns}	-0.001^{ns}	0.012^{ns}	-0.05^{ns}	-0.012^{ns}	-0.01^{ns}	-0.05^{ns}	-0.05^{ns}
Mat			0.028^{ns}	-0.001^{ns}	0.012^{ns}	-0.05^{ns}	-0.012^{ns}	-0.01^{ns}	-0.05^{ns}	-0.05^{ns}
Height				0.127*	0.128*	-0.10*	0.128*	-0.02^{ns}	0.11*	0.11*
Tnum					0.96***	-0.25**	0.052^{ns}	-0.3**	0.02^{ns}	0.023^{ns}
Pnum						-0.45***	0.052^{ns}	-0.3**	0.024^{ns}	0.024^{ns}
InfTi							-0.052^{ns}	0.20**	-0.04^{ns}	-0.04^{ns}
Tpwt								0.31**	1***	1***
Pwt									0.33**	0.33**
Gwt										1***
Yield										

*, **, ***= significant respectively at 0.05, 0.01 and 0.001 probability level, ns=non significant. Pwt= panicle weight, InfTi= infertile tiller, Tpwt = total panicle weight, Gwt = grain weight, Pnum = panicle number, Tnum= tiller number, Mat = time to maturity and Flw= time to flowering.

At Saga yield was significantly and positively associated with time to flowering, time to maturity and height (Table 6.13). A highly strong association existed between yield and total panicle weight, panicle weight and grain weight. But the yield was significantly and negatively associated with infertile tiller number. Thus, yield decreased whenever the infertile tillers increased. The association between yield and tiller number was negative but not significant. The tallest plant had not only a high number of tillers and panicle but also low number of infertile tillers. The families that exhibited good tillering ability had more panicles.

Table 6.13: Trait correlation coefficient at Saga

	Flw	Mat	Height	Tnum	Pnum	InfTi	Tpwt	Pwt	Gwt	Yield
Flw		1***	-0.06^{ns}	-0.26**	-0.38**	0.50***	-0.12*	-0.05ns	0.14*	0.14*
Mat			-0.06^{ns}	-0.26**	-0.38**	0.50***	-0.12*	-0.05ns	0.14*	0.14*
Height				0.22**	0.26**	-0.21**	0.14*	0.01^{ns}	0.14*	0.14*
Tnum					0.9***	-0.21**	-0.04^{ns}	-0.3**	-0.04^{ns}	-0.04^{ns}
Pnum						-0.58***	0.02^{ns}	-0.3**	0.03^{ns}	0.03^{ns}
InfTi							-0.18*	0.06^{ns}	-0.19*	-0.19*
Tpwt								0.8***	1.0***	1.0***
Pwt									0.8***	0.8***
Gwt										1.0***
Yield										

*, **, ***= significant respectively at 0.05, 0.01 and 0.001 probability level, ns=non significant. Pwt= panicle weight, InfTi= infertile tiller, Tpwt = total panicle weight, Gwt = grain weight, Pnum = panicle number, Tnum= tiller number, Mat = time to maturity and Flw= time to flowering.

The across sites results (Table 6.14) showed that the duration (time to flowering and time to maturity) was significantly and negatively correlated with all the other traits except infertile tiller number. In other words plants with long duration were dwarf had less tiller and panicle, bear light panicle and yielded lower. The yield positively co-varied with height, tiller and panicle number.

Table 6.14: Trait correlation coefficient across sites

	Flw	Mat	Height	Tnum	Pnum	InfTi	Tpwt	Pwt	Gwt	Yield
Flw		0.99***	-0.22**	0.38**	-0.40**	0.30**	-0.34**	-0.10*	-0.07*	-0.07*
Mat			-0.24**	0.42**	-0.43**	0.27**	-0.37**	-0.11*	-0.13*	-0.13*
Height				0.24**	0.24**	-0.16*	0.22*	0.02^{ns}	0.20*	0.20*
Tnum					0.96***	-0.18*	0.21*	-0.18*	0.18*	0.18*
Pnum						-0.40**	0.21*	-0.20*	0.18*	0.18*
InfTi							-0.12*	0.13*	-0.12*	-0.12*
Tpwt								0.49**	1.0***	1.0***
Pwt									0.50**	0.50**
Gwt										1.0***
Yield										

*, **, ***= significant respectively at 0.05, 0.01 and 0.001 probability level, ns=non significant. Pwt= panicle weight, InfTi= infertile tiller, Tpwt = total panicle weight, Gwt = grain weight, Pnum = panicle number, Tnum= tiller number, Mat = time to maturity and Flw= time to flowering.

6.3.6. Traits regression

Multiple linear regression analysis was done using proc reg in SAS. All the other traits were regressed on yield. The model was: Yield = *f* (Mat + height + tiller number + panicle number + infertile tillers + panicle weight).

The traits that explained significantly the yield were the height, panicle weight and infertile tillers. The latter influences negatively the yield (Table 6.15). A unit increase of infertile tiller leads to a decrease of yield about 158%. Yield increased of about 33% of a unit increase panicle weight. But the duration influenced negatively the yield. Thus, as the duration was long the yield decreased. But this influence was not significant. The relationship among yield and the other traits is resumed by the following model:

Yield = 1.78 – 0.005Mat + 0.022height + 0.015tiller number -0.009panicle number -1.58infertile tillers +0.024panicle weight.

Consequently, the high yielding families under salt stress had short duration, less infertile tillers and bore heavy panicles.

Table 6.15: Traits regression

Variable	DF	Parameter Estimate	S E	Probability
Intercept	1	1.78	0.77	*
Mat	1	-0.005	0.0064	ns
Height	1	0.022	0.0048	***
Tnum	1	0.015	0.011	ns
Pnum	1	-0.009	0.013	ns
InfTi	1	-1.58	0.77	*
Pwt	1	0.33	0.024	***

Pwt= panicle weight, InfTi= infertile tiller, Pnum = panicle number, Tnum= tiller number, Mat = time to maturity. *, ***= significant respectively at 0.05, and 0.001 probability level, ns=non significant.

The duration was also negatively influenced by the tiller number. The higher was the tiller number, the shorter was the duration. Thus, the plants that had good tillering ability under salt stress did not have their duration affected by salt (Figure 6.1).

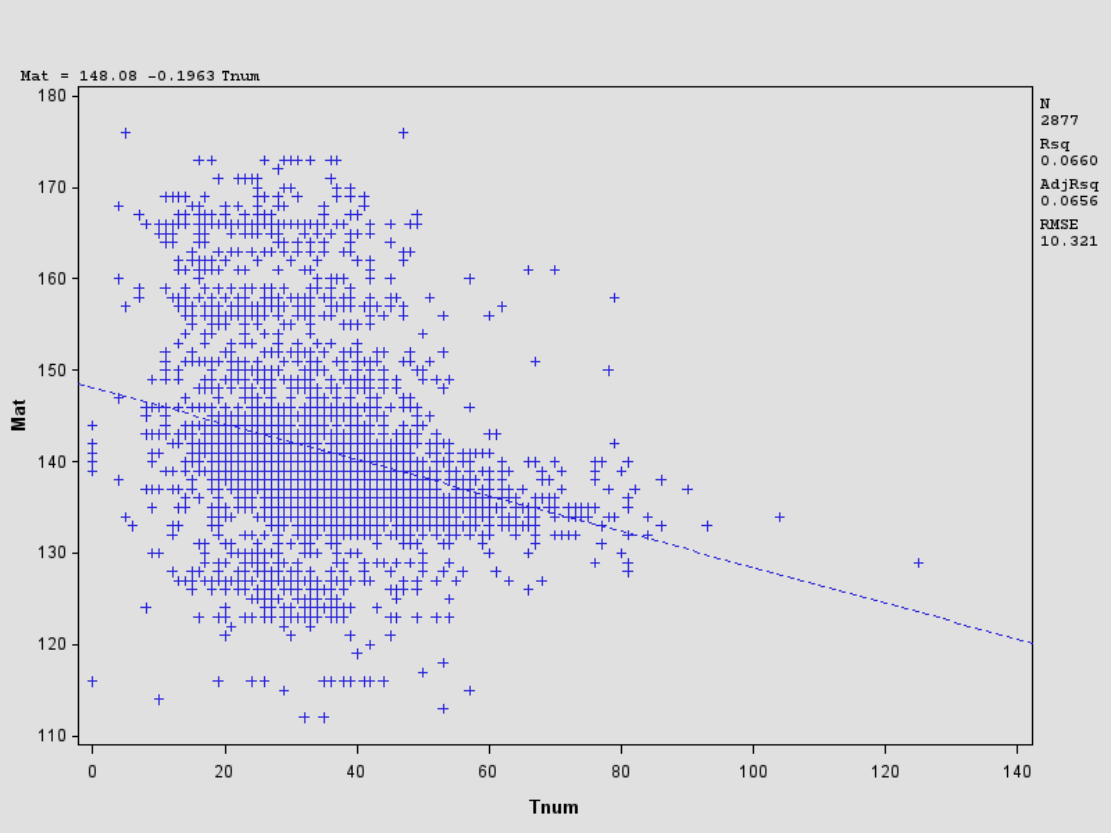

Figure 6.1: regression of tiller number on duration (time to maturity).

6.3.7. Principal component analysis (PCA)

The objective of PCA was to construct 2 to 3 macro-variables that will take into consideration the information contained in the original 8 variables (Figure 6.2). This descriptive method to describe variables relationship showed that more than 50% of the variance was explained by two components. Here, variable relationship is described from correlation table. Only the significant correlation was taken. The first two principal components (PC1 and PC2) explained 43% and 33% of the total variation, respectively. The main traits that had the highest loading scores (and

hence contributed most for the differences) were selection index, total panicle weight, grain weight for component 1, tiller number and panicle number for component2. The Biplot (Fig.6.3) showed the two grouping patterns.

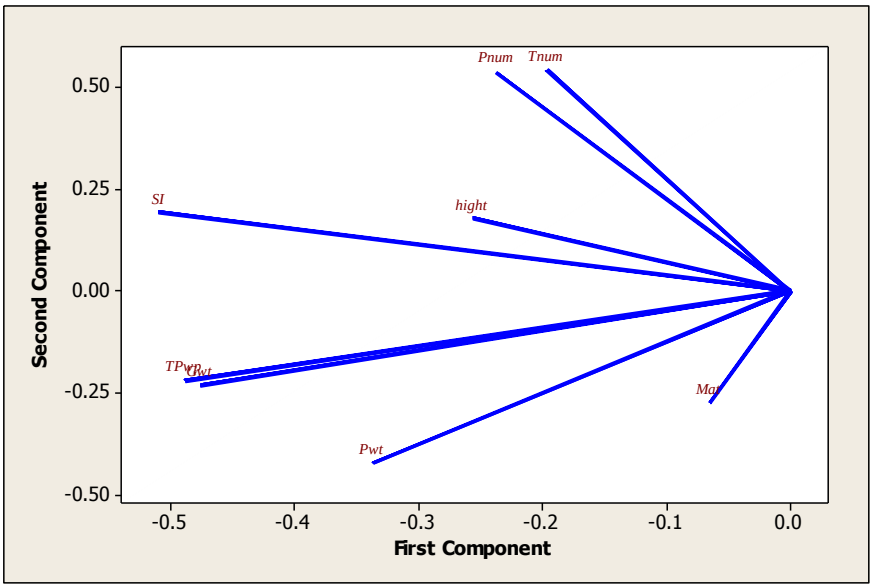

Figure 6.2: The loading plot of eight traits

Pwt= panicle weight, Pnum = panicle number, Tnum= tiller number, Mat= time to maturity, Gwt=grain weight, TPwt=total panicle weight, SI=selection index, hight=height.

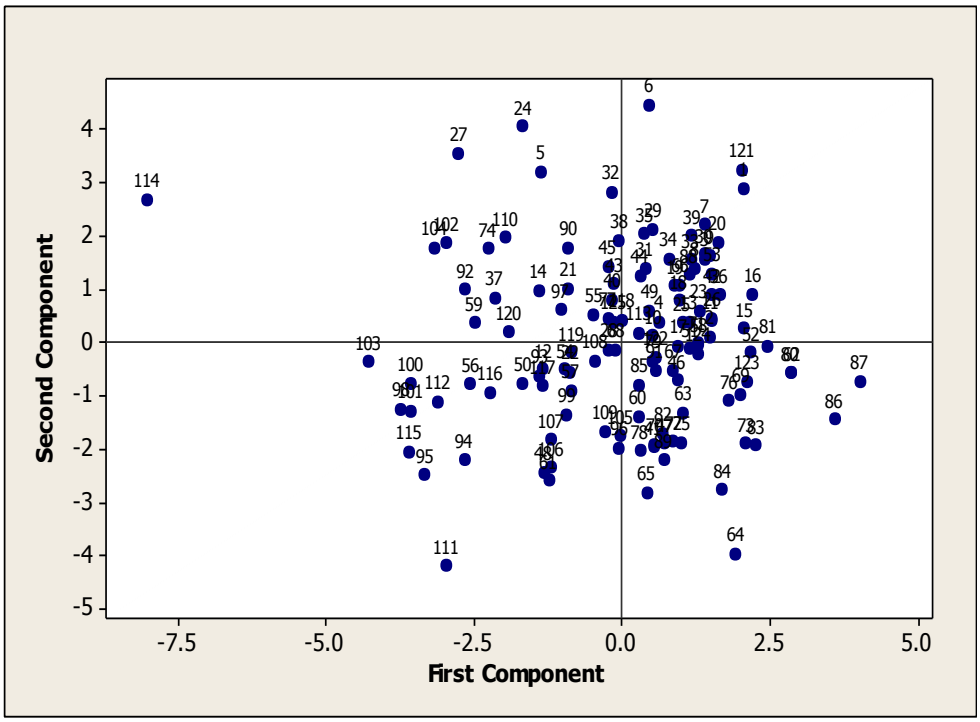

Figure 6.3: Principal components analysis of F_3 population (individuals graphic)

6.4. Discussion

6.4.1. Yield performance and selection indices

The top 20 families across sites yield ranged from 2.52 to 4.17 t/ha under salt stress. While the average national yield was about 4-5 t/ha (Sido, 2010) and has decreased last decade from 4 to 1 t/ha (INS and Mda, 2012). Hence it would be a tremendous opportunity for farmers to produce up to 4 t/ha on fields that were abandoned because of salinity. However, within family maximum yield ranged from 6.8 t/ha to 15 t/ha. These may generate high yielding salt tolerant cultivars if the performance can be maintained over segregating generations up to the lines fixation. The high CV indicated a huge intra family variation which may be due to segregation of the F_3 families. No two individuals in the same family are genetically alike. Generally F_3 families

yielded more at Sekoukou than at Saga. This may be caused by salt stress that was more severe at Saga than Sekoukou, particularly because water shortage that induced drought episodically at Saga.

Certainly, yield is essential but is not the exclusive condition for a variety to be adopted by farmers. Others traits were also preferred in addition to yield. This range of characters was weighted according to farmer's preferences to have the selection index (Table 7.7). The top performing families according to their selection index had values that ranged from 526 to 874. These families containing most of farmer's preferences should be advanced to have them fixed.

6.4.2. Traits association

Most of the F_3 families exhibited higher tillering ability, a character that is significantly correlated with weed competitiveness (Fofana *et al.*, 1995). The F_3 families behaved differently and significantly under salt stress because they derived from different parents and were segregating population. The choice of the incomplete block design was justified by significant block effect. This means that the soil was highly heterogeneous. The average height across sites and families ranged from very dwarf (20 cm) to medium (135 cm). This may due to salt effect on the plant growth (Zeng and Shannon, 2000b). Medium height may be a good trait for farmers. This is because they do not like too tall plant because of it susceptibility to logging. Farmer also do not like too dwarf because it is difficult to harvest.

Across sites, correlation analysis among traits showed that yield was significantly positively associated with height, tiller and panicle number, and panicle weight. Families that could withstand salt stress, had good tillering ability under such condition, grew well and had high yield. This means that those families were tolerant. In the opposite case the susceptible families

had poor tillering ability were dwarf and yielded poorer under salt stress. This is because according to Zeng and Shannon (2000a) salinity reduces growth rate, damages meristems in growing shoots, reduces yield components. This significantly positive association may be exploited to do indirect selection for yield under salt stress. For instance if we knew that height, tiller number and panicle number were always associated with high yield we can at the vegetative stage predict families with high yielding potential.

Yield was negatively correlated with duration and infertile tiller number. The more the time to maturity was long the more the yield was reduced. As salinity reduced the growth rate of susceptible families, this led to a delay of the panicle initiation and elongated the duration. Thus, the susceptible families had their duration too long and their yield reduced because of salt affect. Generally, salinity affects the growth of rice plants at all stages of its life duration. But it is more pronounced on the reproductive stage than on the vegetative stage consequently decreased the grain yield (Afridi and Ali, 1988). The yield decrease at the reproductive stage is the result of tiller infertility. So the higher was the infertile tiller number the lower would be the yield. This was showed in the results by a negative correlation between yield and infertile tillers number. Tiller and panicle number were positively associated with yield components and plant height. This was because according to Abdullah et *al* (2001) plant height, total number of tillers, panicle length, grain weight per panicle, 1000-seed weight and quality and quantity of grains decreased progressively with increase in salinity levels.

The results showed that under salt stress only plant height and panicle weight explained positively and significantly the yield. Higher plants in saline condition means more vigor, more photosynthesis, thus, more organic matter and more filled grains.

6.5.Conclusion

The 120 F_3 families evaluated on farmer's fields under salt stress showed good agronomic performances. These were yield, high tillering ability, high panicle number, and medium height. However, salt effect impacted on the duration that was long. The average families yield across the two sites ranged from 2.53 to 4.11 t/ha. The maximum attained yield within family was 15.97 t/ha. Even though this yield was estimated on an individual basis it supposes that these families may have good high yield potential. However, the plant duration was elongated and the height reduced because of salt stress. Results showed yield, tiller number, panicle number and plant height, panicle weight co-varied significantly and positive. This can lead to other criteria to select indirectly salt tolerant families even at vegetative stage. Selection indices allowed us to work out 20 potential F_3 candidates for farmer's adoption.

CHAPTER SEVEN

7.0. MOLECULAR CHARACTERIZATION OF RICE GENOTYPES USING SNP MARKERS

7.1. Introduction

Genetic markers used in genetics and plant breeding can be classified into two categories: classical markers and DNA markers (Xu, 2010). Classical markers include morphological markers, cytological markers and biochemical markers. DNA markers have developed into many systems based on different polymorphism detecting techniques or methods (Collard *et al.*, 2005), such as RFLP, AFLP, RAPD, SSR, SNP, etc. Conventional breeding selects genotypes indirectly through phenotypes, which is generally effective for qualitative traits only but not for quantitative traits. Over the past few decades, advances of molecular markers have exerted far-reaching influences on the concept and means of conventional rice breeding (Rao *et al.*, 2014). DNA MAS has been seen as a means of improving the speed and efficiency of plant breeding programs because it is growth stage independent, unaffected by environment; no dominance effect and efficient to use in early generations (Singh, 2009). DNA sequence variation accounts for a large fraction of observed differences between plant individuals or varieties, including plant development, yield, stress tolerance, and nutritional quality (Syvanen, 2001).

In rice, Flowers *et al.* (2000) identified AFLP markers for ion transport and selectivity. In the tolerant variety Pokkali, a major gene, possibly SalT (Causse, 1994), has been mapped (IRRI, 1998), as were QTLs governing high K^+ and low Na^+ absorption, and high K^+/Na^+ ratio (Gregorio *et al.*, 2002). A common major QTL was found on chromosome 1 for three traits

associated with salt tolerance mapped (IRRI, 1998),. Other markers have been identified for traits associated with productivity in saline environments, although many are associated with tolerance to submergence, and micronutrient deficiency or toxicity (Gregorio *et al.*, 2002). Other QTLs for salt tolerance have been mapped by Koyama *et al.* (2001), Lin *et al.* (2004) and Takehisa *et al.* (2004). The bulk of natural genetic variation in organisms is represented by SNPs or small insertions or deletions. The potentially large number of SNPs in the genomes of individuals within a population or species (Schafer and Hawkins, 1998; Cargill *et al.*, 1999; Kwok, 2001; Syvanen, 2001) provides the foundation for novel approaches to genomic mapping of quantitative trait loci. SNP is a single nucleotide base difference between two DNA sequences or individuals (Edwards *et al.*, 2007). SNPs are co-dominant markers, often linked to genes and present in the simplest/ultimate form for polymorphism, and thus, they have become very attractive and potential genetic markers in genetic study and breeding (Guo-Liang, 2013). Single nucleotide polymorphism (SNP) is the most abundant polymorphism in the genome. SNPs could be found in both intron and exon regions that affect the translated product or amino acid sequence and induce alteration of normal function (Sasaki *et al.*, 2005). This indicates that SNPs may be important in the expression of quantitative traits that are controlled by a combination of multiple genes. The identification of SNPs within the target genomic region of a QTL is necessary for efficient breeding of quantitative traits such as salt tolerance. For this purpose this study was carried out with following objectives to:

- ⇨ characterise four rice parental genotypes using SNP markers.
- ⇨ identify the polymorphic SNPs for salt tolerance QTL study in offspring.

7.2. Material and Methods

7.2.1. Plant material

The plant material used in the genotyping was composed of four parents among which we had two salt tolerant (IRRI113 and NSIC RC106) varieties imported from IRRI and two farmer preferred varieties (Gambiaka and IR1529) (Table 7.1).

Table 7.1: plant material used for the genotyping

Genotypes	Origin	Characteristic
IRRI113	IRRI	Salt tolerant
NSIC RC106	IRRI	Salt tolerant
Gambiaka	Niger	Susceptible
IR1529	Niger	Susceptible

7.2.2. Leaf sampling and DNA extraction

Leaf sampling was done at heading stage (Figure 7.1). The samples were taken from the young leaves and put into 96 well plates. The plates were then sufficiently sealed to prevent sample leakage and contamination. All DNA sample plates were clearly labeled. The physical plate name must match the name given to the plate in the submitted DNA sample plate file. The samples plates were then sent to LGC Genomics laboratory for DNA extraction. The leaf samples were sent to LGC genomics for DNA extraction and SNP genotyping using an internal protocol.

Figure 7.1: Leave sampling at Sebery.

7.2.3. Data analysis

One thousand eight hundred ninety six (1896) SNPs markers were used to genotype the parental genotypes. Genotypic data were analyzed using MEGA6 and GGT2 software. The following procedures were used:

- ⇨ Homogeneity of Substitution Patterns between Sequences

The probability of rejecting the null hypothesis that sequences have evolved with the same pattern of substitution, as judged from the extent of differences in base composition biases between sequences (Kumar and Gadagkar, 2001). A Monte Carlo test was used to estimate the P-values (Kumar and Gadagkar, 2001). Evolutionary analyses were conducted in MEGA6 (Tamura *et al.*, 2013).

- ⇨ Maximum likelihood Estimate of substitution

Each entry is the probability of substitution (r) from one base (row) to another base (column). Substitution pattern and rates were estimated under the model of Tamura and Nei (1993). %. For estimating ML values, a tree topology was automatically computed. Relative values of instantaneous r were considered when evaluating them. For simplicity, sum of r values was made equal to 100, the nucleotide frequencies were A = 25.12%, T/U = 22.21%, C = 25.88%, and G = 26.79.

- ⇨ Estimates of Evolutionary Divergence between Sequences analysis were conducted using the Maximum Composite Likelihood.

7.3. Results

7.3.1. Statistics on individuals

The heterozygous frequencies were null meaning that all the parents were homozygous (Table 7.2.). All the genotypes had four alleles per locus. The 'A' allele was stable across genotypes with a frequency of about 23%. The allele B frequency ranged from 20.7 in IRRI113 genome to 22.4 in Gambiaka genome with an average of 21.8%. The following values: 25.1, 26.4, and 25.32 respectively were the minimum, maximum and average allele D frequency across genotypes. The most frequent allele's average percentage ranged from 21.8 to 25.32% across genotypes. The average missed values percentage equaled to 4.62. The recombinant ranged from 1439 for IR1529 to 1485 for NSIC RC106. The average distance occupied one the chromosome were 446.27, 413.11, 467.59, 479.91cM by allele A, B, C, and D respectively.

Table 7.2: Individuals statistics

Genotype	- (%)	A (%)	B (%)	C (%)	D (%)	Total (cM)	Recombinant	H frequency
IR1529	4	23.9	22.3	24.7	25.1	1895	1439	0
IRRI113	4.3	23.9	20.7	26	25.1	1895	1447	0
Gambiaka	3.7	23.3	22.4	24.1	26.4	1895	1452	0
NSIC RC106	6.5	23.1	21.8	23.9	24.7	1895	1485	0
Average	4.625	23.55	21.8	24.675	25.325	1895	1455.75	0

- = missed values, A, B, C, and D are the most frequent alleles, H frequency = heterozygous frequency.

Clear evidence of genetic diversity was showed by the linkage group (Figure 7.2). This figure showed the allele frequencies in chromosome1 of the four genotypes.

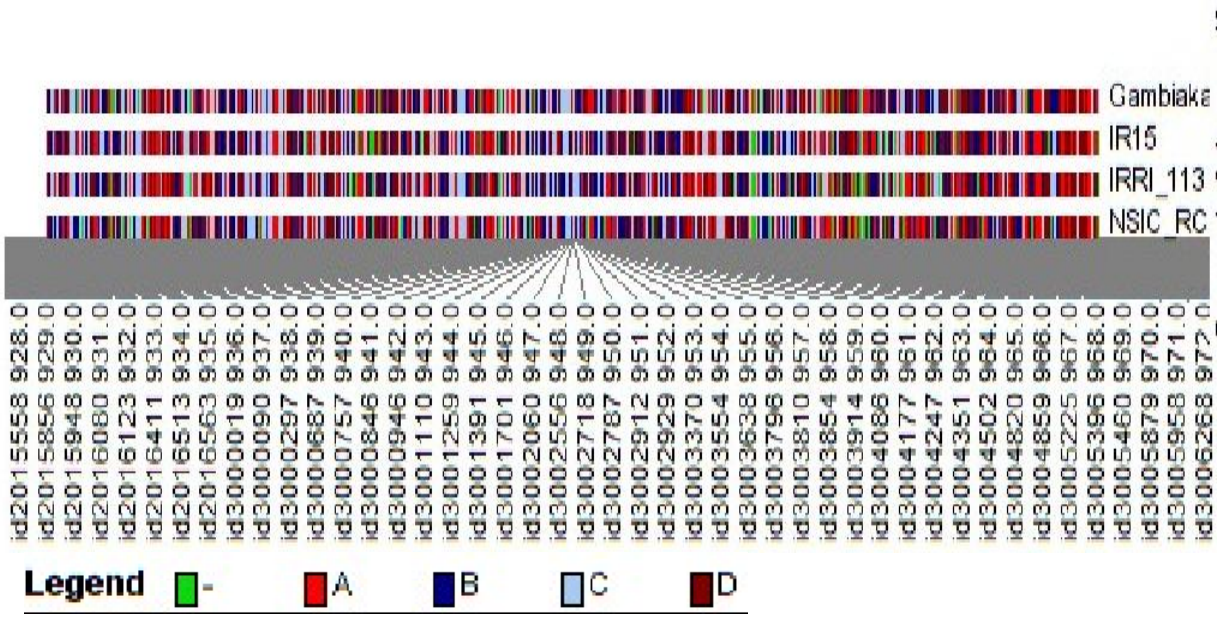

Figure 7.2: Markers and alleles frequencies on chromosome1 of each variety.

7.3.2. Homogeneity of Substitution Patterns between Sequences

The estimates of homogeneity of substitution per genotype are shown above the diagonal (Table 7.3). This table showed that Gambiaka and IR1529 on one hand and Gambiaka and NSIC RC 106 on the other hand did not have sequences involved the same pattern of substitution ($P< 0.05$). This was also true for IR1529 and NSIC RC106. The estimates of the disparity index per genotype are shown for each sequence pair below the diagonal. The disparity index for sequences was high between the two local varieties (Gambiaka and IR1529) and between the local varieties and NSIC RC 106. The substitution pattern was not significant between the 2 imported genotypes (NSIC RC106 and IRRI103) and between the local varieties and IRRI113.

Table 7.3: Test of the Homogeneity of Substitution Patterns between Sequences

	Gambiaka	IR1529	IRRI113	NSICRC106
Gambiaka		0.000	0.371	0.000
IR1529	1.000		0.120	0.000
IRRI113	0.080	0.202		0.081
NSIC RC106	1.000	1.000	0.280	

7.3.3. Maximum likelihood Estimate of substitution

Rates of different transitional substitutions are shown in bold and those of transversionsal substitutions are shown in italics (Table 7.4). A transition is a point mutation that changes a purine nucleotide to another purine (A ↔ G) or a pyrimidine nucleotide to another pyrimidine (C ↔ T). Approximately 68% of nucleotide polymorphisms (SNPs) were transitions and 32 % were

transversion. Transversional substitution is a change of purine nucleotide to pyrimidine or vice versa. The nucleotide frequencies were A = 25.12%, T/U = 22.21%, C = 25.88%, and G = 26.79.

Table 7.4: Maximum likelihood estimate of substitution matrix

From\To	A	T	C	G
A		*3.5965*	*4.1912*	**17.0363**
T	*4.0674*		**18.6243**	*4.3391*
C	*4.0674*	**15.9814**		*4.3391*
G	**15.9697**	*3.5965*	*4.1912*	

7.3.4. Estimates of net base composition disparity between sequences

Disparity Index per genotype is shown for all sequence pairs in Table 7.5. The difference in bases composition between IRRI113 and the other genotypes (IR1529, Gambiaka and NSIC RC106) were larger than can be expected based on evolutionary divergence between sequences and by chance alone (values greater than 0). So in terms of base composition IRRI113 was significantly different from the others.

Table 7.5: base disparity index

	Gambiaka	IR1529	IRRI113	NSIC RC106
Gambiaka				
IR1529	0.00			
IRRI113	0.37	0.12		
NSIC RC106	0.00	0.00	0.08	

7.3.5. Estimates of divergence between sequences

The numbers of base substitutions per genotype from between sequences are shown in Table 7.6. Significant divergence between sequences was noted among all the genotypes.

Table 7.6: Evolutionary Divergence between Sequences

	Gambiaka	IR1529	IRRI113	NSIC RC106
Gambiaka				
IR1529	0.32			
IRRI113	0.39	0.31		
NSIC RC106	0.35	0.34	0.35	

7.3.6. Estimates of base composition difference between sequences

The difference in base composition bias per genotype is shown in Table 7.7. Note that even when the substitution patterns are homogeneous among lineages, the compositional distance will correlate with the number of differences between sequences.

Table 7.7: Base composition difference between sequences

	Gambiaka	IR1529	IRRI113	NSIC RC106
Gambiaka				
IR1529	0.10			
IRRI113	0.66	0.37		
NSIC RC106	0.06	0.02	0.35	

7.3.7. Polymorphic SNPs

Out of 1896 markers 200 were polymorphic and were selected. The tables 7.8, 7.9, 7.10, and 7.11 showed the polymorphic markers on chromosomes and their positions. The average distance of SNPs in chromosome1 was 93.21 cM with a maximum of 186 cM and a minimum of 0.87 cM. In the chromosome2, the average distance was 62.89 cM with a maximum of 161 and a minimum of 4.08. In chromosome3 the mean distance was 51.05 with a maximum of 160 and a minimum of 0.89 cM. The distance ranged from 1.78 to 115 cM for in chromosome4, from 6.35 to 107 cM in chromosome 5 and from 0.62 to 116cM in chromosome6.

Table 7.8: Polymorphic markers on chromosomes 1, 2 and 3

Chromosome1		Chromosome2		Chromosome3	
SNP IDs	Position	SNP IDs	Position	SNP IDs	Position
id1000051	0.867353	id2000428	4.081324	id3000019	0.888419
K_id1004420	29.64	K_id2000835	6.47	K_id3000111	3.03
id1005129	33.47513	id2001523	14.05745	id3000946	9.080839
K_id1006604	46.56	K_id2002229	20.26	id3002060	18.42179
id1006896	49.81913	K_id2002914	28.23	id3003554	29.04366
K_id1007975	61.27	id2003338	33.60295	id3004247	39.31101
K_id1009867	73.57	id2003908	37.68662	K_id3004571	43.52
id1010840	77.43971	wd2000409	41.84493	id3004927	45.12897
K_id1011568	80.19	wd2000520	48.19358	K_id3005216	48.58
id1011905	85.26086	K_id2006621	58.02	K_id3006808	63.69
K_id1012481	93.39	id2006996	58.71465	id3007458	69.84012
K_id1013967	101.46	id2007428	68.78314	id3007751	72.62585
id1016322	119.4759	id2007503	77.52977	ud3001297	93.48196
id1017357	123.6085	K_id2007526	78.71	id3011141	117.6122
id1026169	164.4011	K_id2008158	82.51	K_id3012786	124.95
id1027121	172.879	id2015558	151.9569	id3013874	131.8275
dd1002159	178.3815	id2016411	160.1955	K_id3014586	140.33
K_id1028615	186.14	K_id2016584	161.18	id3015964	149.713
				K_id3017469	160.32

Table 7.9: Polymorphic markers on chromosomes 4, 5 and 6

Chromosome4		Chromosome5		Chromosome6	
SNP IDs	Position	SNP IDs	Position	SNP IDs	Position
K_id4000170	1.78	id5000567	6.348898	id6000009	0.618279
K_id4000429	7.21	id5001182	17.2626	id6000130	2.632115
id4001205	10.24293	id5002216	28.43346	K_id6000134	2.84
id4002026	13.9091	id5002971	37.46984	id6000317	4.267816
K_id4002286	16.6	id5003312	41.58522	id6000402	4.559965
id4002844	20.21566	id5003661	44.58519	K_id6000911	7.25
id4003819	21.68426	K_id5003785	48.18	K_id6001206	8.44
K_id4004294	26.73	id5006085	55.33758	id6001397	9.881325
id4004639	32.04812	K_id5006470	61.38	K_id6001535	11.18
K_id4005120	38.95	K_id5006603	62.16	id6002930	15.69284
id4006940	64.82605	id5007323	67.84047	K_id6002884	15.74
id4007322	68.67374	id5008574	77.80628	K_id6003341	19.23
id4008090	76.83215	id5008964	82.85562	id6005661	54.78933
id4008670	83.66964	id5009481	89.19636	ud6001082	96.70861
K_id4009920	100.41	id5011512	100.8186	K_id6013720	97.94
id4010800	108.3113	id5013010	107.7037	id6013772	98.08077
id4010934	111.3078			id6016361	114.4668
id4011530	115.5442			id6016476	116.3189

The average distance of the 17 SNPs in chromosome 7 was 47.91 cM with a maximum of 113 cM and a minimum of 0.34 cM. In chromosome8 the markers were distributed with a mean distance of 57.75 cM a maximum of 121.7 cM and a minimum of 5.25 cM (Table 7.10). The SNP marker the most distanced in chromosome 9 was at 92.8 cM where the nearest was at 1.31 cM, the average being 52.67 cM. In chromosome 10 SNPs distances ranged from 1.09 cM to 83.2 cM (Table 7.11). The average distance of the 6 SNP markers was 55.5 cM with a maximum of 100 and a minimum 43.6 cM.

Table 7.10: Polymorphic markers on chromosomes 7, 8 and 9

Chromosome7		Chromosome8		Chromosome9	
SNP IDs	Position	SNP IDs	Position	SNP IDs	Position
id7000022	0.33541	id8000118	5.25447	id9000064	1.307157
K_id7000063	2.08	id8000251	8.419736	id9002252	14.34419
id7000178	4.656701	K_id8000876	25.38	K_id9002255	14.53
K_id7000142	5.25	id8001168	29.84783	K_id9002751	32.55
K_id7000304	8.53	wd8000422	35.65761	id9002784	33.49743
id7000357	8.73894	K_id8001641	40.87	K_id9003188	38.31
K_id7000337	9.28	K_id8001908	43.85	id9003470	42.03134
K_id7000384	10.18	K_id8002554	49.55	K_id9003471	42.22
wd7002642	62.17065	K_id8002954	52.07	id9004297	54.76428
K_id7002907	64.06	id8004845	62.76122	K_id9004727	58.87
id7003043	68.6151	K_id8004986	66.01	id9004766	59.10942
wd7002824	68.78491	id8005359	71.59807	K_id9004788	59.51
id7003593	76.83936	id8005704	77.40867	id9004968	61.87891
K_id7003459	76.84	id8007471	114.7086	K_id9006377	70.28
id7005614	110.9133	id8007627	118.8559	id9006995	78.06786
K_id7005611	111.68	id8007913	121.7728	K_id9007001	78.36
id7005631	113.6204			K_id9007171	81.52
				id9007315	86.69069
				id9007525	92.8057

Table 7.11: Polymorphic markers on chromosomes 10, 11 and 12

Chromosome10		Chromosome11		Chromosome12	
SNP IDs	Position	SNP IDs	Position	SNP IDs	Position
K_id10000028	1.09	id11000855	14.56748	ud12000534	43.60153
id10000090	1.488983	id11001777	26.08108	id12004082	44.51469
K_id10001706	13.95	id11002903	36.18737	id12004491	45.85779
wd10001251	15.74153	ud11000372	39.41541	id12005205	48.24597
id10002302	15.96039	id11003441	49.49086	K_id12003862	50.43
K_id10002912	19.82	id11003686	53.93942	id12009798	100.7084
id10003618	27.74746	K_id11004148	55.74		
id10003686	29.53404	id11004812	57.15901		
id10004327	38.09736	id11005900	64.72559		
id10004942	45.39211	id11006682	75.53145		
id10004968	45.61131	K_id11008862	89.98		
id10005369	51.33837	K_id11008951	91.37		
id10005979	55.8158	id11009197	97.59037		
id10006250	57.92678	id11009699	100.894		
id10006378	60.84147	K_id11010335	109.39		
K_id10006910	75.47	id11010893	114.8913		
K_id10006963	77.51	id11011243	116.5388		
id10007012	83.27446				

7.3.8. Markers distribution across rice genome

A list of 200 Polymorphic SNPs was selected. The SNPs were approximately evenly distributed (Figure 7.3) across the rice genome. There was a good number of polymorphic SNP markers in the chromosomes 1 to 11, but only 6 SNPs were polymorphic in the Chromosome 12. Thus, the number of SNPs markers ranged from 6 to 19 per chromosome.

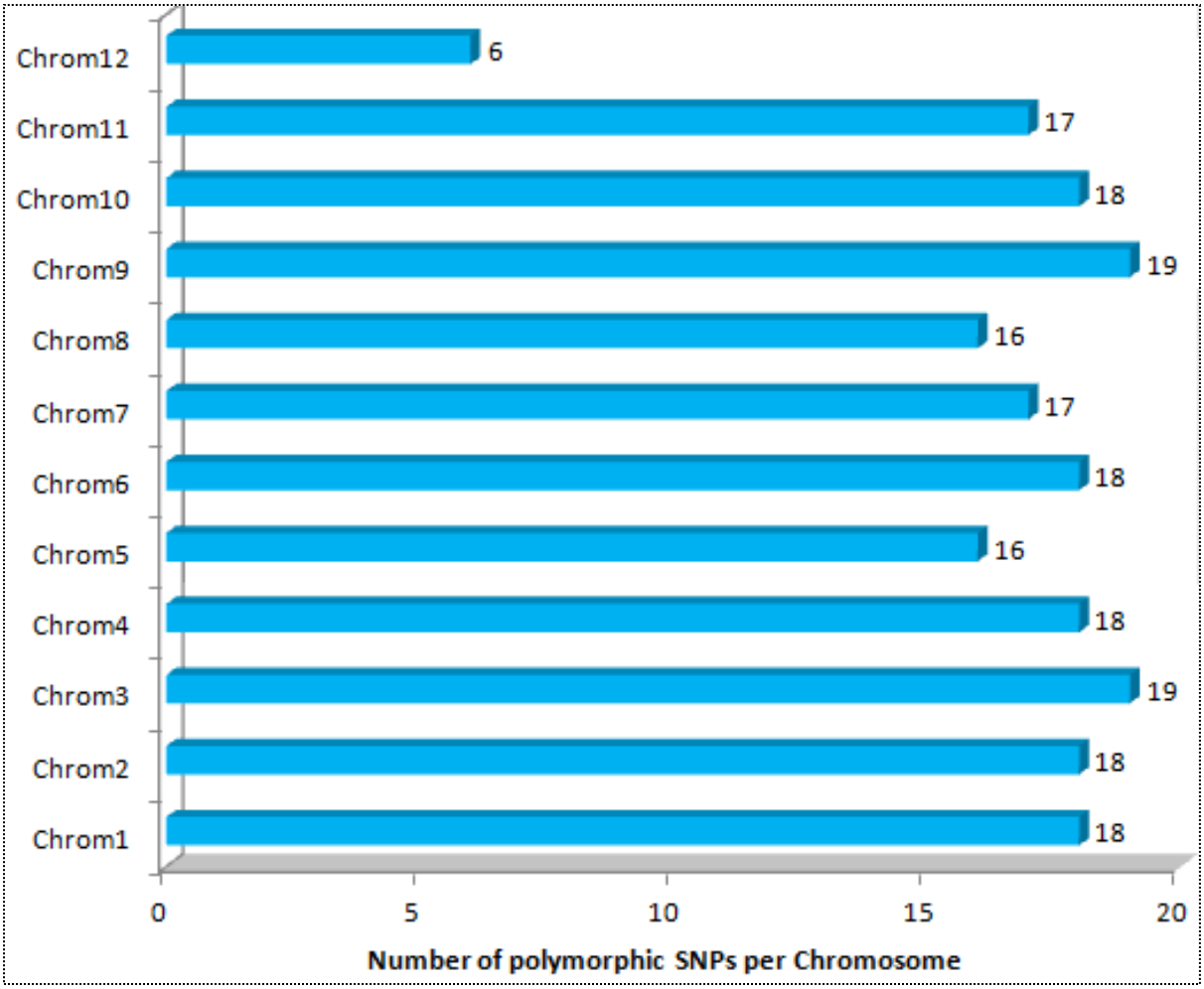

Figure 7.3: Number of polymorphic SNPs markers per Chromosome

The marker distribution across genome (Figure 7.4) showed that the higher distance mean was observed on chromosome 1 and the lower average distance was noticed in chromosome 6. A minimum distance less than 1 was observed in chromosome 1, 3, 6 and 7.

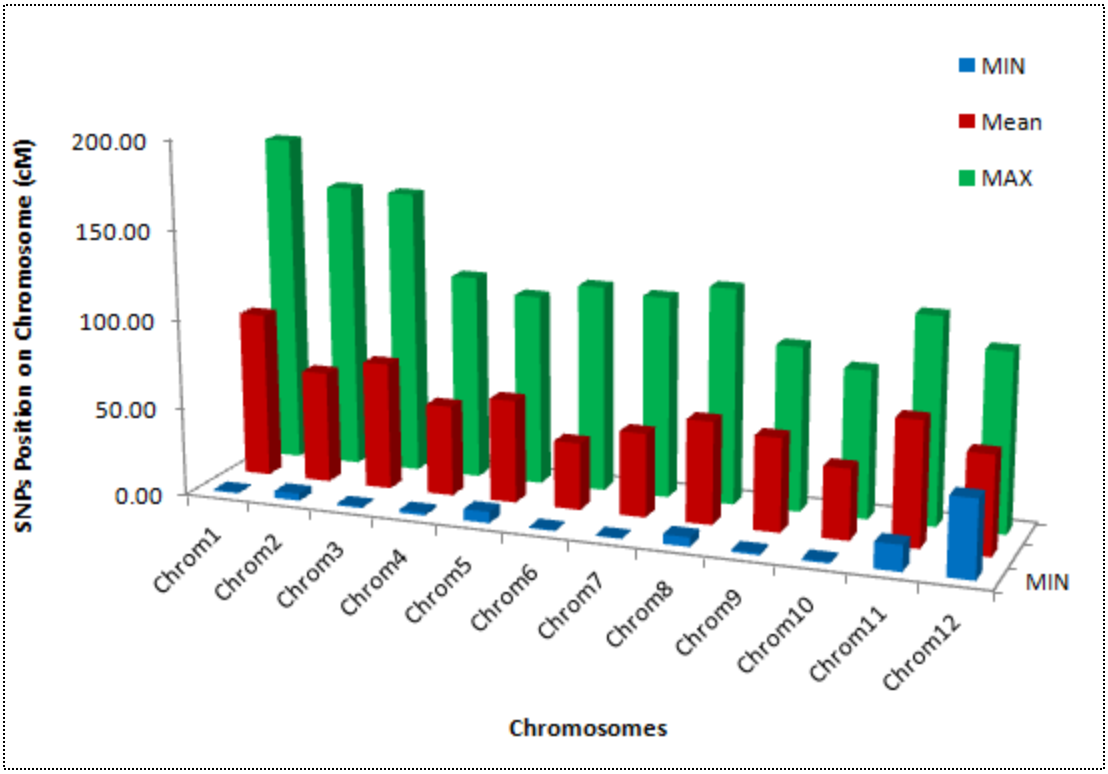

Figure 7.4: Mean, minimum and maximum of polymorphic SNPs distances in rice genome

7.4. Discussion

Results showed that the heterozygous frequency was zero for all the genotypes. This meant that the genotypes used in crossing were highly homozygous with 100% homozygosity. Thus, these genotypes were pure fixed lines. The most frequent allele differed from genotype to genotype except for the allele A which was slightly stable across genotypes. These differences may be due to genotypic diversity of the 4 parents.

The results showed significant difference in substitution pattern between the two farmer preferred varieties: IR1529 and Gambiaka, and between the local varieties and NSIC RC106. This was confirmed by a high disparity index. This may also be due to the genetic diversity of these genotypes (IR1529, Gambiaka and NSIC RC 106). But our method failed to show significant difference of substitution pattern and high disparity between IRRI113 and the 3 others

genotypes. The results showed that 68% of the substitutions were transition and 32% were transversion. This confirmed the results of Tamura *et al.* (2013) who showed that about two third (2/3) of the substitutions were transitions. Although there are twice as many possible transversions, because of the molecular mechanisms by which they are generated, transition mutations are generated at higher frequency than transversions. As well, transitions are less likely to result in amino acid substitutions (due to "wobble"), and are, therefore, more likely to persist as "silent substitutions" in populations as single nucleotide polymorphisms (SNPs) (Steven, 2014).

The result showed that in terms of base composition IRRI113 was significantly different from the others genotypes (IR1529, Gambiaka, NSIC RC106). Thus, the only one that was not significantly different from the others in terms of substitution pattern was in terms of base composition. This means that all the genotypes were genetically different either in terms of base composition or in terms of substitution pattern or both. The results showed also that sequences diverge significantly among all the genotypes. This corroborated the above results.

The 200 polymorphic SNPs markers were evenly distributed across rice genome. However, chromosomes did not have the same number of polymorphic SNPs. This was because each of the 12 rice chromosomes has a characteristic genome structure. The rice chromosomes range in size from 45 Mb (chromosome 1) to 24 Mb (chromosome 10) and the gene density varies from 8.7 (chromosome 3) to 11.6 (chromosome 12) genes per kilobase pair (Wu *et al.*, 2004; Zhang *et al.*, 2004). According to Guo-Liang (2013) SNPs may be present within coding sequences of genes, non-coding regions of genes or in the intergenic regions between genes at different frequencies in different chromosome regions.

7.5. Conclusion

Molecular characterization of parental genotypes allowed us to know the homozygosity level. Thus, all the genotypes were 100% homozygous and genetically diverse. The substitution pattern and disparity index between sequences showed differences between local varieties and one exotic parental genotype. However, divergence between sequences and base composition difference between sequences gave evidence of genetic diversity of the 4 parents. Two hundred out of 1896 SNPs were polymorphic and were distributed evenly across the rice genome. The SNPs number and position on chromosomes differed from one chromosome to another. The two hundred polymorphic SNPs will be used to genotype the rice generations derived from the cross of those parents to find QTL for salt tolerance. This will facilitate the selection of salt tolerance lines.

CHAPTER EIGHT

8.0. GENERAL DISCUSSION

8.1. Introduction

The goal of this chapter is to review and conclude the completed research, and draw out some of its implications for breeding. The research objectives were to:

i. determine farmers' perceptions on influence of salinity in rice production and their preference for rice varieties.
ii. identify potential sources of genes for salt tolerance from local and exotic rice germplasm.
iii. determine genetic control of salt tolerance in rice.
iv. evaluate yield performance of early segregating generations of rice.
v. determine molecular characteristics of parental genotypes used in the crossing.

8.2. Findings of the study and their implications

Germplasm improvement requires an understanding of farmers' preferences and criteria for rice varietal selection in fragile environments. We need to understand what "enabling inputs" may be required to accelerate and widen adoption. These requirements necessitate the use of participatory research approaches (Tozawa, 2005; Rusike *et al.*, 2006). The participatory rural appraisal methodology was adopted to interact with villagers in rice production area of Niger. This was to understand them and learn from them about rice production constraints, their

perception of the salt problem and their coping strategies, their preferences about rice varieties and traits.

According to AFDB and OECD (2007) nearly all cultivated land of Niger is devoted to rain fed crops, mostly millet, sorghum and cowpea, and to a lesser extent, cassava. But the study showed that rice was the most preferred crop in the study area before millet and sorghum known to be the major crop of the country. This confirmed a previous study of Mounirou *et al* (2013) who reported that rice is the favorite crop of the people along the Niger River, especially in the western part of Niger. This justifies the need for resources to improve the crop. The rice production is constrained by many factors, among them salinity was the third most serious after lack of fertilizer and diseases. Thus, farmers were aware of salt problems and its effect on plant growth and yield. But the only attempts to fight against this scourge have been through agronomic measures (use of manure, straw, non application of urea). There have been no attempts to develop varieties with tolerance to salt stress. Among the most preferred rice characteristics were cited yield, tolerance to diseases and salinity high tillering ability and height. This is very useful in the breeding programme to have the improved developed varieties be adopted by farmers. Thus, in the generation advancement the selection must be made for high tillering ability, good height and yield under salt stress conditions. No programme at the national level had been set to improve rice for these traits. All the improved varieties have been developed elsewhere (Africa Rice center Mali, IRRI etc...) and adopted in Niger after multi-location trials and homologation.

A very important thing to know after interacting with farmers was to the extent of salinity in their fields and the contribution of irrigation to the problem. So more precise data were needed

beyond farmer's information. This was the rationale of farmer's fields' soil and irrigation water analysis. Few studies have been conducted before but not a large scale on the irrigated scheme.

Jacques *et al* (1994) have studied the mineralogy of salt efflorescence in paddy field soils of Kollo. When the process of soil physic-chemical degradation was thesis subject of Guero (2000). This study showed that the irrigated soils were characterized by a pH less than 7, an EC (Electrical conductivity) above 4 a SAR (Sodium adsorption Ratio) below 13 an ESP (Exchangeable sodium percentage) below 15 and flocculated structure. These are the typical characteristics of saline soil (Horneck *et al.*, 2007). Moreover, the situation is getting worst with irrigation. Because the results showed that, salts are added to the soil with irrigation, and a huge quantity of sodium from irrigation water is deposited in rice fields. This was clear evidence that salinity is a real problem and that we need salt tolerance rice varieties for the sustainability of irrigation in the country.

Significant variability existed among varieties for response to salt stress. Some genotypes performed well under salt stress when other did not. The response depended on the salt conditions. Therefore, selection index was used to select the best performed along with the farmers preferred varieties to cross. The genetic diversity was confirmed by molecular analysis. Genetic diversity is an important tool for a crop improvement programme, as it helps in the development of superior recombinants (Manonmani and Fazlullah Khan, 2003). Genetic divergence among the genotypes play an important role in selection of parents having wider variability for different traits (Nayak *et al.*, 2004).

Heritability is responsible for the resemblance among relatives due to the transmission of traits from parents to offspring and also for accuracy of the prediction of the genetic gain from the selection. However, heritability estimates are always based on the target population in both space

and time, and the environment where it is estimated (Singh *et al.*, 2010a). The trait heritability was high or low in relation to the nature of the common parent of the population.

If the heritability was high selection can be made in the early generation on a plant basis through few environments. But if the heritability was low, early-generation population size should be large; replications and locations over years should be increased, if possible, to screen the right genotypes; and selection should be done in the later generations. However, if the trait associated with salt tolerance has high heritability, then early selection could be practiced.

Thus, to get more reliable estimates of the heritability, a compromise between resources and the experimental parameters needs to be made. Since salinity is highly variable in the soil because of the dynamic state of soluble salts, more blocks at different locations over the years in accordance with a judicious use of available resources are recommended for the precise estimation of heritability. The additive gene action was reported for tiller number, number of panicle and weight. This indicated their high breeding values, good condition for their selection in the next generations.

The within and inter family mean yield was variable, it ranged from 0 to 15 t/ha. Grain yield of the F_3 families under salt stress in relation to their parent yield has been considered as good criteria for salt tolerance. The family with high mean, under multiple stress environments was adjudged as suitable, stable and adaptable genotypes for sustainable productivity in problem soils. Selection for high mean yield is a fundamental selection criterion for all the varieties.

Selection indices were used to identify the best progenies in early generation of the breeding programme under salt stressed conditions. Twenty progenies were identified with high selection indices. From these families high yielding salt tolerant cultivars are expected if the yield potential were maintained over generations.

Under salt stress, yield was positively associated with height, tiller number, panicle number, panicle weight, and negatively associated with duration and tiller infertility. Association studies are important for indirect selection for a desired trait(s) using associated traits if phenotyping of the latter is relatively easy. The association could mainly be due to two reasons: either the traits are closely linked or the gene has pleiotropic effects (Mishra, 1994). Salt stress affects most growth parameters; therefore, by selecting the most closely associated trait, a desirable genotype can be selected. An altered effect due to salt stress is much more evident in salt-sensitive genotypes than in tolerant ones. Therefore, traits such as height, tiller number, panicle number, panicle weight are good indicators for selecting high yield tolerant families.

8.3. Conclusion and the way forward

Traits relationships under salt stress conditions are very important for salinity stress breeding programme. Their interaction and association pattern must be well understood to enhance salt tolerant genotype breeding programme. Information about traits associations with yield is very useful for developing an efficient breeding strategy for evolving high yielding salt tolerant varieties. Further investigation is required in this area in order to find out or to confirm if significant association exists between morpho-physiological traits and yield under salt stress. The relative contribution of each trait to yield under saline condition should be clearly established. The F_3 families indentified as salt tolerant were: Kol21-18, Kol21-1, kol23-11, Kol25-28, Kol5-14, Kol21-14, Kol23-9, Kol4-13, Kol25-16, Kol25-21, Kol21-19, Kol23-25, Kol25-30, kol23-13, Kol25-8, Kol21-26, Kol5-16, Kol25-22, kol23-10, Kol14-37. Theses progenies should be advanced and evaluated in multi-environment trial to select the best lines.

Farmers have many preferences so specific breeding programmes should be set to respond to their needs. In addition, the segregating population should also be screened for others traits such

as high yield in salt free conditions, resistance to diseases and other abiotic stress. This is because if these families combine other useful preferred traits with salt tolerance, it will facilitate a wide adoption by farmers.

Salinity is strongly influenced by environment so there is a strong need for refinement of phenotyping techniques and use of marker assisted selection to facilitate breeding for salinity tolerance in rice. In this aim the polymorphic SNPs makers should be used genotype the F_2 populations to find QLT for salinity tolerance.

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APPENDICES

Appendix 1: Mean, Cv and maximum of F3 families and parents yield at Saga

Family	Num	Mean	CV (%)	Max	Family	Num	Mean	CV (%)	Max
Kof21-18	114	4.5	78.2	9.0	kof23-10	102	1.7	101.5	4.8
Kof23-9	101	4.3	46.1	9.7	Kof4-7	54	1.7	77.4	3.8
Kof25-21	95	4.3	47.4	7.3	Kof23-31	110	1.7	104.0	4.9
Kof25-28	98	4.2	56.3	7.5	Kof27-22	85	1.7	121.3	6.2
kof23-11	103	4.2	44.4	7.4	Kof5-11	45	1.7	75.3	3.8
Kof5-14	48	4.1	53.0	10.3	Kof29-15	74	1.7	153.3	7.0
Kof21-1	111	4.0	40.2	6.3	Kof23-24	105	1.7	93.6	4.1
KOf4-13	56	3.9	60.4	7.5	Kof27-30	90	1.6	133.7	5.8
Kof21-14	112	3.9	53.4	6.8	Kof31-36	70	1.3	146.8	7.2
Kof25-8	92	3.6	67.6	9.9	Kof25-7	91	1.3	112.7	4.7
Kof25-16	94	3.6	66.2	6.7	Kof4-16	58	1.1	146.5	4.7
Kof21-19	115	3.4	76.8	9.8	Kof5-8	43	1.1	125.1	4.1
Kof4-17	59	3.3	80.0	9.7	Kof31-22	63	1.1	150.4	4.1
Kof23-25	106	3.3	49.9	6.8	Kof23-27	108	1.1	146.8	4.9
Kof21-26	117	3.3	59.2	5.7	Kof21-15	113	1.0	167.6	4.8
Kof21-30	120	3.0	86.4	9.0	Kof11-16	40	0.9	140.1	3.4
Kof21-24	116	2.9	99.7	8.3	Kof25-22	96	0.9	147.3	3.5
Kof23-26	107	2.6	114.6	9.1	Kof11-8	35	0.9	200.7	6.8
Kof21-27	118	2.5	95.1	7.8	Kof14-47	28	0.9	158.5	3.6
Kof21-28	119	2.5	93.6	6.1	Kof27-7	82	0.9	142.9	4.2
Kof31-51	61	2.5	114.1	7.0	Kof14-37	22	0.8	211.0	5.4
Kof5-16	50	2.5	62.4	5.6	Kof29-23	77	0.8	168.1	3.9
kof23-13	104	2.5	111.7	8.9	NERICA L49	125	0.8	162.8	4.9
Kof5-12	46	2.5	73.2	5.3	Kof27-27	88	0.8	188.6	5.3
kof5-13	47	2.4	68.4	5.7	Kof14-42	25	0.8	154.8	3.8
Kof15-3	12	2.4	111.2	9.0	Kof11-9	36	0.8	151.2	4.3
Kof25-27	97	2.4	75.1	4.8	Kof5-6	42	0.7	215.5	6.5
Kof25-30	100	2.3	120.1	7.8	NSIC RC 106	122	0.7	130.9	2.6
Kof23-28	109	2.2	87.4	5.4	Kof2-2	2	0.7	170.7	3.6
Kof5-2	41	2.1	108.5	7.5	Kof27-29	89	0.7	305.7	10.6
Kof4-18	60	2.0	96.0	5.9	kof27-15	84	0.7	207.0	3.8
Kof25-11	93	2.0	110.8	6.2	Kof31-28	68	0.6	247.0	5.1
Kof25-29	99	1.9	102.3	5.7	Kof29-19	76	0.6	151.1	3.3
Kof11-12	37	1.9	102.6	5.4	Kof14-31	21	0.6	151.2	2.3
Kof4-15	57	1.9	94.8	5.2	Kof11-13	38	0.5	190.7	3.1
Kof14-45	27	1.9	61.8	4.3	KOf15-13	14	0.5	301.1	7.2
Kof5-15	49	1.8	102.8	6.7	Kof15-27	18	0.5	268.4	4.9
Kof4-12	55	1.8	95.3	5.4	kof11-7	34	0.5	205.8	2.4
Kof5-10	44	1.8	79.9	4.5	Kof4-3	51	0.4	312.7	5.4
Kof2-5	5	0.4	310.9	4.9	Kof2-1	1	0.2	279.2	1.9
Kof4-6	53	0.4	308.9	4.9	Kof31-29	69	0.2	382.2	2.6
Kof31-25	65	0.4	306.6	4.7	Kof11-6	33	0.2	231.5	1.2
Kof14-41	24	0.4	276.4	4.5	Kof29-14	73	0.1	414.9	2.1
Kof29-16	75	0.3	265.8	3.3	Kof2-4	4	0.1	390.7	1.4
Kof29-24	78	0.3	277.6	3.3	Kof27-25	87	0.1	547.7	2.6
Kof2-3	3	0.2	526.3	6.9	Kof15-15	16	0.1	547.7	1.7
Kof2-15	9	0.2	349.7	4.0	Kof29-31	80	0.1	547.7	1.8
Kof29-25	79	0.2	306.4	2.6	Kof27-6	81	0.1	384.5	0.9
Kof4-5	52	0.2	384.8	3.7	Kof29-11	71	0.0	547.7	0.9

Appendix 2: Mean, Cv and maximum of F3 families and parents yield at Sekoukou

Family	Num	Mean	CV (%)	Max	Family	Num	Mean	CV (%)	Max
Kol25-22	96	4.3	57.5	11.6	Kol14-39	23	2.8	83.6	6.3
Kol14-37	22	4.2	65.8	10.6	Kol5-16	50	2.8	71.0	7.6
Kol29-25	79	3.9	54.8	7.9	Kol23-27	108	2.8	102.6	8.1
KOl15-13	14	3.9	80.5	9.2	Kol21-19	115	2.8	116.3	10.1
Kol15-16	17	3.9	50.1	8.3	Kol25-16	94	2.8	85.5	7.5
Kol21-18	114	3.8	91.1	14.9	Kol14-43	26	2.7	83.5	6.9
Kol2-17	10	3.7	75.5	9.8	Kol11-6	33	2.7	78.5	7.8
Kol31-25	65	3.7	66.9	6.9	Gambiaka	123	2.7	85.4	6.8
Kol29-24	78	3.7	112.1	16.0	Kol21-14	112	2.7	85.5	6.6
kol23-10	102	3.5	66.5	9.8	Kol23-28	109	2.6	96.4	7.2
Kol11-1	31	3.5	65.5	6.8	Kol25-29	99	2.6	95.7	10.4
Kol15-30	19	3.5	70.5	8.6	Kol15-33	20	2.6	75.5	7.6
Kol2-5	5	3.4	82.9	9.6	Kol25-28	98	2.6	118.1	15.2
Kol29-12	72	3.4	99.7	10.0	Kol23-25	106	2.6	106.2	8.7
Kol29-14	73	3.4	68.3	9.6	Kol5-14	48	2.6	79.6	7.6
Kol31-28	68	3.3	70.2	8.3	Kol14-31	21	2.5	101.6	7.5
Kol29-16	75	3.3	87.3	9.9	KOl4-13	56	2.5	69.2	7.3
Kol25-30	100	3.3	93.5	10.7	Kol15-3	12	2.5	106.2	10.3
Kol31-27	67	3.3	86.2	7.7	Kol15-1	11	2.5	107.0	8.4
Kol11-5	32	3.3	88.7	10.3	Kol14-42	25	2.5	70.6	6.7
Kol27-29	89	3.3	85.1	9.4	kol11-7	34	2.4	83.0	7.1
Kol29-11	71	3.3	58.8	10.5	Kol21-15	113	2.4	86.7	7.2
NSIC RC 106	122	3.3	78.1	10.1	Kol14-78	30	2.4	81.4	6.6
Kol14-41	24	3.3	79.5	8.4	Kol11-12	37	2.4	121.1	9.0
Kol21-1	111	3.3	89.8	11.3	Kol5-11	45	2.4	116.0	10.4
Kol14-47	28	3.3	74.6	10.6	Kol15-27	18	2.4	105.3	8.1
Kol27-7	82	3.2	70.6	7.6	Kol1114	39	2.4	93.5	8.3
kol23-13	104	3.2	84.6	9.1	Kol27-6	81	2.4	98.0	8.3
Kol31-26	66	3.2	95.0	9.8	Kol25-7	91	2.4	70.7	7.4
Kol2-4	4	3.2	89.3	7.6	Kol2-14	8	2.4	103.1	7.1
Kol31-23	64	3.1	75.1	9.5	Kol31-36	70	2.4	100.6	7.0
Kol4-7	54	3.1	98.5	11.9	Kol31-51	61	2.4	123.6	10.2

Appendix 2: Mean, Cv and maximum of F3 families and parents yield at Sekoukou (continued)

Family	Num	Mean	CV (%)	Max	Family	Num	Mean	CV (%)	Max
kol23-11	103	3.1	75.4	7.8	Kol31-21	62	2.3	97.0	7.2
Kol11-16	40	3.1	86.1	8.1	Kol11-8	35	2.3	89.0	7.1
Kol15-8	13	3.0	78.3	7.7	Kol5-2	41	2.3	91.0	6.0
Kol29-23	77	2.9	72.3	6.1	Kol4-3	51	2.3	101.6	6.4
Kol11-13	38	2.9	96.4	8.8	Kol27-23	86	2.3	74.3	5.5
Kol14-45	27	2.9	70.8	7.7	Kol4-18	60	2.3	64.5	5.1
Kol5-8	43	2.9	93.8	10.9	Kol29-31	80	2.3	87.5	7.9
kol27-15	84	2.9	77.3	7.4	Kol29-19	76	2.3	93.4	6.1
Kol4-15	57	2.9	84.8	6.7	Kol23-26	107	2.3	94.2	7.7
Kol27-9	83	2.9	86.2	8.2	Kol14-63	29	2.2	95.9	7.2
Kol31-29	69	2.8	52.7	5.9	Kol25-11	93	2.2	116.3	7.5
Kol23-9	101	2.2	112.6	7.9	Kol5-15	49	2.0	81.7	6.5
Kol15-15	16	2.2	64.1	4.9	Kol2-3	3	2.0	107.7	7.5
Kol5-6	42	2.2	66.6	5.5	Kol15-14	15	2.0	133.8	7.5
Kol21-28	119	2.2	91.2	6.2	Kol25-8	92	1.9	94.1	5.3
Kol31-22	63	2.2	95.9	6.1	Kol25-21	95	1.9	120.0	6.8
Kol27-30	90	2.2	99.2	8.9	Kol4-16	58	1.8	85.6	6.6
NERICA L49	125	2.2	104.1	7.4	Kol11-9	36	1.8	101.9	5.8
Kol29-15	74	2.1	127.4	9.0	Kol2-15	9	1.8	120.4	7.2
Kol4-6	53	2.1	72.5	6.1	Kol21-24	116	1.8	157.8	9.9
Kol23-31	110	2.1	128.4	8.6	Kol4-5	52	1.7	119.4	6.7
kol5-13	47	2.1	109.2	10.6	Kol4-17	59	1.7	120.1	6.8
Kol2-13	7	2.1	98.2	6.5	Kol21-30	120	1.6	140.0	7.0
Kol23-24	105	2.1	125.7	8.8	Kol5-12	46	1.6	111.5	7.8
Kol2-2	2	2.1	102.5	5.9	Kol5-10	44	1.5	135.6	9.4
Kol21-26	117	2.1	96.7	6.8	Kol27-27	88	1.5	100.8	5.2
IR15	124	2.0	117.0	7.6	Kol27-25	87	1.5	101.1	4.5
Kol27-22	85	2.0	89.6	7.3	Kol2-1	1	1.4	113.8	4.5
Kol25-27	97	2.0	102.5	6.1	IRRI113	121	1.3	102.0	4.3
Kol2-12	6	2.0	88.6	5.1	Kol21-27	118	1.2	107.1	3.3
Kol4-12	55	2.0	109.6	7.0					

Appendix 3: Mean, Cv and maximum of F3 families and parents yield across sites

Family	Num	Mean	CV (%)	Max	Family	Num	Mean	CV (%)	Max
Kol21-18	114	4.17	83.78	14.88	Kol25-29	99	2.25	99.73	10.35
Kol21-1	111	3.66	65.26	11.29	KOl15-13	14	2.2	135.69	9.2
kol23-11	103	3.64	59.12	7.77	Kol5-2	41	2.19	98.57	7.49
Kol25-28	98	3.39	83.33	15.2	Kol25-27	97	2.18	87.58	6.12
Kol5-14	48	3.32	66.92	10.33	Kol4-18	60	2.16	79.56	5.94
Kol21-14	112	3.28	68.58	6.81	Kol11-12	37	2.15	115.12	8.96
Kol23-9	101	3.27	75.8	9.7	Kol25-11	93	2.13	113.2	7.54
KOl4-13	56	3.2	67.72	7.45	Kol29-25	79	2.09	117.72	7.87
Kol25-16	94	3.19	75.13	7.51	Kol14-47	28	2.06	111.62	10.6
Kol25-21	95	3.08	79.52	7.31	Kol31-25	65	2.05	124.38	6.87
Kol21-19	115	3.07	94.97	10.09	Kol27-7	82	2.05	106.34	7.62
Kol23-25	106	2.95	76.99	8.71	Kol5-11	45	2.03	106	10.4
Kol25-30	100	2.83	105.26	10.67	Kol11-16	40	2.02	116.43	8.1
kol23-13	104	2.83	96.43	9.11	Kol5-12	46	2.02	90.09	7.7
Kol25-8	92	2.78	82.88	9.93	Kol5-8	43	2	116.08	10.92
Kol21-26	117	2.68	76.65	6.83	NSIC RC 106	122	2	116.49	10.0
Kol5-16	50	2.63	67.17	7.56	Kol27-29	89	1.99	139.82	10.61
Kol25-22	96	2.63	99.63	11.6	Kol31-28	68	1.98	120.92	8.26
kol23-10	102	2.63	85.63	9.77	Kol29-24	78	1.97	171.7	15.97
Kol14-37	22	2.52	113.42	10.57	Kol2-5	5	1.93	138.43	9.63
Kol4-17	59	2.5	99.5	9.74	Kol15-16	17	1.93	122.89	8.31
Kol21-30	120	2.47	103.61	9.03	Kol23-27	108	1.92	126.88	8.05
Kol15-3	12	2.44	107.53	10.26	Kol5-15	49	1.91	91.32	6.74
Kol31-51	61	2.43	117.78	10.16	Kol4-12	55	1.91	102.8	6.95
Kol23-26	107	2.43	105.89	9.07	Kol23-31	110	1.91	119.53	8.63
Kol23-28	109	2.43	92.56	7.18	Kol29-15	74	1.9	138	9.03
Kol4-7	54	2.42	101.01	11.85	Kol29-23	77	1.87	110.12	6.13
Kol14-45	27	2.38	72.83	7.72	Kol27-30	90	1.87	113.91	8.88
Kol4-15	57	2.35	91.45	6.7	Kol23-24	105	1.87	114.57	8.77
Kol21-28	119	2.35	92.33	6.16	Kol31-36	70	1.85	120.15	7.17
Kol21-24	116	2.3	123.13	9.9	Kol27-22	85	1.85	103.62	7.27
kol5-13	47	2.25	88.26	10.64	Kol2-17	10	1.84	146.21	9.77

Appendix 3: Mean, Cv and maximum of F3 families and parents yield across site (continued)

Family	Num	Mean	CV (%)	Max	Family	Num	Mean	CV (%)	Max
Kol14-41	24	1.8	133.8	8.4	Kol27-9	83	1.4	157.4	8.2
Kol29-16	75	1.8	142.8	9.9	Kol14-39	23	1.4	154.7	6.3
Kol21-27	118	1.8	109.6	7.8	Kol4-3	51	1.4	154.7	6.4
Kol25-7	91	1.8	90.3	7.4	Kol2-2	2	1.4	133.5	5.9
kol27-15	84	1.8	121.2	7.4	Kol14-43	26	1.4	154.6	6.9
Kol15-30	19	1.7	141.2	8.6	Gambiaka	123	1.3	156.6	6.8
Kol11-1	31	1.7	136.4	6.8	Kol15-33	20	1.3	146.2	7.6
Kol29-14	73	1.7	134.1	9.6	Kol4-6	53	1.3	130.3	6.1
Kol21-15	113	1.7	117.7	7.2	Kol11-9	36	1.3	125.3	5.8
Kol11-13	38	1.7	141.0	8.8	Kol15-1	11	1.2	180.8	8.4
Kol29-12	72	1.7	172.3	10.0	Kol27-6	81	1.2	165.9	8.3
Kol11-5	32	1.7	160.1	10.3	Kol14-78	30	1.2	152.3	6.6
Kol5-10	44	1.7	106.9	9.4	Kol1114	39	1.2	165.4	8.3
Kol31-27	67	1.7	157.5	7.7	Kol2-14	8	1.2	176.3	7.1
Kol29-11	71	1.7	128.5	10.5	Kol29-31	80	1.2	154.5	7.9
Kol11-8	35	1.6	127.6	7.1	Kol31-21	62	1.2	169.3	7.2
Kol31-22	63	1.6	118.7	6.1	Kol27-23	86	1.2	145.0	5.5
Kol2-4	4	1.6	156.1	7.6	Kol27-27	88	1.1	133.9	5.3
Kol14-42	25	1.6	105.8	6.7	IR15	124	1.1	173.4	7.6
Kol31-26	66	1.6	167.1	9.8	Kol15-15	16	1.1	131.2	4.9
Kol31-23	64	1.6	145.8	9.5	Kol14-63	29	1.1	168.1	7.2
Kol14-31	21	1.6	138.1	7.5	Kol2-3	3	1.1	175.3	7.5
Kol15-8	13	1.5	149.1	7.7	Kol2-13	7	1.1	170.6	6.5
Kol31-29	69	1.5	117.6	5.9	Kol2-12	6	1.0	160.0	5.1
NERICA L49	125	1.5	131.3	7.4	Kol2-15	9	1.0	177.5	7.2
Kol4-16	58	1.5	110.6	6.6	Kol15-14	15	1.0	213.0	7.5
Kol5-6	42	1.5	114.7	6.5	Kol4-5	52	1.0	178.5	6.7
kol11-7	34	1.4	128.3	7.1	Kol2-1	1	0.8	166.7	4.5
Kol15-27	18	1.4	153.9	8.1	Kol27-25	87	0.8	166.7	4.5
Kol11-6	33	1.4	138.6	7.8	IRRI113	121	0.7	175.0	4.3
Kol29-19	76	1.4	127.1	6.1					

Appendix 4: selection index of the evaluated populations

S I. Sekoukou			S I. SAGA			Pooled SI		
	family	SI		family	SI		family	SI
Kol21-18	114	1047.9	Kol21-18	114	701.89	Kol21-18	114	874.915
kol23-10	102	921.43	Kol25-28	98	604.93	kol23-11	103	636.4825
Kol14-41	24	906.69	Kol23-9	101	561.89	kol23-13	104	624.6925
Kol2-5	5	865.84	Kol25-21	95	557.675	Kol14-45	27	619.0325
kol23-13	104	863.64	KOl4-13	56	546.34	kol23-10	102	597.78
Kol23-31	110	824.91	kol23-11	103	544.47	Kol25-28	98	593.4525
Kol29-15	74	819.3	Kol4-17	59	510.625	Kol25-30	100	589.9
Kol14-45	27	816.83	Kol25-30	100	502.505	Kol25-8	92	586.3025
Kol11-12	37	813.01	Kol25-16	94	501.98	Kol23-9	101	583.6575
Kol31-26	66	790.07	Kol5-14	48	499.65	Kol14-41	24	579.1625
Kol11-5	32	770.76	Kol25-8	92	488.2	Kol21-14	112	562.8525
Kol2-12	6	762.95	Kol21-1	111	475.525	Kol2-5	5	557.3475
Kol27-30	90	760.99	Kol21-14	112	471.875	Kol21-19	115	555.88
Kol23-27	108	747.92	Kol21-24	116	464.855	Kol4-17	59	554.3725
Kol14-31	21	745.21	Kol21-19	115	461.035	Kol29-15	74	549.87
Kol5-8	43	738.59	Kol15-3	12	429.59	KOl4-13	56	549.3275
KOl15-13	14	735.43	Kol14-45	27	421.235	Kol23-31	110	548.9125
Kol31-28	68	732.99	Kol31-51	61	411.58	Kol11-12	37	547.4125
kol23-11	103	728.5	Kol21-26	117	407.35	Kol25-21	95	535.365
Kol29-25	79	721.54	Kol23-26	107	403.955	Kol21-30	120	526.6375
Kol29-23	77	707.49	Kol21-30	120	401.87	Kol25-16	94	523.46
Kol29-24	78	700.4	kol23-13	104	385.75	KOl15-13	14	519.0775
Kol5-11	45	700.26	Kol23-25	106	384.875	Kol21-24	116	507.4975
Kol4-7	54	696.4	Kol21-28	119	382.77	Kol27-30	90	502.985
Kol25-8	92	684.41	Kol21-27	118	347.57	Kol5-16	50	500.125
Kol31-27	67	679.22	Kol25-27	97	345.2	Kol11-5	32	497.1225
Kol25-30	100	677.3	Kol25-11	93	334.695	Kol21-1	111	496.6375
Kol29-12	72	676.8	Kol5-16	50	324.43	Kol5-11	45	493.41
Kol5-16	50	675.82	Kol5-12	46	319.115	Kol2-12	6	491.79
Kol2-17	10	675.21	Kol23-28	109	315.86	Kol15-3	12	488.9625
Kol14-37	22	671.05	kol5-13	47	311.735	Kol25-27	97	487.525
Kol11-13	38	670.78	kol11-7	34	309.935	Kol21-26	117	486.045
Kol25-22	96	665.11	Kol11-16	40	307.64	Kol5-14	48	485.6

Appendix 4: selection index of the evaluated populations (continued)

S I. Sekoukou			S I. SAGA			Pooled SI		
	family	SI		family	SI		family	SI
Kol4-12	55	656.21	KOl15-13	14	302.725	Kol25-11	93	482.415
Kol21-14	112	653.83	Kol4-15	57	292.49	Kol5-8	43	479.3525
Kol21-30	120	651.41	Kol15-27	18	290.575	Kol11-13	38	474.9
Kol21-19	115	650.73	Kol5-2	41	290.08	Kol4-7	54	472.895
Kol1114	39	641.73	Kol5-11	45	286.565	Kol14-31	21	472.8225
Kol29-11	71	640.99	Kol25-29	99	286.53	Kol14-37	22	471.95
Kol29-16	75	640.09	Kol11-8	35	285.805	Kol21-28	119	470.1925
Kol11-1	31	639.71	Kol14-63	29	282.17	Kol11-16	40	461.3775
Kol27-27	88	636.54	Kol14-47	28	282.125	Kol11-8	35	460.4
Kol2-1	1	635.62	Kol11-12	37	281.815	Kol4-12	55	459.5575
Kol11-8	35	635	Kol5-10	44	281.38	Kol5-10	44	457.81
Kol15-1	11	634.92	Kol29-15	74	280.44	Kol23-25	106	457.1175
Kol14-78	30	634.75	Kol11-13	38	279.02	Kol31-51	61	453.53
Kol5-10	44	634.24	Kol27-22	85	276.47	Kol4-15	57	452.6275
Kol25-11	93	630.14	kol23-10	102	274.13	Kol23-27	108	451.865
Kol25-27	97	629.85	Kol23-31	110	272.915	Kol23-26	107	447.1425
Kol11-6	33	621.69	Kol14-37	22	272.85	Kol5-15	49	443.1575
Kol14-39	23	618.48	Kol5-15	49	269.9	Kol29-23	77	442.05
Kol27-29	89	618.09	Kol4-18	60	266.125	Kol11-1	31	441.5625
Kol31-25	65	616.49	Kol2-2	2	264.7	Kol31-28	68	437.9025
Kol5-15	49	616.42	Kol23-24	105	264.61	Kol14-47	28	435.3875
Kol11-16	40	615.12	Kol4-12	55	262.91	Kol23-28	109	432.6725
Kol4-15	57	612.77	Kol11-9	36	261.205	Kol14-63	29	432.2525
NSIC RC 106	122	609.27	Kol2-4	4	254.835	Kol21-27	118	431.295
Kol23-9	101	605.43	Kol15-33	20	254.83	Kol25-29	99	429.8525
Kol2-15	9	601.69	Kol14-41	24	251.635	NSIC RC 106	122	423.67
Kol15-30	19	600.55	Kol4-7	54	249.395	Kol31-26	66	423.09
Kol2-14	8	598.22	Kol2-5	5	248.855	Kol2-4	4	421.0775
Kol4-17	59	598.12	Kol4-6	53	247.58	kol11-7	34	419.4625
Kol21-15	113	594.01	Kol14-42	25	246.525	Kol21-15	113	419.2225
IRRI113	121	590.45	Kol27-30	90	244.985	Kol2-17	10	418.74
Kol27-6	81	589.83	Kol21-15	113	244.44	Kol15-30	19	415.085
Kol14-47	28	588.65	Kol11-1	31	243.42	Kol25-22	96	412.56

Appendix 4: selection index of the evaluated populations (continued)

S I. Sekoukou			S I. SAGA			Pooled SI		
	family	SI		family	SI		family	SI
Kol25-28	98	581.98	Kol2-3	3	229.91	Kol5-12	46	405.4225
Kol5-6	42	576.42	Kol15-30	19	229.62	Kol15-16	17	403.2875
Kol14-43	26	575.8	Kol2-13	7	229.21	Kol15-27	18	402.7475
Kol25-29	99	573.18	Kol11-5	32	223.49	NERICA L49	125	401.2125
NERICA L49	125	571.6	Kol27-7	82	221.09	Kol2-13	7	398.895
Kol2-13	7	568.58	Kol2-12	6	220.63	Kol25-7	91	398.81
Kol15-33	20	567.96	Kol15-16	17	220.59	Kol14-78	30	396.84
Kol21-26	117	564.74	Kol4-3	51	220.175	Kol27-22	85	396.1025
Kol29-14	73	564.04	Kol5-8	43	220.115	Kol5-2	41	395.04
Kol31-29	69	562.49	Kol31-36	70	217.26	Kol2-1	1	394.32
Kol25-7	91	558.97	Kol4-16	58	203.74	Kol2-3	3	393.91
Kol2-3	3	557.91	Kol15-14	15	202.71	Kol27-27	88	392.2675
Kol21-28	119	557.62	Kol14-31	21	200.435	IRRI113	121	390.7825
KOl4-13	56	552.32	Kol11-6	33	192.95	Kol2-14	8	390.5525
Kol21-24	116	550.14	IRRI113	121	191.12	Kol4-6	53	388.9975
Kol23-28	109	549.49	IR15	124	184.975	Kol14-39	23	388.3375
Kol15-3	12	548.34	Kol15-15	16	183.63	Kol14-42	25	387.76
Kol4-16	58	546.19	Kol2-14	8	182.89	Kol4-18	60	386.5575
Kol25-16	94	544.94	Kol5-6	42	182.16	Kol15-8	13	386.45
Kol31-22	63	542.87	Kol29-23	77	176.61	kol5-13	47	385.875
Kol27-9	83	542.56	Kol1114	39	175.28	Kol23-24	105	385.865
Kol4-3	51	541.67	Kol31-22	63	171.79	Kol29-24	78	384.0525
Kol15-8	13	537.67	Kol14-43	26	167.38	Kol2-15	9	383.4625
Kol29-19	76	530.54	Kol2-15	9	165.24	Kol31-27	67	383.4175
Kol27-7	82	530.52	Kol2-17	10	162.27	Kol4-3	51	380.9225
Kol4-6	53	530.42	Kol25-22	96	160.015	Kol5-6	42	379.2875
Kol23-25	106	529.36	Kol4-5	52	159.025	Kol27-7	82	375.8025
Kol14-42	25	529	Kol14-78	30	158.93	Kol4-16	58	374.965
kol11-7	34	528.99	Kol14-39	23	158.2	Kol14-43	26	371.5875
Kol31-36	70	519.99	Kol23-27	108	155.81	Kol31-36	70	368.6225
Kol21-1	111	517.75	Kol2-1	1	153.025	Kol2-2	2	366.8075
Kol27-22	85	515.74	Kol27-27	88	147.995	Kol15-1	11	366.665
Kol21-27	118	515.02	Kol31-21	62	147.66	Kol11-9	36	365.74
IR15	124	514.97	Kol31-28	68	142.815	Kol27-29	89	365.5025

Appendix 4: selection index of the evaluated populations (continued)

S I. Sekoukou			S I. SAGA			Pooled SI		
	family	SI		family	SI		family	SI
Gambiaka	123	588.25	Kol25-7	91	238.65	Kol15-33	20	411.395
Kol2-4	4	587.32	NSIC RC 106	122	238.07	Kol1114	39	408.5025
Kol15-16	17	585.99	Kol15-8	13	235.23	Kol29-25	79	407.92
Kol14-63	29	582.34	NERICA L49	125	230.83	Kol11-6	33	407.32
Kol15-27	18	514.92	Kol29-19	76	123.44	Kol29-11	71	365.0925
kol27-15	84	514.39	Kol27-29	89	112.915	Kol31-22	63	357.3275
Kol25-21	95	513.06	Kol15-1	11	98.41	Kol29-12	72	356.645
Kol23-24	105	507.12	Kol29-25	79	94.305	Kol31-25	65	354.3225
Kol4-18	60	506.99	kol27-15	84	92.29	IR15	124	349.97
Kol27-23	86	501.06	Kol31-25	65	92.155	Kol29-16	75	348.1
Kol5-2	41	500	Kol29-11	71	89.2	Kol15-14	15	342.115
Kol29-31	80	498.84	Kol31-27	67	87.62	Kol15-15	16	337.0775
Kol31-23	64	498.57	Kol29-31	80	75.62	Kol27-6	81	327.14
Kol31-51	61	495.48	Kol29-24	78	67.705	Kol29-19	76	326.9875
Kol5-12	46	491.73	Gambiaka	123	64.52	Gambiaka	123	326.3825
Kol15-15	16	490.53	Kol27-6	81	64.45	Kol4-5	52	323.4175
Kol23-26	107	490.33	Kol31-26	66	56.11	kol27-15	84	303.3375
Kol4-5	52	487.81	Kol29-16	75	56.11	Kol29-14	73	302.5225
Kol15-14	15	481.52	Kol29-14	73	41.01	Kol31-29	69	300.375
Kol5-14	48	471.55	Kol31-29	69	38.265	Kol31-21	62	296.0325
Kol11-9	36	470.28	Kol27-25	87	38	Kol29-31	80	287.2275
Kol2-2	2	468.92	Kol29-12	72	36.49	Kol27-9	83	282.1375
kol5-13	47	460.02	Kol27-9	83	21.72	Kol27-23	86	248.06
Kol31-21	62	444.41	Kol31-23	64	-4.42	Kol31-23	64	247.0725
Kol27-25	87	440.74	Kol27-23	86	-4.94	Kol27-25	87	239.3675