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GENETIC IMPROVEMENT OF ALKALINITY TOLERANCE IN RICE IN OFFICE DU NIGER IN MALI

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

Alkalinity stress is one of the most important abiotic stresses limiting rice production in irrigated systems. Alkalinity stress during seedling stage widely affects irrigated rice production of Office du Niger in Mali. The development of rice cultivars that tolerate alkalinity stress condition has been hindered by the lack of an elaborate breeding program. The objectives of this study were: (i) to evaluate and validate production constraints and varietal preferences of rice farmers in alkalinized zones of Office du Niger; (ii) to identify alkaline tolerant accessions and their association with SSR markers and, (iii) to characterize phenotypically F_2 progenies at seedling stage in alkaline hydroponic solution.

Participatory rural appraisal was conducted in three (3) highly alkalinized zones of Office du Niger. Two (2) villages per zone were involved in the study. Fertilizer cost, water management, inadequate agricultural equipments and declining soil fertility were identified as the main constraints of rice production. Overall, alkalinity was the most important abiotic stress affecting rice production. Crop rotation; use of organic matters; ploughing followed by flooding and use of tolerant varieties were the strategies developed by farmers to overcome alkalinity. Taste and swelling for home consumption; grain color and size for marketing were farmers preferred traits. Kogoni 91-1, BG 90-2 and Adny 11 were identified as farmer preferred varieties. Research priorities important to the farmers, included: alkalinity tolerance at seedling and reproductive stages combined; yield and taste and yield alone. Improvement of these characters in new varieties with alkalinity tolerance would enhance productivity with likely positive impact on small scale farmers' food security, incomes and livelihoods. Farmers had high interest in participatory varietal selection and participatory plant breeding.

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The morphological and physiological analysis relationships between different rice accessions revealed four accessions (Sahel 210, Damodar India (IRGC 17038), CSR 10 and CSR11) in the same group of the tolerant check (Pokkali).

Genetic diversity and association of Simple Sequence Repeat (SSR) markers with alkalinity tolerance were detected in a set of 26 rice genotypes. Among SSR markers used for morpho-physiological characters, RM208 was significantly (P-value = 0.01) associated with the potassium content in the flag leaves at 37% correlation coefficient. Phenotypic and genotypic association studies identified the accessions Sahel 210, Damodar India (IRGC 17038), CSR 10 and CSR11 in the same clustering as Pokkali (salt tolerant check).

A protocol based on an alkaline hydroponic solution was validated and adopted. The pH 9.0 was superior to separate alkaline tolerant and sensitive rice at early seedling stage. A total of 1,800 individual F_2 genotypes were screened and 1,652 were phenotyped in alkaline hydroponic solution. The total number of surviving F_2 individuals at twenty five (25) days after transplantation was 34 and varied from one cross combination to another.

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DEDICATION

This thesis is dedicated to my wife Mrs COUMBA SAGARA and Kids SOUMAILA, SOUTOURA and MOUSSA. Your love and support made it easier for me to persevere to the end.

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LIST OF ABREVIATIONS

ANOVA: Analysis of variance

bp.: base pair

Chr.: Chromosome

cM: centi Morgan

CTAB: Cetyl-Triméthyl-Amonium Bromide

CV: Coefficient of Variation

DAASA: Days After Alkaline Stress Application

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DAT:	Day After Transplantation
DNA:	Deoxyribo-Nucleic Acid
ECe:	Electrical conductivity (mmhos/cm)
ESP:	Exchange Sodium Percentage
FAO:	Food and Agriculture Organisation of the United Nations
FAOSTAT:	Food and Agriculture Organisation Statistical Database
FGD:	Focus Group Discussion
GGT-2:	Graphical Geno-Typer-2
IER:	Institute Economy Rural
IFDC:	International Fertilizer Development Center
IRRI:	International Rice Research Institute
LSD:	Least Significance Difference
MAS:	Marker Assisted Selection
NARI:	National Agricultural Research Institute
PAGE:	Poly-Acrylamide Gel Electrophoresis
PCR:	Polymerase Chain Reaction
pH:	potential Hydrogen
PIC:	Polymorphism Information Content
PPB:	Participatory Plant Breeding
PSI:	Irrigated System Pole

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PRA: Participatory Rural Appraisal

PVS: Participatory Varietal Selection

QTL: Quantitative Trait Loci

RYMV: Rice Yellow Mottle Virus

SAR: Sodium Absorption Ratio

SAS: Statistical Analysis System

SES: Standard Evaluation System

SSR: Simple Sequence Repeat

UPGMA: Unweighted Pair Group Method with Arithmetic Mean

USAID: United States Agency for International Development

USDA: United States Department of Agriculture

UVP: Ultra-Violet Products

WARDA: West Africa Rice Development Association

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CHAPTER I

GENERAL INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food for half of the world's population and the second most important crop in the world after wheat, with more than 98% currently grown in Asia (USDA, 2012). Globally it is cultivated on about 158.83 million ha and 464.87 million tons of paddy are harvested annually (USDA, 2012). Africa produces an average of 23.4 million tons of rice per year (2005-2011) on 9.7 million ha respectively equivalent to 3.5% and 6.1% of the world's total production and rice area (FAO, 2012). Rice is the most important food crop in Mali, and is grown in all regions of the country. It is increasingly favored by consumers primarily in urban areas but also in the rural areas where it is produced. In 2011, 13.34% of the 6,221,422 ha of West African rice area were harvested in Mali (FAO, 2012).

Rice production in Mali requires large amounts of water due to the high evaporative demand of the hot and dry climate. While the irrigated water is not highly mineralized, excessive concentration through evaporation can become dangerous as these waters often possess a positive calcite residual alkalinity (Bertrand *et al.*, 1993; Vallès *et al.*, 1992). The inventory of damaged soils in the Office of Niger reported that 62%, 54%, 32% and 27% of soils are affected by salinity and alkalinity, in Molodo, Niono, N'Debougou and Macina` respectively (N'Diaye, 1998).

In Mali and particularly in Office du Niger, sodic and alkali soils are reclaimed for agricultural use particularly for rice production. Reclamation commonly starts with soil tilling followed by harrowing and treatment with gypsum and organic manures and then flooding of the fields in which the rice crop is planted.

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The use of inputs such as fertilizers, pesticides and weed control is seen by farmers as being risky (Cassman, 2004; Sophia, 2010). Therefore the adoption of new agronomic technologies has been limited. The most economic solution to improve crop productivity in unfavorable environments is through breeding for tolerance to the existing stress (Efisue *et al.*, 2008; Sophia, 2010). The effects of salinity and alkalinity on the growth of rice have been found to be related to the stage of plant development, salt concentration and type, duration of rice plant exposure to salt, soil pH, water regime, temperature, humidity and solar radiation (Deepa *et al.*, 2011). Tolerant genotypes of rice have a high germination percentage, root length, shoot height, less salt injury, amylase and dehydrogenase activity with less accumulation of anthocyanin in their roots (Janaguiraman *et al.*, 2003). Deepa *et al.* (2006) have ranked salt tolerant rice lines based on germination and seedling growth under salt stress conditions. Breeding rice varieties with in-built salt tolerance is the promising, less resource consuming, economically viable and socially acceptable approach.

In Mali, increased rice production can be achieved by in non alkalinized soil environments using improved cultivars and improved agronomic practices. However, such an approach may exclude many small and poor farmers in marginal environments who represent the majority of the farmers in the country and, breeders do not have a clear understanding of these farmers' requirements and preferences (Banziger and Cooper, 2001; Cleveland and Soleri, 2002). Most of the rice varieties produced in Mali has been cultivated by farmers' for traits such as grain size and color, taste, cooking qualities and high yielding ability. However all of these cultivated varieties are sensitive to alkalinity stress. The transfer of these preferred traits into the tolerant accessions would be the breeding program objective to develop new tolerant alkaline genotypes accepted by farmers.

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Screening and developing rice with increased salt tolerance and better ability to grow at seedling stage in alkaline soil will be necessary to improve rice production in Mali. The use of morphological and physiological parameters in breeding could enable rapid selection of genotypes with tolerance. A combination of selection criteria could give a good indication of the salt response of crop plants morphologically (Singh, 2002). Factors like germination, seedling stage response, vegetative vigor (tillering ability and plant height), spikelet fertility; stress rating (growth and tillering), panicle exsertion and pH of environment should be considered. To obtain accurate heritability estimates in salt stress tolerance breeding, precise phenotyping and genotyping is required.

Molecular marker technology provides a powerful tool in the assessment of genetic relationships within and among species, in which differences among accessions can be revealed at the DNA level (Chakravarthi and Naravaneni, 2006). The applications of SSR markers in rice research include studies on genetic diversity of stress tolerance (Islam *et al.*, 2012), estimation of genetic diversity in rice genotypes using SSR markers and morphological characters (Seetharam *et al.*, 2008) and genetic diversity analysis of traditional and improved Indonesian rice germplasms (Thomson *et al.*, 2003). The molecular characterization information as well as genetic diversity analysis could be helpful to Malian breeders for planning programs for improving several traits in rice. Thus, the seedling and reproductive stages are the most vulnerable to salt stress and plants experience greater damage from salt stress mostly in these specific stages. In this study, the seedling and reproductive stages were used to select for tolerant genotypes in alkalinized soils. The F_2 individual rice plants from different cross combinations were screened at seedling stage in hydroponic alkaline solution for identification of F_2 survival plants and their molecular characterization.

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The objectives of this study were to:

- evaluate and validate production constraints and varietal preferences of rice farmers in alkalinized zones of Office du Niger;
- identify alkaline tolerant accessions and establish their genetic association using SSR markers;
- characterize F_2 progenies phenotypically at seedling stage in alkaline hydroponic solution.

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CHAPTER II

2. LITERATURE REVIEW

2.1 Taxonomy and origin of rice

The genus *Oryza* belongs to the tribe Oryzeae of the family Poaceae (*http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi*). There are 12 genera within the Oryzeae tribe (Vaughan, 1994). The genus *Oryza* contains nearly 22 species of which 20 are wild species and two, *Oryza sativa* and *Oryza glaberrima*, are cultivated (Vaughan, 1994). *Oryza sativa* is the most widely grown of the two cultivated species.

The origin of rice can be grouped into two classes: polyphyletic origin and monophyletic origin. Polyphyletic origin refers to cultivated species that originated from several species. Monophyletic refers to cultivated species that originated from common specie. Some believe that Asian rice, *Oryza sativa*, and African rice, *Oryza glaberrima*, have evolved independently in their respective regions from several species. But others believe that both Asian rice and African rice arose from a common parent (*O. perennis*). The second option is the most accepted one because both Asian rice and African rice are similar except in glumes pubescence, ligules size and colour of pericarp which is red in African rice. The progenitors of *Oryza sativa* are considered to be the Asian AA genome diploid species (Randhawa *et al.*, 2006).

2.2 Climatic conditions for rice production (Production environment)

Most of the rice growing areas in the world are located in the tropics (Yoshida, 1981). These areas are between the tropics of Cancer (23.5^0N) and the tropic of Capricorn (23.5^0S) and extend to as far as 49^0N and 35^0S of the latitudes. Rice also grows from sea level to an altitude of 2,500m or more (Khush, 1997). Worldwide, there are about 150 million hectares of rice, which provide around 550–600 million tons of rough rice annually (Maclean *et al.*, 2002). Rice is

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unique among the major food crops in its ability to grow in a wide range of hydrological situations, soil types, and climates.

Although, rice is primarily a tropical and subtropical crop, the best grain yields have been obtained in the temperate region. This is attributed to lower temperature during ripening, which gives more time for grain filling, long day lengths and high levels of solar energy during the ripening period (Yoshida, 1981). These phenomena are also conducive to lower grain yield obtained during the wet season versus the dry season. However, due to the prevalence of biotic and abiotic factors during dry seasons, lower grain yield is obtained in most cases as compared to the wet season.

Sensitivity to high levels of salt is a major problem for rice but degree of salt tolerance is known. Temperature and relative humidity are the most important climatic factors affecting salt tolerance (Singh *et al.*, 2009). Temperature regime greatly influences 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 greatly affect plant growth, under salt stress. This is because high temperature enhances depletion of internal water status of the plant through high transpiration. Plant transpiration increases to maintain the plant's internal temperature and, in the process, more salt enters into plant tissues at a rapid pace (Deepa *et al.*, 2011). Over-accumulation of salts ultimately leads to severe injury or death of the plant (Singh *et al.*, 2009). Relative humidity also plays an important role under salt stress. Dry weather and very low humidity increase the evapotranspiration rate of plants while the reverse is true with high humidity. Low humidity in a stress environment is detrimental to plant growth because of higher ion uptake (Singh *et al.*, 2005).

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2.3 Rice ecosystem

Rice ecologies are classified according to source of water supply into two broad terms as rain-fed and irrigated. In West Africa, Buddenhagen (1978) classified rice ecologies into four main types and eight sub-types based on climate, soil conditions, water regime and management practices. WARDA (1997) current AfricaRice defined six ecological areas favorable for rice production in West Africa. These ecologies are: rainfed upland, rainfed lowland, lowland irrigated, Sahelian irrigated, mangrove swamp and deep water/floating. This current study will focus on the Sahelian irrigated rice ecology. This is because it is important rice production ecology, particularly in Mali where it accounts for more than 50% (Mendez de Villar *et al.*, 2011) and also where alkalinity stress is more pronounced and has limited rice production without soil reclamations.

2.4 Role of farmer in development of new technology

Participatory rural appraisal (PRA) is one of the families of approaches and methods which enable communities to share; develop and analyze their own knowledge of life and conditions (Chambers, 1997). Essentially, participatory appraisal is employed for its adaptability and some of the non-traditional ways in which needs assessment is undertaken (Tock, 2001). It involves the researcher identifying community priorities and can include varietal development with rural people defining their ideotype. There is a wealth of knowledge and skills within a community that essentially goes unused during traditional research (Tock, 2001). The participatory approach is a rapid and cost effective method of identifying farmers-preferred rice cultivars in India and it also revealed a number of important characters that would have not been identified in breeders' experiments (Joshi and Witcombe, 1995). To study socio-economic factors influencing the adoption of sawah rice production technology in Nigeria PRA was practical and used (Fashola *et*

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al., 2007). In Namibia farmers were engaged in pearl-millet (Pennisetum glaucum L.) cultivar selection (Monyo *et al.*, 2000). Efisue *et al.* (2008) use also the PRA to identify early maturing and tall varieties among the most important traits by farmers in the rainfed production ecologies in Sikasso region of Mali. Sophia (2010) identified through PRA rice farming in Tanzania more active participation of women than men and noted that woman's involvement and utilization of their indigenous knowledge and understanding their preferences for rice varieties may increase adoption of new varieties. Therefore PRA is considered as a tool to understand the farmers' important information on constraint, needs and preferences and considered as vital means to collect information on farmers' knowledge on the soil affected stress. Understand farmers' characteristics and their involvement in the technology development process is central for success in the adoption of farm technologies (Fashola *et al.*, 2007). The adoption or not of farmers decision will depend on their attitude to the innovation, farming experience, household size and visits by extension agents (Azuma, 2004).

Neglecting farmers need may lead to low adoption rate of developed cultivars. According to Efisue *et al.* (2008), the low adoption rate of rice varieties by the farmers could be due to the fact that scientists are working in isolation from farmers and other stakeholders and therefore, might not identify the unique requirements for the subsistence farmers in particular niches of the diverse environments.

2.5 Alkali soil characterization and effect on rice yields

Salt-affected soils are considered as degraded soils that contain an excess of water-soluble salts (saline soils), exchangeable sodium (sodic soils) or both an excess of salts and exchangeable sodium (saline-sodic soils or alkaline) (Siyal *et al.*, 2002).

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Soil salinization is the accumulation of the soluble salts of sodium, calcium, and magnesium in the soil root zone (Siyal *et al.*, 2002). The effect of these salts can degrade soils to the extent that crop production such as rice is severely decreased. High levels of salt limit plant growth by increasing osmotic pressure of the soil solution, which reduces the plant's capacity to withdraw water from the soil (Siyal *et al.*, 2002). Depending on salt concentration (Electrical Conductivity), Exchangeable Sodium Percentage (ESP) and pH of the soil solution, salt affected soil is divided into three categories, saline, alkali and saline-alkali soils (Table 2.1.).

Table 2.1: Classification of salt-affected soils

Classification	Electrical conductivity mmhos/cm (ECe)	Exchangeable Sodium Percentage (ESP%)	pH
Saline soils	> 4.0	< 15	< 7.05
alkali soils	< 4.0	> 15	> 8.5
Saline – alkali soils	< 4.0	> 15	> 8.5

Source: (Siyal *et al.*, 2002)

In alkali soils, a sodium ion disperses the minerals colloids, which form the tight soil structure. This structure slows the infiltration and percolation of water (Siyal *et al.*, 2002). The sodium excess effect in alkali soil does not allow soil particles to attach one another resulting in dispersion and non friability of soil. The low permeability causes by alkali soils, makes difficult water movement due to dispersed clay and humus. The alkali soils can be identified from the black color of the soil surface (Siyal *et al.*, 2002).

Salt stress disrupts the nutrient balance in the plant. In rice, a deficiency of one nutrient causes increased uptake of a nutrient of the same valence (Singh, 2001). Rice production in alkali soil is complex (Condom, 2000). Low use efficiency of nitrogen and low potential yielding of the varieties were due to the impact of alkalinity on rice production (Wopereis *et al.*, 1999). The volatilization process of ammonia is the most noticeable effect which in reproductive stage

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affects spikelet fertility (Dicko, 2005). Under salt stressed conditions, K^+ deficiency increases and Na^+ uptake increases (Dobermann and Fairhurst, 2000) resulting in plant injury, sterility and yield reductions. Excess Na^+ may compete with K^+ in membrane transport and when accumulated in the cytoplasm, it inhibits many enzymes. Devitt *et al.* (1981) found that Na^+/K^+ imbalance adversely affects grain yield.

Due to high levels of these salts in soils, the genetic potential of the plant is not realized to its maximal extent to target grain yield (Tollenaar and Lee, 2002), hence reduction in yield. The majority of farmers in salt affected soils areas have low level of understanding of degraded soils, symptoms and their impacts in plant growth (Mnkeni, 1996). Dicko (2005) revealed further in alkalinity stress environments, the difficulty to differentiate symptoms due to salt affected soils and nutrient stresses. Thus, there is the need to understand farmers' perception and behavior under salt affected soils community for proper planning on mitigation measures.

2.6 Sensitivity of Rice to salt stress

Alkaline soils are defined as soils which contain sufficient exchangeable sodium to impair crop growth. Excess sodium results in poor soil physical properties and nutrient imbalances. The high pH causes low availability of essential nutrients such as P, Ca, Fe, and Zn and rice crop sensitivity start from or above the pH 7.5, which hinders or affects the normal plant growth. The alkalinity constraint is measured in terms of the exchangeable sodium percentage (ESP) or the sodium absorption ratio (SAR) and pH of saturated soil paste extract (Singh *et al.*, 2011). Therefore, sodic and alkaline soils are those with ESP equal to or more than 15 % and a pH above 8.5 (Abrol, 1986; Szabolcs, 1994; Waisel, 1972). Alkalinity causes a drastic decrease in potassium content of salt-sensitive rice varieties; the salt susceptible rice cultivars have the low ratio of K^+/Na^+ in leaves and high grain yield reduction under alkalinity (Asch *et al.*, 2000). Salt-

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tolerant cultivars of rice accumulate less Na^+, Cl^-, Zn and proline and more K^+ at root and shoot levels than salt-sensitive varieties. Phosphorus transport from root to shoot is inhibited in the salt-sensitive cultivars. Accumulation of Na^+, and Cl^-, and decrease in K^+ content at the shoot level are restricted to the oldest leaves in salt-tolerant genotypes while proline is accumulated in the youngest leaves in all cultivars (Asch *et al.*, 2000).

The mechanism of salt tolerance fall into three categories: tolerance to osmotic stress, sodium exclusion from leaf blades and tolerance of tissue to accumulated sodium or chloride (Munns and Tester, 2008). The osmotic stress reduces cell expansion in root tips and young leaves and causes stomatal closure. A reduced response to the osmotic stress would result in greater leaf growth and stomatal conductance. However, the increased leaf area would benefit only plants that have sufficient soil water. Sodium exclusion by roots ensures that sodium does not accumulate to toxic concentrations within leaves. A failure in sodium exclusion manifests its toxic effect after days or weeks, depending on the species, and causes premature death of older leaves. Tolerance requires compartmentalization of sodium and chloride at cellular and intracellular level to avoid toxic concentrations within the cytoplasm, especially in mesophyll cells in the leaf. The toxicity occurs with the time, after leaf sodium increases to high concentrations in older leaves.

Rice is differentially affected by alkalinity at different stages (Munns, 2002). The effects of alkalinity on the growth of rice were found to be related to the stage of plant development, salt concentration, type of salt, duration of exposure to salt, soil pH, water regime, temperature, humidity and solar radiation (Akbar, 1986). Rice is relatively tolerant to salinity and alkalinity during germination stage. The germination though affected by salinity can be appreciably higher germination percentage, root length, Shoot height, vigor index and amylase and dehydrogenase activity with less accumulation of anthocyanin in their roots (Janaguiraman *et al.*, 2003). Salt

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tolerant rice lines based on germination and seedling growth has been ranked under salt stress conditions (Deepa *et al.*, 2006). Rice is very sensitive during the early seedling stage, gains tolerance during the active tillering stage, but becomes sensitive during panicle initiation, anthesis, and fertilization, and then becomes relatively more tolerant at maturity (Rao *et al.*, 2008).

2.7 Breeding for alkalinity stress tolerance

A successful breeding program should have a wide spectrum of genetic variability for the desired trait (good donors), and a robust, repeatable, and reliable screening technique (Singh *et al.*, 2009). A phenotype with tolerance or sensitivity is the overall manifestation of the sum of the different tolerance mechanisms operating in the genotype. Rice is an important crop and experimental evidence suggests that most rice varieties exhibit salt sensitivity, but that varietal differences tend to manifest themselves at different salt concentrations (Yeo and Flowers, 1984). A wide range of variation of sensitivity exists among different rice cultivars. There are a number of salt tolerant rice varieties available for different levels of soil stresses *viz*., CSR 10 for very high stress; CSR 13, CSR 27, Narendra Usar 2 and Narendra Usar 3 for moderate to high stress and a basmati CSR 30 rice for moderate stress. These are being utilized for land reclamation programs in Uttar Pradesh in India (Deepa *et al.*, 2011).

The level of salt tolerance found so far in rice is inadequate, however, the transfer of this trait to combine desirable characters has had only limited success because the knowledge of genetic basis of salt tolerance is limited (Ansari *et al.*, 2003). The main expectation from plant physiologists is the identification of "salt markers" as defined by (Ansari *et al.*, 2003; Epstein *et al.*, 1979). Such markers include characters that are associated with salt stress, easily identifiable, and can be used for screening salt-tolerant plants in large breeding populations.

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The development of salt-tolerant varieties has been proposed as a means of expanding agriculture into the regions affected by salinity (Epstein, 1980). Breeding rice varieties with in-built salt tolerance is the most promising, less resource consuming, economically viable and socially acceptable approach.

There is a need to develop rapid screening methods to evaluate tolerant plants and subsequently combine desirable characters, and identify, at an early stage of development, some markers that can play an important role in identifying superior performance at later growth stages. Some characters which manifest themselves at an early stage but are directly related to later performance of plants under salt conditions have been identified. These characters are leaf mortality, seedling vigor, leaf chlorophyll, Na^+ uptake, Na^+/K^+ ratio and distribution of Na^+ among leaves (Ansari *et al.*, 2003).

Leaf senescence is a well-known symptom of stress and it indicates leaf tissue death resulting from the excessive accumulation of ions (Ansari *et al.*, 2003). This can serve as an indicator of salt tolerance even at a very early stage of plant development. Salt stress through osmotic effects reduces the ability of plants to take up water and salts rise to a toxic level in older transpiring leaves causing premature senescence, and reduce the photosynthetic leaf area of a plant to a level that cannot sustain growth (Munns, 2002).

Rice cultivars differ considerably in their growth rate with the most vigorous lines being the traditional varieties. Dwarfing genes were incorporated into most of the modern varieties and breeding lines to increase harvest index and reduce lodging (IRRI, 2006). Naturally occurring salt resistant varieties invariably belong to these modern varieties. Vigorous growth has a dilution effect (IRRI, 2006). Differences in vigor among rice cultivars accounted for much of the variation in survival under high salt levels. The vigorous growth is a selection criteria for

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alkalinity tolerance because of the good yield potential associated with short plants that do not lodge. Thus, early seedling vigor is desirable due to high sensitivity during this stage coupled with high salt levels encountered at the beginning of the season (Sexcion *et al.*, 2009).

Tolerance of one rice variety was found to be associated with its ability to restrict potentially toxic ion uptake like Na^+ and associated with preferential uptake of the balancing ion like K^+ (IRRI, 2006). It is an adaptation for the survival of plants so that the vital metabolic activities are not affected. There are larger differences in ion (Na^+ and K^+) uptake between the species in comparison to the genotypic differences within a crop species. These are the most studied parameters for salt tolerance in crop plants. The tolerant varieties maintained lower Na^+ concentrations and maintaining higher K^+ concentrations under high salt concentrations (Sexcion *et al.*, 2009). One factor may be the overall control mechanism (before flowering) of sodium uptake through root properties and its subsequent distribution in different vegetative and floral parts especially in leaves where it causes leaf mortality thereby reducing transportation of total assimilates to the growing region (Munns, 2002). Tolerant genotypes like CSR1 showed regulation of distribution and accumulation of Na^+ taken up by the plants i.e. the delicate and vital organs like young and photosynthetically active leaves as well as the reproductive organs like panicles are kept relatively free of Na^+, besides having an assured supply of K^+, even under higher salt concentrations (IRRI, 2006). Thus the differences in the distribution of ions in individual plant organs and with the age and position of these organs on the plant are more important indicators of its tolerance potential than the difference in the average salt content of the plant.

The uptake of both Na^+ and K^+ are entirely independent, but lower Na^+/K^+ ratio is considered a as desirable trait as it maintains the ion balance. Na^+ is transported to shoots usually through

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apoplastic pathways (passive transport) while K^+ transport takes place through symplastic pathways (active transport). Younger leaves have relatively lower Na^+ than K^+ as compared to the older leaves, which in turn results in higher Na^+/K^+ ratio in the older leaves. Thus Na^+/K^+ ratio increases precipitously with salt concentration and leaf age (Munns, 2002). Alkalinity stress causes a drastic decrease in potassium content of salt-sensitive rice varieties; the salt susceptible rice cultivars have low ratio of K^+/Na^+ in leaves and high grain yield reduction under alkalinity stress (Asch *et al.*, 2000). A tolerant variety keeps its leaves relatively free of the toxic ions besides having assured K^+ supply. This factor, along with the higher number of leaves and higher leaf area, probably contributes to success under high salt concentration. This parameter is not a universal phenomenon hence should be used with caution as selection criteria (IRRI, 2006).

2.8 Molecular markers for alkalinity tolerance in rice

A good deal of progress has been made in agriculture using conventional methods of breeding crop varieties despite non-availability of knowledge about the physiological and biochemical mechanisms. However, in some situations, genetic advance through breeding plants has been slow due to complex and ambiguous natures of the traits, like salts stress tolerance. A more comprehensive understanding of physiological and biological mechanisms would contribute to efficient breeding of crops suitable for stress conditions. Alkalinity tolerance in rice (low Na^+/K^+ ratio in shoot) is governed by both additive and dominance gene effects (Ramayya *et al.*, 2009). However, it is difficult to identify these genes, known as quantitative trait loci (QTLs), by using morphological and physiological markers because each of these genes singly has a relatively small effect on the phenotype (Ramayya *et al.*, 2009).

Molecular analysis of the genome at the DNA level can provide a greater advantage because DNA molecules are the same in all of the living cells of a plant, regardless of physiological or

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developmental state of the tissue (Mohan *et al.*, 1997). DNA marker technology has provided a new source of information and an impetus for modifying some plant breeding methods. DNA marker technology is a new source of information, and now is being integrated into existing plant breeding programs all over the world to facilitate transferring and combining desirable genes at a rate and precision not previously thought possible (Mohan *et al.*, 1997).

Marker-assisted selection (MAS) can be used successfully to pyramid the major genes for salinity/alkalinity tolerance (Bohnert and Jensen, 1996).

Simple Sequence Repeat (SSR) and Quantitative Trait Loci (QTL) analyses have been developed as advanced techniques to obtain meaningful data about the gene complement governing tolerance phenotypes. SSR or microsatellite markers are ideal for making genetic maps (Islam, 2004); assisting selection (Bhuiyan, 2005) and studying genetic diversity in germplasms. SSR markers play also an important role for identifying gene for salt tolerance or introgressing the genes to develop new cultivars.

Genes/QTLs have been mapped for several important traits such as yield, quality and tolerance against biotic stresses, and abiotic stresses including drought and salinity/alkalinity. A major QTL was identified conferring salt tolerance on chromosome 1 and designated *Saltol* (Bonilla et al., 2002; Zhou et al., 2013). This QTL has been the target of marker assisted selection (Thomson *et al.*, 2010). A chromosome 1 QTL *SKC1* that appears to be a part of this locus was isolated by positional cloning and determined to be a protein that functions as a Na^+ selected transporter (Ren *et al.*, 2005; Zhou *et al.*, 2013). Many salt tolerant rice varieties have been developed and have also been adopted, especially in the inland saline/sodic areas of India. An association between SSRs and the alkaline tolerance has been indicated by the presence of genetic polymorphism between parents and segregation of SSRs in an F_2 mapping population

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(Ramayya *et al.*, 2009; Rao *et al.*, 2008). They observed also the occurrence of transgressive segregants and demonstrated the feasibility of finding more tolerant progenies than the tolerant parent by employing proper selection from tolerant extremes of the population distribution. These techniques have the potential to make effective selection of complex traits in early generations.

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CHAPTER III

3. PRODUCTION CONSTRAINTS AND VARIETAL PREFERENCES OF RICE FARMERS IN THREE HIGH ALKALINITY ZONES OF OFFICE DU NIGER OF MALI

3.1 Introduction

In Mali, rice is grown in every region of the country and is increasingly favored by consumers, primarily in urban zones but also in the rural areas where it is produced predominantly by resource-poor farmers. Rice production in Mali relies on large amounts of irrigation water is the main reason for soil alkalinization in Office du Niger. Water, soil and fertilizer management are the main factors used as remedies to solve the alkalinity constraint, but they were and are insufficient. Little work has been done on breeding for tolerance to alkalinity. Breeding for alkalinity tolerance is, however, regarded as an important means of bridging the yield gap between potential and actual yield.

Inclusion of farmers in developing tolerant varieties is an important requirement. Farmers have preferences for varietal characteristics such as grain size and color, taste, cooking qualities and high yield. Consideration of farmer preferences in the varietal development process can help the breeders to select appropriate genetic materials (Witcombe *et al.*, 1996; Zhou *et al.*, 2013). Unfortunately, formal research systems in developing countries are highly centralized and do not target the problems of resource-poor farmers (Zhou *et al.*, 2013). It is important for breeders to clearly understand farmers' constraints and preferences for production, marketing and utilization of crop varieties they produce.

Therefore, to improve rice varieties grown by farmers for alkalinity tolerance, it is imperative that farmer's needs and preferences are understood for integration into the breeding programs.

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Soil degradation through salts concentration is one of the major constraints for rice production and its current status is not well known. The factors that contribute to such constraints may not be known by farmers. Rice varieties that are released in Mali are mostly farmers preferred varieties, but sensitive to alkalinity stress. These varieties are from the national institute research. Those tolerant varieties developed by Africa Rice (WARDA) and IRRI have low adoption by farmers and this could be attributed to the non integration of farmers' needs in the breeding programs.

In this study, a Participatory Rural Appraisal (PRA) was conducted to evaluate farmers' traits preferences, perception in relation to salts stress particularly soil alkalinity and crop management practices in Office du Niger rice production zones of Mali. PRA was conducted in these zones to determine farmers' perceptions and preferences as well as management practices under alkalinity conditions that often occur. The PRA enabled this study to determine ways of ameliorating effects of alkalinity and enhancing irrigated rice production in Office du Niger.

The specific objectives of the PRA were to:

- identify production constraints of rice farmers in areas with high soil alkalinity;
- assess the effectiveness of strategies adopted by farmers in their production environments;
- explore farmer's preferences and adoption criteria for future varietal development.

3.2 Research Methodology

PRA approaches which lead to the interaction between breeders and farmers and mutual exchange of knowledge and experience were used to facilitate the interaction with farmers in three zone of Office du Niger in 2011.

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3.2.1 Site selection

The PRA and survey were conducted in the Office du Niger regions of Mali. Three zones were chosen because they had high rates of degraded soils (alkaline) and also offer an opportunity for comparing farmer preference for rice varieties susceptible to alkalinity in irrigated ecosystems. The selected zones were: Niono, Molodo and N'Debougou. Two villages per zones were selected and focus group discussions and structured interviews were used as tools for PRA and data collection in each village.

3.2.2 Selection of farmers

Twenty farmers were selected from two villages in each zone of Office du Niger (ON). In Niono zone, the study was conducted in Gnoumanke and Kolodougou villages; in Kangaba and Fabacoura villages in Molodo zone and Banissirela and Siengo in N'Debougou zone. In addition to the twenty farmers per village, one group of fifteen to forty farmers per village was formed for focus group discussions. These groups were selected based on their experiences with rice production in alkaline soil conditions.

3.2.3 Data collection

Different PRA techniques were used to obtain information about the farming problems, varietal selection criteria, research priorities and opportunities. A combination of three data collection techniques was employed. These included (1) semi-structured interviews for focus group discussion (FGD); (2) survey questionnaires for individual interviews (Appendix 3.1.); (3) and transect walks for field observation with the groups.

Semi-structured interviews were conducted with 10 rice growers from two villages in each zone of Office du Niger. The discussions were followed by transect walks through the rice fields during which alkalinity problems and other biotic and abiotic constraints were identified and

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rated with the farmers' groups. Group discussions were held with a selected sample of 10 farmers in each zone to confirm results from questionnaires for individual interviews.

Rank matrices were drawn to rank the constraints. Individual farmers ranked biotic and abiotic constraints independently. The constraint with the highest score was considered the most important constraint. Transect walks in the fields of farmers were conducted and observations made. Different traits and plant characters that were considered by farmers in variety selection were recorded. In addition to providing information on variety preferences, special preferences of farmers were identified for home consumption and marketing quality of rice.

3.2.4 Data Analysis

Data from questionnaires for individual interviews were compiled and coded and analyzed using Microsoft excel 2007. The coded information data was imported to Statistical Package for the Social Sciences (SPSS) version 17.0 for further analysis. Microsoft excel 2007 was used to calculate average scores and average ranks for data obtained from both group discussions and structure interviews.

3.3 Results

3.3.1 Rice production constraints

The use of grower association's seed for rice production was the most common practice. In general 45.5% of farmers used grower association seeds followed by seeds obtained through self or farm saved seeds (29.1%), professional private seed companies (21.8%) and research stations (3.6%) (Table3.1). Farmer association seed growers were farmers' groups who came together to produce seeds for contract buyers without any certificate of seed inspectors.

Ten (10) constraints were identified as important in rice production zone of Office du Niger. Fertilizer costs were ranked as the most important constraint (15.22%) for farmers in rice

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production in Office du Niger (Table 3.2). Water management (12.08 %) was ranked as the second most important constraint, followed by inadequate agricultural materials (11.80%). Varietal quality was ranked low at almost all zones.

Eight causes of alkalinity were identified as being important in affecting rice production although their importance varied among locations. Nature of soil ploughing (22.5%) and sandy soils (21.4%) were identified by farmers as the first and second most important causes of alkalinity in Office du Niger (Table 3.3). Water quality (surface and ground) and non drainage of irrigated water were third and fourth alkalinity causes. At Gnoumanke, clay soils and poor drainage were the important causes besides nature of ploughing.

Soil alkalinity is variable and occurs as several alkalinity types. Four types were identified in Office du Niger (Table 3.4). The most representative in Office du Niger according to farmers is the white salt (33.8%). Except for Kolodougou, black soil (23.3 – 62.5%) was high at all other locations.

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Table 3.1: Seed source of rice farmers in three zones of Office du Niger (%) in Mali in 2011

Source of seeds	N'Debougou[a]		Niono[a]		Molodo[a]		Average
	Siengo[b]	Banissirila[b]	Kolodougou[b]	Gnoumankè[b]	Kangaba[b]	Fabacoura[b]	
Farm saved seed	14.3	61.9	7.7	30.4	20.0	33.3	29.1
Farmer association seed growers	71.4	33.3	38.5	56.5	40.0	16.7	45.5
Private companies	9.5	4.8	46.2	8.7	350	50.0	21.8
Research institutes	4.8	0.0	7.7	4.3	5.0	0.0	3.6

[a] Zones, [b] Villages

Table 3.2: Rice production constraints and their relative importance (%) ranked in Office du Niger in 2011

Constraints	N'Debougou[a]		Niono[a]		Molodo[a]		Average	Ranking
	Siengo[b]	Banissirala[b]	Kolodougou[b]	Gnoumankè[b]	Kangaba[b]	Fabacoura[b]		
Fertilizer cost	11.29	12.63	18.29	17.54	16.09	15.50	15.22	1
Water management	12.60	11.61	9.03	14.45	16.09	8.68	12.08	2
* Inad. agricultural equip.	11.29	9.78	12.83	14.45	13.79	8.68	11.80	3
Declining soil fertility	13.65	7.74	9.03	16.59	9.20	10.33	11.09	4
Land shortage	16.27	9.78	9.03	7.11	10.34	13.84	11.06	5
Disease	8.66	14.46	9.03	11.37	9.20	11.98	10.78	6
Seed cost	12.60	12.63	9.03	5.21	9.20	11.98	10.11	7
Delay in land preparation	3.67	8.76	10.93	3.08	3.45	10.33	6.70	8
Weeds	4.99	6.72	5.46	7.11	10.34	5.17	6.63	9
Lack of improved varieties	4.99	5.91	7.36	3.08	2.30	3.51	4.52	10

[a] Zones, [b] Villages, *inadequate agricultural equipments

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Table 3.3: Causes of alkalinity affecting rice production in (%) in 2011 in Office du Niger

Causes of alkalinity	N'Debougou[a]		Niono[a]		Molodo[a]		Average
	Siengo[b]	Banissirela[b]	Kolodougou[b]	Gnoumanke[b]	Kangaba[b]	Fabacoura[b]	
Continuous cropping	9.7	23.7	12.1	2.7	2.6	5.6	9.4
Water Quality	19.4	7.9	9.1	16.2	15.8	11.1	13.2
[*]Non-drainage of Irrig. Water	12.9	5.3	9.1	24.3	15.8	5.6	12.2
Clay soils	6.5	5.3	12.1	24.3	5.3	0.0	8.9
Sandy soils	12.9	21.1	21.2	2.7	26.3	44.4	21.4
Silt soils	9.7	10.5	6.1	0.0	2.6	0.0	4.8
Fertilizer application	3.2	10.5	12.1	8.1	0.0	11.1	7.5
Nature of soil plowing	25.8	15.8	18.2	21.6	31.6	22.2	22.5

[*]Non drainage of irrigated water: Surface and Ground water; [a] Zones, [b] Villages

Table 3.4: Alkalinity types in rice production zones of Office du Niger in (%) in 2011

Alkalinity (type)	Zone N'Debougou[a]		Zone Niono[a]		Zone Molodo[a]		(%)
	Siengo[b]	Banissirela[b]	Kolodougou[b]	Gnoumanke[b]	Kangaba[b]	Fabacoura[b]	
White salt	33.3	32.3	33.3	30.0	57.1	12.5	33.1
Black salt	28.6	38.7	0.0	23.3	23.8	62.5	29.5
Oil salt	38.1	29.0	41.7	40.0	14.3	12.5	29.3
Red salt	0.0	0.0	25.0	6.7	4.8	12.5	8.2

[a] Zones, [b] Villages

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3.3.2 Importance of soil alkalinity in Office du Niger

There were differences between the six villages in terms of impact of alkalinity type on rice production in Office du Niger (Table 3.5). However, according to the farmers, the white salt represented 36.1 %, followed by black (27.9%) and oil (27.7%) salts.

Table 3.5: Impact of alkalinity types on rice production in Office du Niger in 2011

	Zone N'Debougou[a]		Zone Niono		Zone Molodo		
Salts	Siengo[b]	Banissirela	Kolodougou	Gnoumanke	Kangaba	Fabacoura	(%)
White salt	42.1	38.7	33.3	30.8	59.1	12.5	36.1
Black salt	26.3	32.3	8.3	15.4	22.7	62.5	27.9
Oily salt	31.6	29.0	33.3	46.2	13.6	12.5	27.7
Red salt	0.0	0.0	25.0	7.7	4.5	12.5	8.3

[a] Zones, [b] Villages

In Gnoumanke, the oil salt has the greatest impact of 46.2% and at Fabacoura the black salt had 62.5% (Table 3.5). Farmers' adopted various strategies for alkalinity impacts control including crop rotations (25%), use of organic matter (24%) and puddling followed by flushing (22%). The adoptions of the above strategies were higher than the use of tolerant varieties (17%) and pre flooding (12%) (Table 3.6).

Table 3.6: Farmers' strategies for alkalinity control across Office du Niger villages in 2011

Strategies	Percent
Puddling following by flushing	22
Pre-flooding	12
Use of organic matters	24
Crop rotation	25
Tolerant varieties	17

Kogoni 91-1 variety was adopted by farmers as being tolerant to alkalinity in all villages except Kolodougou and Fabacoura (Table 3.7). On average, 19% of farmers selected Kogoni 91-1 as

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tolerant to alkalinity; and 66%, with a range of 54 to 76% did not consider any variety as being tolerant to alkalinity.

Table 3.7: Proportion of farmers (%) perception growing tolerant varieties in alkaline soils in 2011

	N'Debougou[a]		Niono		Molodo		(%)
Varieties	Siengo[b]	Banissirela	Kolodougou	Gnoumanke	Kangaba	Fabacoura	
Kogoni 91-1	24	33	0	22	35	0	19
Nionoka	0	0	23	0	0	0	4
Adny11	0	0	8	9	0	25	7
Nerica L2-IER	0	0	8	0	0	0	1
Gambiaka dian	0	0	8	0	0	0	1
Nerica7	0	0	0	0	0	8	1
no variety	76	67	54	69	65	67	66

[a] Zones, [b] Villages

3.3.3 Farmers' varietal preference and selection criteria

There are a number of rice varieties grown by Farmers in Office du Niger although they vary from one village to another (Table 3.8). Kogoni 91-1 (35.3%) is grown more than the other varieties. ''Nionoka'' with an average of 13.4% is also grown in all six villages.

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Table 3.8: Percentage of farmers growing different rice varieties in Office du Niger in 2011

varieties	N'Debougou[a]		Niono zone		Molodo zone		
	Siengo[b]	Banissirela	Kolodougou	Gnoumanke	Kangaba	Fabacoura	(%)
Kogoni 91-1	36.2	25.6	27.6	40.4	46.5	40.7	35.3
BG 90-2	5.2	1.3	13.8	12.3	0.0	11.1	6.2
Sambala	19.0	10.3	0.0	10.5	0.0	14.8	9.9
Nerica L2-IER	0.0	9.0	3.4	1.8	0.0	0.0	3.1
Nerica L1-IER	0.0	1.3	0.0	0.0	0.0	0.0	0.3
Nionoka	24.1	5.1	41.4	7.0	4.7	11.1	13.4
Telimani	0.0	0.0	0.0	8.8	0.0	3.7	2.1
Wassa	5.2	21.8	0.0	5.3	18.6	18.5	12.3
Adny11	8.6	25.6	13.8	14.0	30.2	0.0	17.1
ECIA	1.7	0.0	0.0	0.0	0.0	0.0	0.3

[a] Zones, [b] Villages

Farmer preferences tended to vary based on yield, market and taste (Table 3.9). The non-preference rate per variety is an important parameter to rank farmer preference for varieties. Kogoni 91-1 was the least preferred variety with 13.6%, followed by BG 90-2 (25.5%) and Adny 11 (43.5%). The other varieties had non-preference ratings of more than 50% (Table 3.9). The two Nerica varieties and ECIA were grown at rather limited locations compared to other varieties.

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Table 3.9: Factors affecting farmer preferred rice varieties for production in Office du Niger

Factors	Kogoni 91-1	BG 90-2	Sambala malo	Nerica L2-IER	Nerica L1-IER	Nionoka	Telimani	Wassa	Adny 11	ECIA
Yield	26.4	69.1	10.9	6.4	0.0	13.6	0.0	10.9	40.0	0.9
Market	23.6	2.7	0.9	0.9	0.0	9.1	0.0	2.7	8.2	0.9
Taste	23.6	0.0	0.9	0.0	0.0	0.9	0.9	0.9	4.5	0.0
Late fertilization	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fertilizer response	9.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Grow on sandy soil	0.9	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0
Grain quality	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Maturity	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Swelling after cooking	0.0	2.7	0.9	0.0	0.0	0.0	0.9	0.0	2.7	0.0
Early maturity	0.0	0.0	7.3	0.0	0.0	3.6	1.8	10.9	0.0	0.0
Quality after cooking	0.0	0.0	2.7	0.0	0.0	1.8	0.0	0.0	0.0	0.0
Young seedling vigor	0.0	0.0	0.0	0.9	0.0	0.9	0.9	0.9	0.0	0.0
Low inputs	0.0	0.0	0.0	0.0	0.0	68.2	0.0	0.0	0.9	0.0
No preferences	13.6	25.5	76.4	91.8	90.9	100.0	95.5	73.6	43.6	98.2

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Across the six villages, the results for home consumption criteria showed that taste and swelling after cooking (30.9%) were more appreciated by farmers than other attributes (Table 3.10). Taste (21.8%), swelling after cooking (20%) and the storage after cooking (15.5%) were identified by farmers as good criteria for rice preference for home consumption.

Table 3.10: Criteria for selecting rice for home consumption in Office du Niger in 2011

Consumption criteria	Percent
Ability to store	15.5
Swelling after cooking	20.0
Taste	21.8
Swelling after cooking and storing	10.9
Taste and swelling after cooking	30.9
No response	0.9

For preference for marketing, grain colour and size were identified by farmers as most important (Table 3.11). For color, white (57.3%) and the white yellowish (26.4%) were preferred by rice buyers in the market. The long slender grain and less breaking were identified by 90% of farmers as rice buyer's preference in the market.

Table 3.11: Attributes of grain considered by farmers for selection for the market in Office du Niger in 2011

Grain color	Percent
White bluish	3.6
White yellowish	26.4
White	57.3
White greenish	2.7
No response	10.0
Grain size	
Long slender and less breakage	90.0
Medium and less breakage	0.9
No response	9.1

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3.3.4 Farmer priorities for research needs

Table 3.12 shows farmers' suggestions of traits they prefer to be incorporated into rice varieties. A total of 58.2% of the farmers interviewed wanted vegetative tolerance to salts and spikelet fertility, 13.6% wanted yield and taste, 10% wanted yield and 13.6% did not suggest any specific trait.

Table 3.12: Farmers' preference for rice traits in Office du Niger in 2011

Suggested traits	Percent
Vegetative and spikelet fertility tolerance	58.2
Yield	10.0
Taste	.9
Yield and taste	13.6
Avoid lodging	.9
Swelling	1.8
Store after cooking	.9
No proposition	13.6

3.4 Discussion

Results from Participatory Rural Appraisal revealed that most farmers used seeds purchased from the farmers' association. Farmers in the relatively homogenous irrigated zones used improved varieties because of their high value returns. The varieties grown were not improved for stress tolerance and therefore they succumbed to alkalinity damages. AfricaRice (2010) observed that most farmers selected seeds for planting from the conventional seed (seed from Farmer association seed growers) in irrigated systems. Conventional seed systems are not always well adapted to the specific abiotic stress. Hence, the production of unimproved varieties that are susceptible to abiotic stress is still continued.

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Some farmers obtained their seeds from seed companies because of the proximity of their village to seed company's retail outlet. For example in Office du Niger, there are two seed companies involved in seed certification for good quality and marketing (Dembélé, 2006). One company is in Niono zone where Kolodougou village is located and another one is in Molodo zone which is close to Fabacoura village. Both of the two villages have easy access to the improved seed but the varieties are not tolerant to alkalinity.

A number of factors were listed as constraints to rice production across the three zones in Office du Niger. These included fertilizer costs, water management, insufficient agricultural equipment, declining soil fertility and land shortage. These constraints are in agreement with a previous survey conducted in 1995 (Ouvry *et al.,* 1999). In that study, the nature of plowing the soil was the most important cause of alkalinity curtailing rice production in Office du Niger, followed by sandy soil type, water quality and non drainage of irrigated water. In this study, differences were observed in causes of alkalinity ranking across the three zones of Office du Niger. In N'Debougou and Niono, nature of plowing the soil was the number one cause of alkalinity. This could probably be attributed to three factors. The first one is the impact of cropping systems (Bagayoko *et al.*, 2010). Most farmers have continued to use the local plow for rice field plowing and this is unable to level the soil surface efficiently. It creates shallow areas in the field where salt concentration is high. This situation can explain the constraint of inadequate agricultural equipment mentioned by farmers. The second factor is the effect of soil types on evolution of water table in terms of dynamics and quality (Bagayoko *et al.*, 2010; N'Diaye, 1998; Ouvry *et al.,* 1999). The soil type in these two zones is largely clay soil. Sodicity and alkalinity are environmental factors that greatly affect plant growth and development in clay soils. Harmful high alkalinity is related principally to the presence of sodium carbonate and sodium bicarbonate

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(Bagayoko *et al.*, 2010; Ouvry *et al.*, 1999). Accumulation of these salts in the soil and soil water occurs when groundwater with a high content of bicarbonate ions evaporates. Initially, calcium and magnesium carbonates precipitate upon evaporation, immobilizing part of bicarbonate ions. If excess bicarbonate is present (called residual alkalinity), high pH values will develop, even at initially low concentrations of sodium carbonate. In strongly alkaline soils, sodium carbonate and bicarbonate become prevalent salts, and sodium will replace calcium and magnesium in the clay complex (sodic soils). This leads to the formation of sodium clays; those are strongly dispersed and highly impermeable to water. The third factor is the lack of tolerance to alkalinity in improved rice varieties being grown. Alkalinity has been an abiotic stress of importance in Office du Niger but there has not been any active breeding programme in Mali specifically addressing tolerance to alkalinity stress. The environmental conditions in the breeding area have not favored the selection for tolerance against alkalinity. Hence most of the materials released do not have the genetic tolerance against soil degradation by alkalinisation. In Molodo zone, the sandy soil type is the main cause of alkalinity. This situation could be attributed to soil permeability factors (Bagayoko *et al.*, 2010; Dicko, 2005; N'Diaye, 1998). The previous studies (N'diaye *et al.*, 2002) showed the sensitivity of sandy soil types to soil degradation and the impact of drainage as means of controlling of the ascent by capillarity.

Soils of irrigated areas in Sub Sahara African countries have continued to be degraded as a result of poor irrigation management practices. In the Office du Niger zones, for example, producers and extension workers are concerned about emerging soil degradation symptoms such as salinisation/alkalinisation or sodisation. Whereas the above problems require urgent attention, the phenomenon has been treated as localised and therefore not very important. From 1995 to 1999, studies were conducted by "the Pole regional de recherche sur les Systèmes Irrigués (PSI)"

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which was a regional networking project, to determine the nature, importance and dynamics of soil degradation processes in irrigated zones of Mali. The results clearly show soil geochemical changes of the irrigated areas (Marlet *et al.*, 1998). However it is important to note that, during these studies, the breeding program was not included and this could be the main reason for the lack of rice genotypes tolerant to alkalinity, although all rice varieties produced are improved for yield, grain quality and taste.

Alkalinity tolerance is a polygenic trait acting both additively and with interactions between the alleles at some loci (Singh *et al.*, 2001), which implies that there will always some symptoms even when improved materials that carry the tolerant genes are used. For farmers, most improved varieties suffer alkalinity damage and among them Kogoni 91-1 is considered as tolerant to alkalinity by 19% of farmers.

All seeds for sale by certification system are considered as commercialized seeds in Mali (Dembélé, 2006). The seed that is grown for many years and not renewed is also considered as commercialized seeds. Often the commercial seeds producers don't observe the precautions that require the maintenance of the purity and the genetic identity. As a result, the commercialized seeds of most of the varieties are not genetically homogeneous. By selecting for seeds from uninfected rice plants, farmers tend to accumulate susceptible materials hence increasing alkalinity damage. Most released varieties grown by farmers were not improved for salinity and alkalinity tolerances and could have a negative impact with alkalinity problems.

Soil degradation control methods being employed by the farmers are limited and consist of crop rotation, use of organic matter, puddling followed by flooding and use of tolerant varieties. Many do not practice any control method, either because they lack knowledge on control technologies, or they could not afford to use chemical control.

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For alkalinity, the effectiveness of these control methods depends on a number of factors including, soil and irrigated water management, crop growth stage at which the susceptibility of plant is shown and the genetic make-up of the plant variety in question. Rice is more sensitive to salts during early seedling stage than at reproductive stages (Flowers and Yeo, 1981). Practices such as pre-flooding, puddling, flushing and use of chemical and organic matters could help control yield reduction (Dicko, 2005). A more effective and practical solution for subsistence farmers is high yielding rice varieties that are tolerant to alkalinity stress (Singh and Flowers, 2010).

Farmers' preferences when choosing varieties for market and home consumption differed. Farmers identified taste and swelling after cooking as selection criteria for home consumption and grain colour and size as criteria for market.

In comparison to many other nationalities in West Africa, the majority of Malian consumers prefer local rice over the imported rice, due to its preferred taste and swelling after cooking (IFDC, 2008). The most popular domestic rice variety is the Kogoni 91-1 variety, which is the dominant variety produced in the controlled irrigated systems. This variety is more appreciated by urban consumers because of its taste and aroma. However BG 90-2 variety is more preferred by rural consumers for its swelling. These two varieties are improved varieties released by Irrigated Rice Programme of National Research Institute of Mali. It is important to note that these two varieties are susceptible to alkalinity stress and to Rice Yellow Mottle Virus (RYMV) disease.

Standards for grain size and shape differ among countries, although the Food and Agriculture Organization (FAO) has developed international standards. Translucency and color are affected by environment and their assessment is largely subjective. For marketing of milled rice, grain

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color and size were the criteria identified by farmers as attractive to rice traders in Office du Niger. The white color was preferred by majority of farmers as criteria for selling rice to the traders. White, long and slender grain rice is more preferred by Malian consumers (IFDC, 2008; USAID, 2007). There is a positive correlation between grain length and grain shape (Gupta *et al.*, 2006) suggesting that breeders could select for both traits simultaneously, or they could choose one of the traits to indirectly select for the other. Grain size is usually evaluated by two criteria: grain weight and shape (Fan *et al.*, 2006; Tan *et al.*, 2000). Grain weight and grain shape (length, width, length/width ratio) are positively correlated characters (Fan *et al.*, 2006).

Besides taste and swelling after cooking for home consumption and grain colour and size for market, farmers had some other preferences that were identified by the survey. These included vegetative and spikelet fertility tolerance to alkalinity, a combination of yield and taste and yield alone. There is need to integrate such special farmer preferences into selection criteria. Involvement of farmers in varietal adoption would help bridge the information gap between farmers and breeders. It has been reported that farmers grow the same varieties for long periods of time. This could probably be because they have failed to find new varieties with special qualities they desire.

Farmers' preferences for home consumption were different from preference for market consumption. Farmer desire for market is driven by the traders and consumers needs or demands. Farmer request is based on consumers. In this regard, different breeding objectives should be formulated with input from the farmers to address these special preferences.

Compared with conventional plant breeding, Participatory Plant Breeding (PPB) is more likely to produce farmer-acceptable products, particularly for marginal environments. While meeting the farmers' needs requires continuous engagement of the farmers in the breeding process, the reality

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of this remains a scaring task to breeders. On the other hand, when a variety fails to meet farmers' needs during participatory varietal selection (PVS), farmers would not adopt it and the effort and resources spent would have been wasted.

3.5 Conclusion

The survey revealed four dominant factors as rice production constraints, namely fertilizer cost, water management, inadequate agricultural materials, and declining soil fertility. The combination of the four factors constitutes the main cause of soil alkalinisation in Office du Niger. Overall, alkalinity was the most important abiotic stress for rice production. The type of alkalinity varies from village to village and from zone to zone, however the white salt was more common. To overcome alkalinity constraints, farmers have developed some strategies like crop rotation, use of organic matters, plowing followed by flooding and use of tolerant varieties. The absence or weakness of a breeding program for alkalinity tolerance was apparent. Farmers have a basic preference for taste and swelling for home consumption and grain colour and size for marketing. Research priorities, as perceived by the farmers, included alkalinity tolerance of vegetative and spikelet fertility combined, combination of yield and taste and yield alone. Farmers had high interest in participatory varietal selection and participatory plant breeding.

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CHAPTER IV

4. IDENTIFICATION OF ALKALINE TOLERANT RICE ACCESSIONS AND THEIR ASSOCIATION WITH SSR MARKERS THROUGH GENETIC DIVERSITY

4.1 Introduction

Rice production in Mali is threatened by several factors including soil degradation, alkalinity and/or use of brackish ground water. Mali experiences huge recurring losses in terms of limited productivity from the salt-affected lands, apart from serious socio-economic repercussions. With the increase in population, effective utilization of these soils has become necessary either by reclamation or by growing salt tolerant rice.

Rice (*Oryza sativa* L.), is quite sensitive to salinity and alkalinity, particularly during early seedling and reproductive stages. Rice yield starts to decline beyond a threshold pH of 7.5, and a drastic reduction in yield occurs at pH 9.0. However rice is one of the few crops that can thrive on salt-affected soils. Being a species native to brackish ground water, it is able to grow well in standing water that can help leach salts from top soils, and therefore rice is recommended as a salts flushing crop (Marlet *et al.*, 1998).

The threat of soil alkalinity to the sustainability of rice agriculture can be overcome by developing and adopting rice-farming practices that can help prevent or arrest soil alkalinization. Soil remediation schemes can also be undertaken to rehabilitate salt-affected rice lands. However, these management and reclamation practices require considerable capital investments that are beyond the capability of the resource-poor farmers whose source of livelihood are these alkaline lands. Longer-term and affordable solutions can be achieved by developing rice varieties that are highly tolerant to salt stress. This can best be attained by profiling salt-management morpho-physiological processes in rice cultivars, which could subsequently facilitate the

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development of conventional and molecular breeding as well as genetic engineering approaches to combine these traits and speed up the development of varieties that are tolerant to salt stress.

Salt tolerance in rice involves several physiological and adaptive mechanisms (Ismail *et al.*, 2007; Peng and Ismail, 2004; Yeo and Flowers, 1986). The physiological bases of salt tolerance during early seedling stage involve traits such as high seedling vigor to dilute salt concentration in plant tissues, selective ion uptake by roots, compartmentalization of harmful ions in older tissues, such as older leaves, stems, leaf sheaths, and roots, responsive stomata that regulate water and salt uptake in response to increasing salt stress in the rhizosphere and re-circulating sodium back to roots to avoid accumulation of toxic concentrations in the cytoplasm. In the reproductive stage, tolerant genotypes tend to exclude salt from flag leaves and developing panicles (Khatun *et al.*, 1995; Yeo and Flowers, 1986).

Despite a wealth of knowledge on physiological mechanisms associated with salt tolerance in rice, little is known about the genetic diversity of these individual traits across rice germplasm. This is because most of the tolerant genotypes characterized to date seem to be superior in only one or a few of these traits (Ismail *et al.*, 2007; Moradi and Ismail, 2007; Yeo and Flowers, 1986). Nonetheless, this information is essential for breeders to select appropriate parental lines by choosing genotypes that are superior in expressing a particular trait when combining or pyramiding these characters to develop highly tolerant varieties.

Rice genotypes may be characterized as tolerant or sensitive based on the various responses examined. Genotypes identified could be selected for subsequent use of specific traits in breeding programs for selecting high yielding salt-tolerant varieties. Genetic characterization of the causal mechanisms related to these morpho-physiological adaptive characters would facilitate their use in developing resilient cultivars with multiple traits associated with salt tolerance.

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The objective of this study was to determine the variability in morpho-physiological and genotypic responses of some rice cultivars from IRRI and Africa Rice to alkalinity stress.

4.2 Materials and methods

4.2.1 Plant materials

4.2.1.1. Identification of salt tolerant accessions under alkaline soil condition

Twenty six (26) rice accessions including, IR 29 (sensitive check) and Kogoni 91-1 (local checks), were used to identify salt tolerant accessions under alkaline soil conditions of Office du Niger in Mali (Table 4.1).

Table 4.1: Rice accessions for phenotypic study

No	Accessions	Sources		Accessions	Sources
1	D14	AfricaRice	14	IR29 (sensible check)	Africa Rice
2	IR 66401-2-B-6-1-3	AfricaRice	15	(tolerant check)	Africa Rice
3	IR 71829-3R-89-1-1	AfricaRice	16	Dasal	IRRI
4	IR 72593-B-3-2-3-14	AfricaRice	17	CSR11	IRRI
5	ROHYB 6	AfricaRice	18	IR4630-22-2-5-1-3	IRRI
6	IR 66946-3R-178-1-1	Africa Rice	19	Damodar Haryana (CSR1)	IRRI
7	IR 71991-3R-2-6-1	Africa Rice	20	Damodar India (IRGC 17038)	IRRI
8	SAHEL 210	AfricaRice	21	Getu (IRGC 17041)	IRRI
9	IR 76393-2B-7-1-13-1	Africa Rice	22	Getu (IRGC 22709)	IRRI
10	IR 65192-4B-11-3	AfricaRice	23	CSR10	IRRI
11	IR 71895-3R-60-3-1	AfricaRice	24	CSR28	IRRI
12	IR 76346-B-B-10-1-1-1	Africa Rice	25	CSR36	IRRI
13	Kogoni 91-1 (local check)	Africa Rice	26	CSR23 (SAL 332)	IRRI

4.2.1.2. Determination of genetic diversity

Twenty eight rice genotypes (28) of which 24 accessions and 4 varieties, were used to determine allelic diversity using molecular markers (Table 4.2).

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Table 4.2: Rice genotypes for allelic diversity study

No	Rice genotypes	Sources	No	Rice genotypes	Sources
1	Nerica L1-IER	IER-Mali	15	IR29 (sensitive check) from student	AfricaRice
2	BG 90-2	IER-Mali	16	Pokkali (tolerant check) from student	AfricaRice
3	IR 66401-2-B-6-1-3	AfricaRice	17	Dasal	IRRI
4	IR 71829-3R-89-1-1	AfricaRice	18	CSR11	IRRI
5	Nerica L2-IER	IER-Mali	19	Damodar Haryana (CSR1)	IRRI
6	WAT 310	IER-Mali	20	Damodar India (IRGC 17038)	IRRI
7	IR 66946-3R-178-1-1	AfricaRice	21	Getu (IRGC 17041)	IRRI
8	IR 71991-3R-6-3-1	AfricaRice	22	Getu (IRGC 22709)	IRRI
9	SAHEL 210	AfricaRice	23	CSR10	IRRI
10	FL478 from AfricaRice	AfricaRice	24	Hassawi from AfricaRice	AfricaRice
11	IR 65192-4B-11-3	AfricaRice	25	CSR23 (SAL 332)	IRRI
12	IR 71895-3R-60-3-1	AfricaRice	26	IR4630-22-2-5-1-3	AfricaRice
13	IR 76346-B-B-10-1-1-1	AfricaRice	27	IR29 (sensitive check) AfricaRice	AfricaRice
14	Kogoni 91-1	AfricaRice	28	Pokkali (Tolerant check) AfricaRice	AfricaRice

4.2.1.3. Characterization of phenotypic and genetic associations

Nineteen (19) accessions were used to study the phenotypic and genotypic association (Table4.3).

Table 4.3: Rice accessions for phenotypic and genotypic association study

No	Rice accessions	Sources	No	Rice accessions	Sources
1	Kogoni 91-1 (local check)	AfricaRice	11	SAHEL 210	AfricaRice
2	CSR11	IRRI	12	IR 71829-3R-89-1-1	AfricaRice
3	CSR10	IRRI	13	IR 71895-3R-60-3-1	AfricaRice
4	Damodar Haryana (CSR1)	IRRI	14	CSR23 (SAL 332)	IRRI
5	IR 76346-B-B-10-1-1-1	AfricaRice	15	IR 71991-3R-2-6-1	AfricaRice
6	IR 66946-3R-178-1-1	AfricaRice	16	Dasal	IRRI
7	Damodar India (IRGC 17038)	IRRI	17	IR 65192-4B-11-3	AfricaRice
8	Pokkali (tolerant check)	AfricaRice	18	IR29 (sensible check)	AfricaRice
9	Getu (IRGC 17041)	IRRI	19	Getu (IRGC 22709)	IRRI
10	IR 66401-2-B-6-1-3	AfricaRice			

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4.2.2. Methodology

4.2.2.1 Identification of salt tolerant rice accessions under alkaline soil condition

Twenty six (26) accessions were screened in Randomized Complete Block Design with two replications. Direct seeding of 3 grains/pot was done after soil crushing. Plants were thinned to two seedlings per pot after germination. The genotypes were sown on June 06, 2010 in the greenhouse of Centre regional de Recherche Agronomique (CRRA) de Gao. The pot used was 20 cm in diameter and 50 cm high with 3 kg alkali soil of Office du Niger with 9.9 of pH, 8.9 mg/100g of Na^+ and 0.43 mg/100g of K^+. The ratio Na^+/K^+ was 20.7. Twenty days after plant emergence, three holes were made in the bottom of each pot and then placed in a hollow plate. After each, irrigation the water which was collected in the plate was returned to the pot to maintain alkalinity level in soil.

Experimental pots were fertilized using: urea = 0.69g/pot (220 kg/ha) and di-ammonium phosphate (DAP) = 0.31g/pot (100 kg/ha). The DAP was used during direct seeding and urea was applied after plants had 3 to 5 leaves and the second application was during panicle initiation.

Soil and plants were characterized to evaluate the nutrient uptake of each genotype. The analyses of soil were done for all genotypes in one composite soil before sowing them individually in pots. The composite soil was obtained from the mixture of soils from all pots. Soils were analyzed before sowing and after the flowering stage for the key parameters of pH, exchangeable cations (K^+ and Na^+) and the ratio of Na^+/K^+. Plant analyses on flag leaves were done on the same elements as in soil after flowering stage. These analyses were done at Soil Science Department of University of Ghana (Legon).

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4.2.2.2. Determination of genetic diversity

Three sources of rice genotypes were used for allelic diversity for alkalinity tolerance. These were sourced from IRRI in Philippine, AfricaRice in Saint Louis (Senegal) and IER in Mali (Table 4.1).

Ten flanking/nearest markers as Simple Sequence Repeat (SSR) covering the chromosomes 1, 2 4, 8 and 9 were selected for the genetic diversity analysis. Table 4.4 shows details on selected makers. The original source, repeat motifs, primer sequences and chromosomal position for these markers can be found in the RiceGenes database (http://www.gramene.org/microsat/RM_primers.html). SSR markers were obtained from AfricaRice Research Genetics and Biotechnology; Saint Louis, Senegal.

Table 4.4: Selected markers used for genetic diversity for alkalinity tolerance

Gene/QTLs	Chr. No	Traits	Flanking/nearest Marker	Size bp	References
qNa$^+$ SV1-1	1	Na$^+$ in stem at vegetative stage	RM572	147 – 181 Na	Ammar *et al.* (2009)
QSst2	2	Score of salt toxicity of leaves and/ survival day of seedling	RM250 RM208	154 – 174 166 – 180	Zang *et al.* (2008)
Trait based QTL	4	K$^+$ absorption	RM537 RM551	230 – 236 168 – 214	Gregorio (1997)
QPh4	4	Plant High	RM142 RM273		Singh and Flower (2010)
qNa$^+$/K$^+$SV 8-1	8	Na$^+$/K$^+$ ratio in stem at vegetative stage	RM477	219 – 223	Ammar *et al.* (2009)
Saltol (as control)			RM493 RM215	211 – 259	

The DNA of the 26 genotypes (accessions and varieties) was extracted from juvenile leaves of 3-week-old plants according to the simplified protocol modified CTAB method, (Bimpong *et al.*, 2010). The Polymerase Chain Reaction (PCR) was performed in 10 μL reactions containing 2 μL of DNA template, 4.5 μL ddH_2O, 1 μL 10X TB buffer (containing 200mM Tris-HCl pH 8.3,

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500mM KCl, 15mM MgCl2), 1 μL of 1mM dNTP, 0.3 μL $MgCl_2$, 0.50 μL each forward and reverse primers, and 0.5 μL of *Taq* DNA polymerase. The thermocycler was used to amplify the PCR product (DNA) with the program Saltol60. The PCR products were checked using the high resolution polyacrylamide gel electrophoresis (PAGE) for the primers size < 200 pb for 2hr 30mn running, and agarose gel for the primers size ≥ 200 bp for 3 hr running. The gels used are polyacrylamide 7.5% and agarose 3%. The gels were stained in 0.5 mg/mL ethidium bromide for 15 – 30 minutes and were documented using Multido lt digital Imaging System UVP.

4.2.2.3. Phenotypic and genotypic association study for alkaline tolerance rice identification

Nineteen rice accessions morpho-physiological characters (% uptake of Na^+ and K^+ in the flag leaves, the ratio Na^+/K^+, % spikelet fertility, weight of 100 grains (g) and the grain weight/plant) were evaluated. Ten simple sequence repeats (SSR) markers (Table 4.4) were used for molecular analysis to validate the markers effects on morpho-physiological characters.

4.2.3. Data collection

4.2.3.1. Identification of salt tolerant rice accessions under alkaline soil condition

The analysis of soil and plant was done in Soil Science Department laboratory of Ghana University (Legon). The data's collected included pH, exchangeable cations (K^+ and Na^+) and the ratio Na^+/K^+.

For morphological and physiological characteristics, the plants were developed in the pot and evaluated for 8 traits:

1) % Na^+ uptake (%Na^+) was calculated as the percent of Na^+ in the flag leaf.
2) % K^+ uptake (%K^+) was calculated as the percent of K^+ in the flag leaf.
3) Na^+/ K^+ ratio was calculated as the ratio Na^+/ K^+ in the flag leaf.

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4) Germination percentage (GM%) was calculated as the number of seeds germinated as a percentage of the total number of seed sowed.
5) Plant vigor at seedling stage (vg) was measured after 21 days after sowing (at 3 to 5) leaves to assess salt injury level in alkaline condition using the Standard Evaluation Score (SES) of IRRI, 2002 (Table 4.5).
6) The spikelet fertility in percent (sp fert%) as the percent of filled grain per plant in alkaline condition at reproductive stage.
7) The filled grain weight per plant (gw) was calculated as the weight of the fully filled grain of the plant in grams.
8) Weight of 100 grains per accession was recorded as the weight of 100 filled grains for each accession in grams.

Table 4.5: Standard Evaluation System score (SES)

Score	Observation	Vegetative Vigor
1	Fast growing; plants at 5 leaf stage have 2 or more tillers	Extra vigorous
3	Fast growing plants at 4-5 leaf stage have 1-2 tillers· in majority of population	Vigorous
5	Plants at 4-leaf stage	Normal
7	Plants somewhat stunted; 3-4 leaves; thin population; no tiller formation	Weak
9	Stunted growth; yellowing of leaves.	Very weak

Source: IRRI (2002)

4.2.3.2. Determination of genetic diversity

The size (in nucleotide base pairs) of the most intensely amplified band for each SSR marker was scored based on its migration relative to a molecular-weight size marker (10 bp DNA ladder).

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4.2.3.3. Phenotypic and genotypic association study for alkaline tolerance rice identification

Refer to 4.2.3.1 and 4.2.3.2 for data collection.

4.2.4. Data Analysis

4.2.4.1. Identification of salt tolerant rice accessions under alkaline soil condition

Analyses of variance were conducted using the procedure General Linear Model from SAS 9.2 software with rice accessions being considered as fixed effects, and replications as random effects. The Least Significant Means of plant vigor at seedling stage, the % spikelet fertility, the grain weight (g), the weight of 100 grains (g) associated with Na^+ and K^+ uptakes and Na^+/K^+ ratio were used to construct the dendrogram representing the phenotypic relationships between accessions and the morpho-physiological characters based on the UPGMA mean cluster tree using the program DARwin 5.0.158.

4.2.4.2. Determination of genetic diversity

The marker data was analyzed using the software Graphical Geno-Typer (GGT 2.0). The frequency of SSR Markers polymorphism was calculated based on presence of bands in each genotype. The dendrogram was constructed by the un-weighted pair-group method with arithmetic average (UPGMA) subprogram of DARwin5.0.158. The polymorphism information content (PIC) value described was calculated using the software PowerMarker V3.25.

4.2.4.3. Phenotypic and genotypic association study for alkaline tolerance rice identification

The genetic associations among genotypes were evaluated by calculating the Jaccard similarity coefficient for pair-wise comparisons based on the proportion of shared bands (alleles) produced by primers. Similarity matrices were generated using the software Graphical Geno-Typer (GGT2.0). The dendrogram was constructed by the un-weighted pair-group method with arithmetic average (UPGMA) subprogram of DARwin5.0.158.

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4.3 Results

4.3.1 Identification of salt tolerant rice accessions under alkaline soil condition

4.3.1.1 Soil characteristics after flowering

Analysis of the soil from pots, in which different rice accessions were grown after flowering (Table 4.6), showed that the pH, Na^+, K^+ and Na^+/K^+ varied from one accession to another after flowering. From sowing to flowering, the pH of the soil of all accessions decreased due to the flushing system used 20 days after sowing and varied from 21.2% for ROHYB 6 and CSR28 to 39.4 for Kogoni 91-1 (local check). The percent reduction of soil pH for Pokkali (tolerant check) was 28.3% and it was 35.4% for IR29 (sensible check). The soil under different rice accessions showed the drastic reduction of Na^+ and K^+ content after flowering. This reduction varied from 81.5% for CSR28 to 97.1 for Damodar India (IRGC 17038) in Na^+ content and 37.2% for IR 65192-4B-11-3 to 62.8% for CSR10 in K^+ content. The Na^+ content reduction for the tolerant check (Pokkali), susceptible (IR29) and local check (Kogoni 91-1) was 95.2%, 85.4% and 96.6%, respectively, whereas the one for K^+ was in the same order 65.1%, 39.5% and 65.1% (Table 4.6).

4.3.1.2 Rice accessions flag leaves analyses

The Na^+, K^+ uptake and Na^+/K^+ ratio varied from one accession to another (Table 4.7).

The uptake of Na^+ varied from 0.07% for IR 72593-B-3-2-3-14 to 0.17% for IR 76346-B-B-10-1-1-1. The tolerant (Pokkali) check uptake of Na^+ was 0.13% and 0.15% for sensitive (IR29) and the local (Kogoni 91-1) check. However, the uptake of K^+ varied from 0.50 % for tolerant (Pokkali) and sensitive (IR29) check to 1.50% for IR 71991-3R-2-6-1 and Getu (IRGC 17041). The local check (Kogoni 91-1) uptake of $\%K^+$ was 1.00%. The ratio of Na^+/K^+ was 0.10 for Getu (IRGC 22709) and IR4630-22-2-5-1-3.

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Table 4.6: The effect of rice accessions on pH, Na^+ and K^+ in alkaline soil of Office du Niger (Mali) in 2011

		pH			Na^+(mg//kg)			K^+ (mg/kg)		
No	Rice accessions	before sowing	flowering stage	% reduction	before sowing	flowering stage	% reduction	before sowing	flowering stage	% reduction
1	D14	9.9	6.7	32.3	0.089	0.0065	92.7	0.0043	0.002	53.5
2	IR 66401-2-B-6-1-3	9.9	6.8	31.3	0.089	0.0035	96.1	0.0043	0.0014	67.4
3	IR 71829-3R-89-1-1	9.9	6.5	34.3	0.089	0.004	95.5	0.0043	0.0015	65.1
4	IR 72593-B-3-2-3-14	9.9	6.4	35.4	0.089	0.0065	92.7	0.0043	0.0018	58.1
5	ROHYB 6	9.9	7.8	21.2	0.089	0.0043	95.2	0.0043	0.0015	65.1
6	IR 66946-3R-178-1-1	9.9	6.4	35.4	0.089	0.003	96.6	0.0043	0.0017	60.5
7	IR 71991-3R-2-6-1	9.9	6.9	30.3	0.089	0.0035	96.1	0.0043	0.0015	65.1
8	SAHEL 210	9.9	7	29.3	0.089	0.004	95.5	0.0043	0.0014	67.4
9	IR 76393-2B-7-1-13-1	9.9	7	29.3	0.089	0.0043	95.2	0.0043	0.0018	58.1
10	IR 65192-4B-11-3	9.9	7.2	27.3	0.089	0.0087	90.2	0.0043	0.0027	37.2
11	IR 71895-3R-60-3-1	9.9	8	19.2	0.089	0.0161	81.9	0.0043	0.0038	11.6
12	IR 76346-B-B-10-1-1-1	9.9	6.6	33.3	0.089	0.0087	90.2	0.0043	0.0022	48.8
13	Kogoni 91-1 (local check)	9.9	6	39.4	0.089	0.003	96.6	0.0043	0.0015	65.1
14	IR29 (sensible check)	9.9	6.4	35.4	0.089	0.013	85.4	0.0043	0.0026	39.5
15	Pokkali (tolerant check)	9.9	7.1	28.3	0.089	0.0043	95.2	0.0043	0.0015	65.1
16	Dasal	9.9	7.1	28.3	0.089	0.0096	89.2	0.0043	0.0026	39.5
17	CSR11	9.9	6.9	30.3	0.089	0.0035	96.1	0.0043	0.0015	65.1
18	IR4630-22-2-5-1-3	9.9	6.8	31.3	0.089	0.0043	95.2	0.0043	0.0015	65.1
19	Damodar Haryana (CSR1)	9.9	6.8	31.3	0.089	0.0035	96.1	0.0043	0.0018	58.1
20	Damodar India (IRGC 17038)	9.9	7	29.3	0.089	0.0026	97.1	0.0043	0.0014	67.4
21	Getu (IRGC 17041)	9.9	6.2	37.4	0.089	0.013	85.4	0.0043	0.0024	44.2
22	Getu (IRGC 22709)	9.9	6.3	36.4	0.089	0.013	85.4	0.0043	0.0031	27.9
23	CSR10	9.9	6.9	30.3	0.089	0.0043	95.2	0.0043	0.0016	62.8
24	CSR28	9.9	7.8	21.2	0.089	0.0165	81.5	0.0043	0.0021	51.2
25	CSR36	9.9	7	29.3	0.089	0.014	84.3	0.0043	0.0027	37.2
26	CSR23 (SAL 332)	9.9	7.2	27.3	0.089	0.014	84.3	0.0043	0.0032	25.6

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Table 4.7: Percent of Na^+, K^+ uptake and Na^+/K^+ ratio in the flag leaves at flowering stage in 2011

No	Rice accessions	% Na^+	% K^+	Na^+/K^+
1	D14	0.11	1.00	0.11
2	IR 66401-2-B-6-1-3	0.08	1.00	0.08
3	IR 71829-3R-89-1-1	0.11	1.00	0.11
4	IR 72593-B-3-2-3-14	0.07	1.00	0.07
5	ROHYB 6	0.13	1.00	0.13
6	IR 66946-3R-178-1-1	0.10	1.00	0.10
7	IR 71991-3R-2-6-1	0.16	1.50	0.10
8	SAHEL 210	0.11	0.97	0.11
9	IR 76393-2B-7-1-13-1	0.11	0.93	0.12
10	IR 65192-4B-11-3	0.11	1.16	0.10
11	IR 71895-3R-60-3-1	0.15	1.00	0.15
12	IR 76346-B-B-10-1-1-1	0.17	1.00	0.17
13	Kogoni 91-1 (local check)	0.15	1.00	0.15
14	IR29 (sensible check)	0.15	0.50	0.30
15	Pokkali (tolerant check)	0.13	0.50	0.25
16	Dasal	0.11	0.70	0.16
17	CSR11	0.12	1.00	0.12
18	IR4630-22-2-5-1-3	0.11	1.50	0.07
19	Damodar Haryana (CSR1)	0.11	0.93	0.12
20	Damodar India (IRGC 17038)	0.11	1.16	0.10
21	Getu (IRGC 17041)	0.12	1.50	0.08
22	Getu (IRGC 22709)	0.11	0.70	0.16
23	CSR10	0.09	1.00	0.09
24	CSR28	0.13	1.00	0.13
25	CSR36	0.11	0.93	0.12
26	CSR23 (SAL 332)	0.11	0.70	0.16

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4.3.1.3 Percent germination, vigor, percent spikelet fertility, and grain weight

The tested accessions responded similarly to alkalinity constraint for percent germination, weight of 100 grains and the total grain weight (Table 4.8). This was evidenced by the no significant effect obtained between the genotypes (rice accession) for these different characteristics. However significant and highly significant differences were obtained respectively for vigor and for percent of spikelet fertility among the 26 rice accessions.

Table 4.8: Mean squares of 26 accessions and varieties' for plant growth and yield characteristics

Source of variation	d.f.	% germ	Vigor (SES)	% spikelet fertility	Weight of 100 grains (g)	Grain weight (g)
Block	1	355.77*	17.31**	245.82	0.68	22.44
Genotype	26	680.76	7.45**	302.43**	0.32	11.12
Residual	25	455.77	3.07	60.48	0.33	11.21
Corrected total	52					

*=P<0.05 and **=P<0.01

The best percent germination of 87.5% was obtained for IR71895-3R-60-3-1 and the worst percent germination (25.0%) was obtained for IR76393-2B-7-1-13-1, ROYB6, CSR36 and CSR23 (SAL332) (Table 4.9). The overall mean for percent germination for all rice accessions was 54.4%, with 38.68 %CV and 43.9% LSD at 5%.

The mean vigor rating of rice accessions in alkaline soil condition is shown in Table 4.7. The best vigor, according to Standard Evaluation System of IRRI, was obtained for the accessions D14, IR 66401-2-B-6-1-3, IR 71829-3R-89-1-1 and IR 71991-3R-2-6-1 (score 2). The worst vigor scoring was obtained from the rice accessions ROHYB6, IR65192-4B-11-3 and IR29 with the score of 8. The mean of the vigor for tolerant, sensitive and local check were respectively 4, 8 and 5. The overall mean for vigor for all rice accessions was 4.89, with 36.40% CV and 3.6% LSD at 5%.

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The best percent spikelet fertility was recorded for Sahel 210 with 74.45% and the poorest spikelet fertility percent was the sensitive check (IR 29) with 31.06% (Table 4.7). However the percent fertility of tolerant and local checks was, respectively, 69.45% and 41.45%. The overall mean for all rice accessions was 49.70%, with 15.37% CV and 16.17% LSD at 5%.

The weight of 100 grains varied from one accession to another (Table 4.7). This variation was from 0.35 g for IR 65192-4B-11-3 and IR 29 (Sensitive check) to 1.99 g for CSR11. The value of the tolerant check was 0.75 g and that of the local check was 1.55 g. The overall mean for all accessions was 1.24g with 46.50% CV and 1.20 LSD at 5%.

The weight of rice accession grains varied from one to another (Table 4.7). The lowest weight was recorded with IR 65192-4B-11-3 and IR 29 (Sensitive check) with 2.44 g and the highest was with CSR10 with 11.52 g. The weight of the tolerant check was 5.03 g and local check was 10.43g. The general mean for all accessions was 7.88 with 42.51%CV and 6.96 LSD at 5%.

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Table 4.9: percent germination, vigor (SES), percent spikelet fertility and grain weight

No	Rice accessions	% Germination	Vg (SES)	% Spikelet fertility	Weight of 100 grains (g)	Grain weight (g)
1	D14	50.00	2	51.10	1.19	8.03
2	IR 66401-2-B-6-1-3	50.00	2	58.10	1.09	6.08
3	IR 71829-3R-89-1-1	62.50	2	52.00	1.08	6.05
4	IR 72593-B-3-2-3-14	75.00	4	40.45	1.27	8.55
5	ROHYB 6	25.00	8	36.26	0.59	4.09
6	IR 66946-3R-178-1-1	37.50	5	56.60	0.86	5.78
7	IR 71991-3R-2-6-1	62.50	2	54.10	0.9	6.08
8	SAHEL 210	50.00	3	74.45	1.6	10.8
9	IR 76393-2B-7-1-13-1	25.00	6	41.45	1.02	6.9
10	IR 65192-4B-11-3	37.50	8	35.26	0.35	2.44
11	IR 71895-3R-60-3-1	87.50	5	46.76	1.17	7.99
12	IR 76346-B-B-10-1-1-1	50.00	6	42.50	1.25	8.4
13	Kogoni 91-1 (local check)	62.50	5	41.45	1.55	10.43
14	IR29 (sensitive check)	75.00	8	31.06	0.35	2.44
15	(tolerant check)	75.00	4	69.45	0.75	5.03
16	Dasal	75.00	5	45.60	1.54	10.35
17	CSR11	62.50	3	70.25	1.99	11.28
18	IR4630-22-2-5-1-3	50.00	6	38.65	0.99	6.676
19	Damodar Haryana (CSR1)	50.00	7	41.05	0.64	4.28
20	Damodar India (IRGC 17038)	75.00	3	72.05	1.97	11.04
21	Getu (IRGC 17041)	62.50	4	48.20	1.29	7.46
22	Getu (IRGC 22709)	50.00	7	37.05	1.22	7.02
23	CSR10	75.00	3	72.00	1.84	11.52
24	CSR28	75.00	4	43.90	1.67	9.95
25	CSR36	25.00	5	49.65	1.73	11.03
26	CSR23 (SAL 332)	25.00	6	43.00	0.96	6.45
Mean		54.4	4.89	49.70	1.24	7.88
CV%		38.68	36.40	15.37	46.50	42.51
LSD 5%		43.97	3.60	16.17	1.20	6.96

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4.3.1.4 Association among traits across rice genotypes

The simple correlation analyses were carried out across the rice accessions. Rice accessions reacted differently to alkalinity stress. Across the rice accessions, the scoring vigor, was significantly (P< 0.001) and negatively associated with the percent spikelet fertility and the grain weight. The percent spikelet fertility was significantly (P< 0.001) and positively associated with the grain weight (Table 4.10).

Table 4.10: Correlation coefficients among traits under alkaline soil of Office du Niger Mali

	Vigor (SES)	% Spikelet fertility
% Spikelet fertility	-0.73***	
Grain weight (g)	-0.52**	0.52**

** and *** significant at 0.01 and 0.001 probability level respectively

4.3.1.5 Morphological and physiological analysis relationships between the rice accessions

The UPGMA mean cluster tree analysis grouped th 27 rice accessions into three major groups, which were further divided into several subgroups (Figure 4.1). Group I is comprised of accessions CSR10, Damodar India (IRGC 17038), CSR11, Sahel 210 and Pokkali (Tolerant check); Group II contained IR71991-3R-2-6-1, IR71829-3R-89-1-1, D14, IR66946-3R-178-1-1 and IR66401-2-B-6-1-3; and Group III contained CSR23(SAL332), IR76393-2B-7-1-13-1, IR76346-B-B-10-1-1-1, Kogoni 91-1 (local check), IR72593-B-3-2-14, Getu (IRGC 22709), IR4630-22-2-5-1-3, Damodar Haryana (CSR1), CSR28, Dasal, Getu (IRGC 17041), IR71895-3R-60-3-1, CSR36, IR65192-4B-11-3, ROYB6 and IR29 (Sensitive check).

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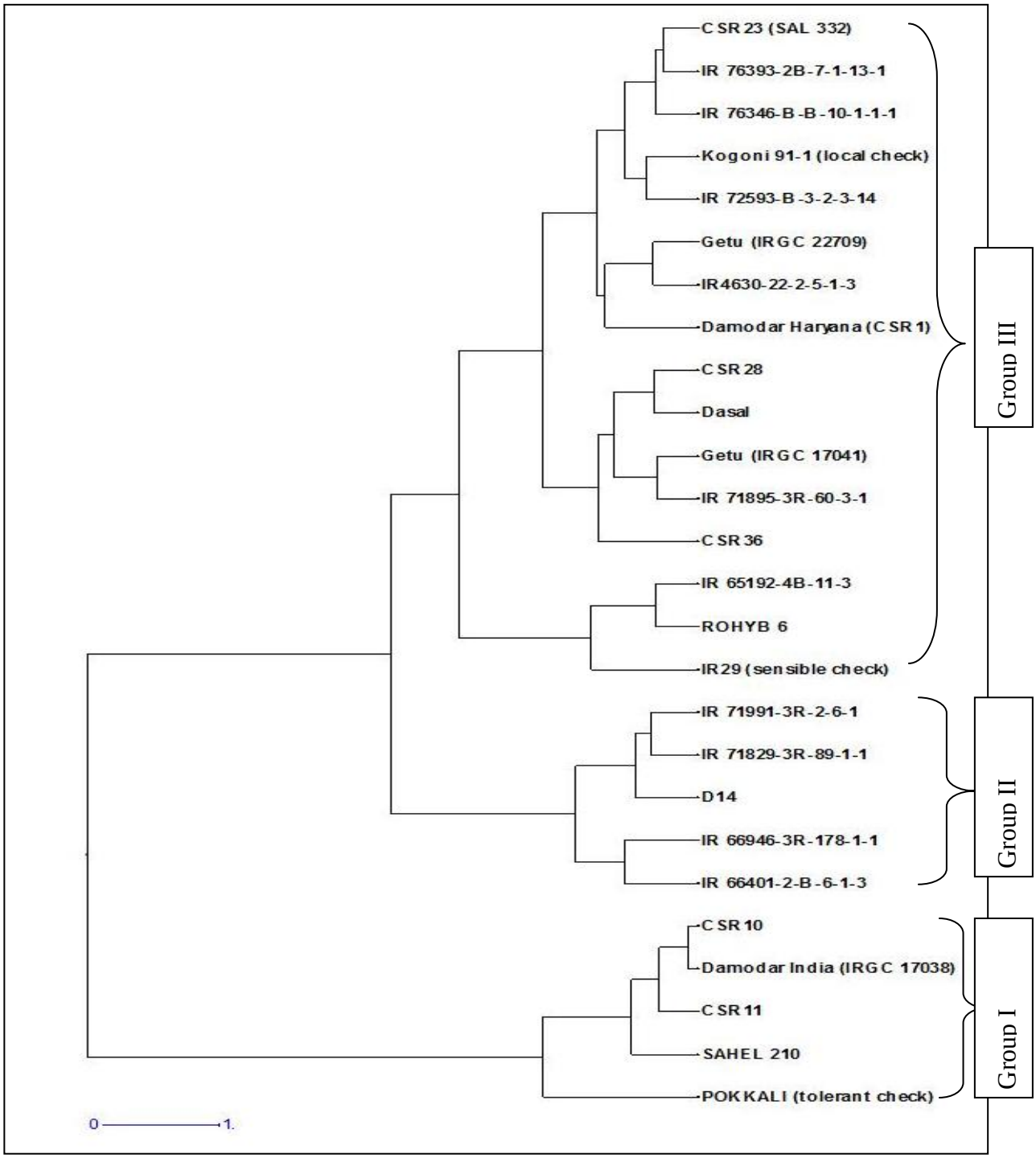

Figure 4.1: Dendrogram of 26 rice accessions based on seedling vigor (SES), %spikelet fertility and grain weight (g) derived from UPGMA cluster analysis.

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4.3.2. Determination of genetic diversity

4.3.2.1 Number of alleles

A total of 76 alleles for 10 SSR loci were detected among 28 rice varieties (Table 4.11). The number of alleles per locus ranged between 3 (RM537) and 15 (RM208) with an average of 7.6 alleles per locus. Loci with di-nucleotide repeat motifs were somewhat more polymorphic (mean number of alleles 7.8, n=6) than the ones with tetra-nucleotide repeat motifs (mean number of alleles 7, n=1) or tri nucleotide repeat motifs (mean number of alleles 4.3 alleles, n=3).

Table 4.11: Allelic diversity among 28 rice genotypes for 10 SSR markers

Markers	Chr location	no of alleles	No of genotypes	Distance min (cM)	Size range (bp)	Diff. (bp)	Frequency of alleles		PIC
							Size (bp)	Freq. (%)	
RM142	4	4	22	68.5	235 – 238	3	240	55	0.47
RM208	2	15	27	186.4	166 -180	14	173	91	0.90
RM215	9	7	28	99.4	147 – 152	5	148	82	0.79
RM250	2	6	22	170.1	154 – 174	20	153	77	0.74
RM273	4	9	26	94.4	199 – 207	8	207	86	0.84
RM477	8	7	28	121.8	219 – 223	4	223	79	0.77
RM493	1	6	23	79.7	211 – 259	48	211	78	0.75
RM537	4	3	13	8.5	230 – 236	6	236	59	0.52
RM551	4	12	26	8.5	168 – 214	46	192	89	0.88
RM572	1	7	17	66.4	147 – 181	34	159	81	0.78
Total		76							
Mean		7.6		90.37				78	0.74

4.3.2.2 Allele size range

The overall size of PCR products amplified using 10 SSR primer pairs ranged from 148 to 240 bp (Table 4.11). The molecular size difference between the smallest and the largest allele for a given SSR locus varied from 3 (RM142 with CGG repeat motif) to 48 bp (RM493 with CTT repeat motif). Maximum variation in allele size was observed for RM markers with CTT repeat

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motifs (RM493, 48 bp), AG repeat motifs (RM551, 46 bp); TC repeat motifs (RM572, 34 bp); CT repeat motifs (RM250, 20 bp; RM208, 14 bp; RM215, 5 bp), GA repeat motifs (RM273, 8 bp); CCG repeat motifs (RM537, 6 bp); AATT motifs (RM477, 4 bp); and CGG motifs (RM142, 3 bp).

4.3.2.3 Frequency of alleles

On average, 78% of alleles were shared among varieties at any given locus. There was considerable range in allele frequency, as illustrated by the fact that at RM208 and RM551 loci, the highest frequency allele was present, respectively, in 91% and 89% of the genotypes and RM142 was less present in 55% of the genotypes (Table 4.11).

4.3.2.4 Polymorphism Importance Content (PIC) values

The level of polymorphism among the 28 varieties was evaluated by calculating PIC values for each of the 10 SSR loci (Table 4.11). PIC values varied among loci and ranged from 0.47 (RM 142) to 0.90 (RM 208), with an average value of 0.74 per locus.

4.3.2.5 Euclidian mean cluster tree dendrogram

The Euclidian mean cluster tree analysis led to the grouping of 26 varieties into three major groups, which were further divided into several small groups (Figure 4.2). Group I is comprised of genotypes Nerica L1-IER, Getu (IRGC 22709), Dasal and IR29 (Sensitive check). Group II is comprised of genotypes IR4630-22-2-5-1-3 (AfricaRice), IR 66401-2-B-6-1-3, Damodar Haryana (CSR1), FL478 (AfricaRice), CSR11, IR 76346-B-B-10-1-1-1, Sahel 210, CSR23 (SAL 332), IR 71895-3R-60-3-1 and Hassawi (AfricaRice). Group III is comprised of genotypes Nerica L2-IER, IR 71991-3R-60-3-1, IR 65192-4B-11-3, CSR 10, BG 90-2, Pokkali (tolerant

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check), Getu (IRGC 17041), IR 66946-3R-178-1-1, IR 71829-3R-89-1-1, Damodar India (IRGC 17038), Kogoni 91-1 (local check) and WAT310.

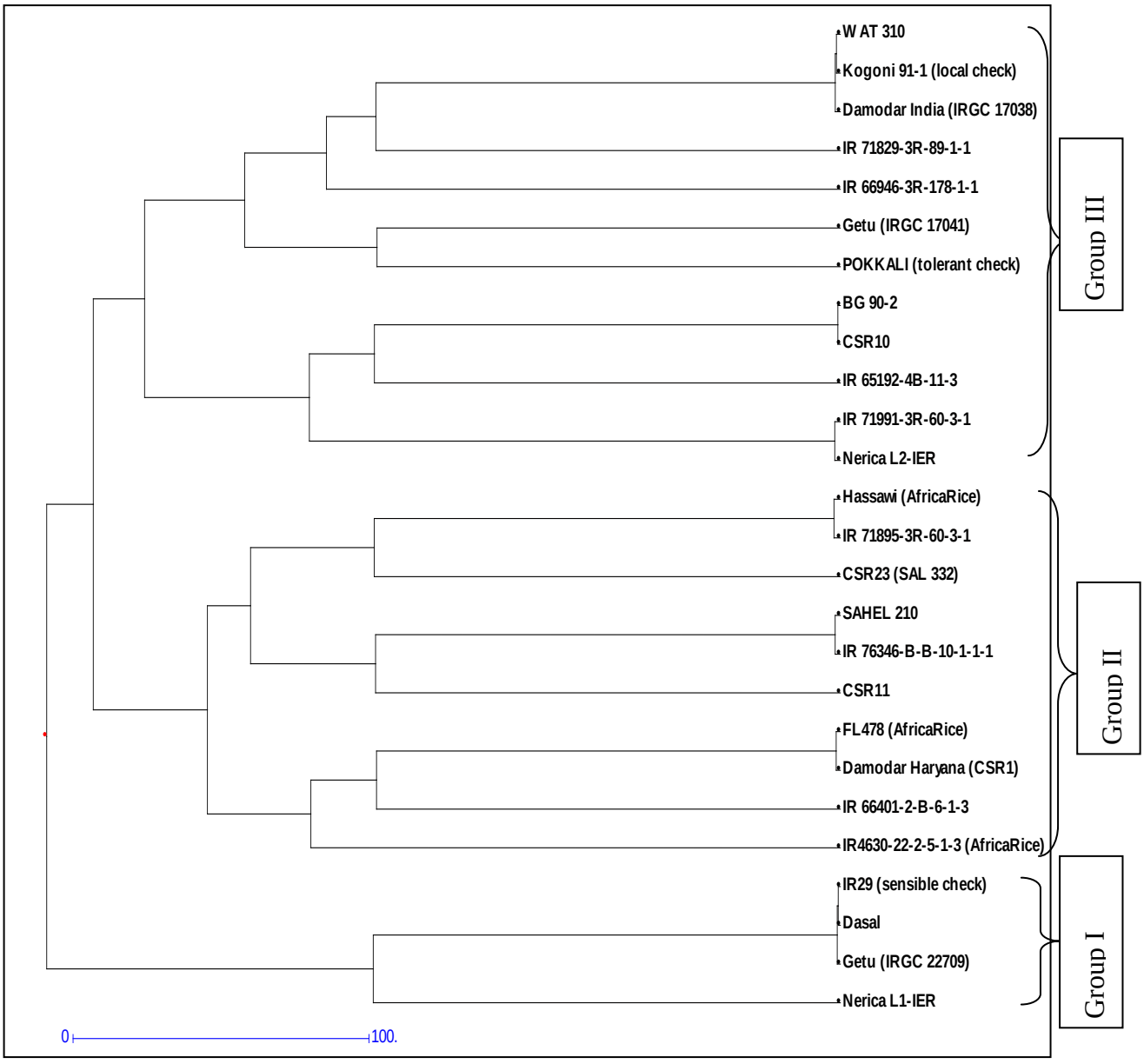

Figure 4.2: Cluster analysis of the 26 rice genotypes using distant coefficient based on 10 SSR markers.

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4.3.3. Phenotypic and genotypic association study for alkaline tolerance identification

Upon association with the morpho-physiological traits, one marker RM208 on chromosome 2 was associated with the percent uptake of K^+ which is associated with salt tolerance (Table 4.12). It accounted for 37% (P-value = 0.01) of the % K^+ uptake in flag leaves of rice accessions. The P-values for the other markers associated with the remaining traits were not significant, but some of them reacted weakly on some traits (Table 4.12). For spikelet fertility, the RM572 and RM142 reacted, respectively, at 17% and 13%. For the weight of 100 grains, the marker RM142 reacted at 14%. For the grain weight, the markers RM493, RM537 and RM142 reacted, respectively, at 13%, 12% and 11%. For per cent Na uptake, the marker RM572 reacted at 13%. For per cent K^+ uptake, the marker RM537 reacted at 28%. For Na^+/K^+ ratio, the markers RM537, RM208, RM551 and RM572 reacted respectively, at 23%, 22%, 18% and 14%.

The dendrogram based on markers RM208 and RM537 for % uptake of K^+, associated with salt tolerance on chromosomes 2 and 4; RM572 and RM142, respectively, at the loci of chromosome 1and 4 for spikelet fertility; the marker RM142 at the locus of chromosome 4 for the weight of 100 grains; the markers RM493, RM537 and RM142, respectively, at the loci of chromosome 1 and 4 for grain weight; the marker RM572 for % uptake of Na^+ at the locus of chromosome 1 and the markers RM537, RM551, RM208, and RM572, respectively, at the loci of chromosome 4, 2 and 1 for Na^+/K^+ ratio revealed three clusters. The four accessions identified with the tolerant check morpho-physiologically were revealed with the same tolerant check genotypically (Figure 4.3).

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Table 4.12: Marker effects on rice accessions morpho-physiological characters

Markers	Chr. no	Loc. cM	% Spikfert		Weight of 100 grains (g)		Grain weight (g)		% Na^+		% K^+		Na^+/K^+ ratio	
			% R^2	P-value	% R^2	P-value	% R^2	P-value	% R^2	P-value	% R^2	P-value	% R^2	P-value
RM493	1	79.7	3	0.19	5	0.42	13	0.16	5	0.41	9	0.27	2	0.59
RM572	1	66.4	17	0.52	2	0.7	1	0.75	13	0.25			14	0.23
RM208	2	186.4	1	0.99	2	0.61	2	0.63	5	0.39	37	0.01	22	0.06
RM250	2	170.1			1	0.72			1	0.78	4	0.49	1	0.72
RM142	4	68.5	13	0.49	14	0.19	11	0.24	4	0.5	5	0.43	5	0.45
RM273	4	94.4	8	0.39	7	0.28	1	0.2	1	0.72	3	0.48		
RM537	4	8.5	6	0.21	9	0.37	12	0.29	4	0.58	28	0.10	23	0.13
RM551	4	8.5	5	0.25	5	0.41	9	0.24	3	0.5	3	0.48	18	0.09
RM477	8	121.8	1	0.73	3	0.5	4	0.42	2	0.59	2	0.55	3	0.48
RM215	9	99.4							1	0.68	1	0.19	3	0.45

Spikfert= Spikelet fertility.

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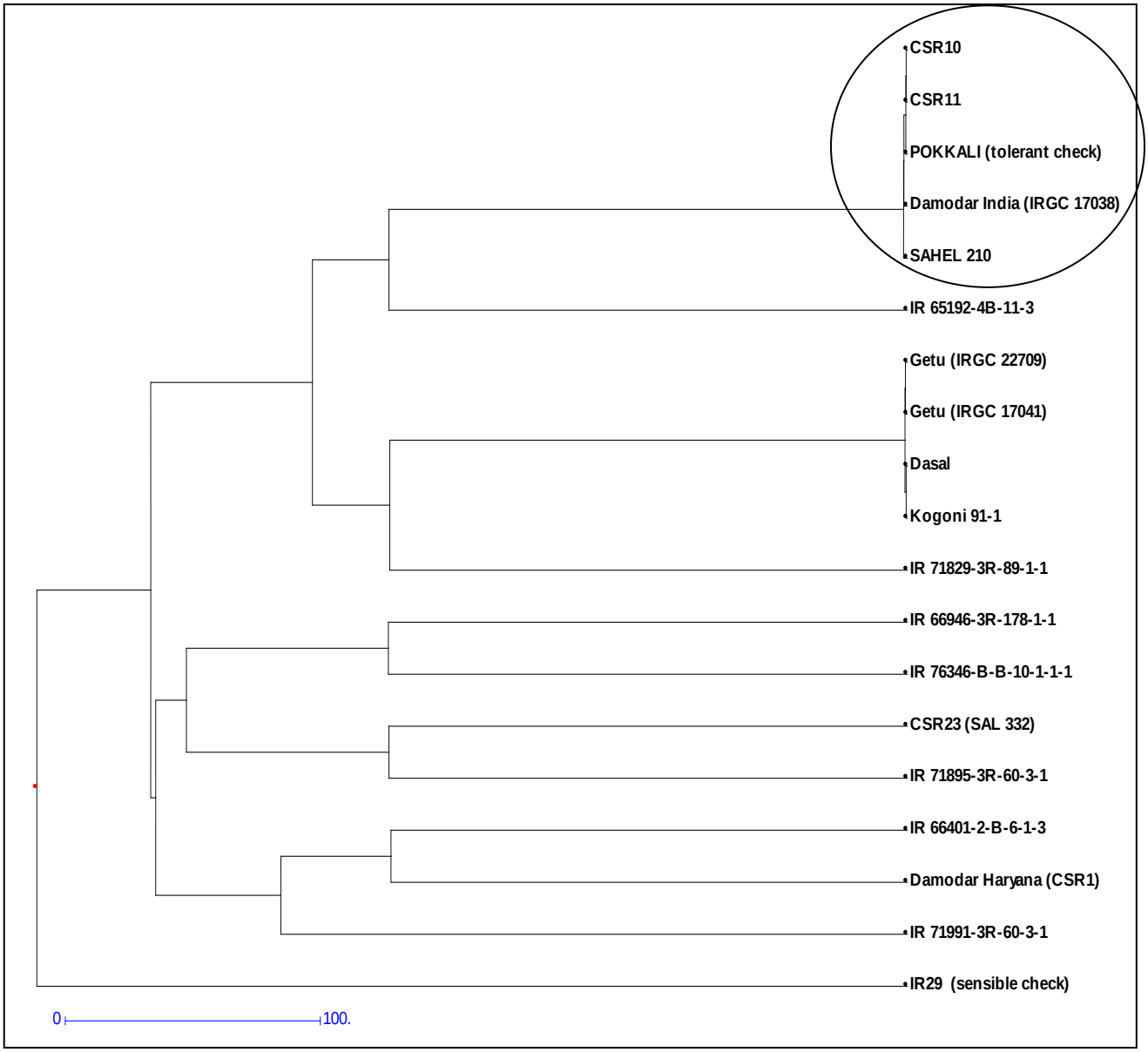

Figure 4.3: Dendrogram of genotypic relationship in rice accessions based on RM208, RM537, RM572, RM142, - and RM493 markers, derived from UPGMA cluster analysis

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4.4 Discussion

The high value of pH, Na^+ and K^+ indicated that these soils are alkaline as observed by Valles *et al.* (1992); Bertrand *et al.* (1993) and N'Diaye (1998). Soil degradation through alkalinization is considered one of the most important threats facing sustainable irrigated rice cropping in the Sahel (Ceuppens *et al.*, 1997). Irrigated rice cultivation is linked directly with soil alkalinization (Condom, 2000). Soil alkalinization is induced by evaporation, poor drainage and low irrigated water quality. Its impact on rice production is important and can cause plant stunting, lower tillering, leaf discoloration, spikelet sterility and yield reduction or loss. While salt stress in a soil generally involves osmotic stress and ion-induced injury (Munns, 2002), there is a high pH effect with alkali stress. The loss of normal physiological functions of rice can be mainly explained by a high-pH environment surrounding the roots due partially to nutrients imbalance in the soil. The soil analysis results for sodium and potassium before the sowing of rice accessions showed an unbalanced nutrient indicator. The relationship between the rice root and its environment is very complex and constitutes an important parameter for genotype characterization for a particular trait. Jones (1986) studied the genetics of saline tolerance in two mangrove varieties, viz. Pokkali and Pa Merr 108A in Sierra Leone using relative root lengths as a criterion for salt tolerance in saline and non saline conditions. The study indicated additive genetic variation effects. The soil pH after flowering decreased and varied from one accession to another. The nutrient uptake by rice roots in unbalanced conditions can be used for salt tolerant genotype identification. The $\%Na^+$ and $\%K^+$ content in the flag leaf varied from one accession to another. Rice plants tolerate salt stress by 3 mechanisms acting singly or jointly (Islam, 2009; Munns *et al.*, 2006). These mechanisms are exclusion, dilution, compartmentalization or maintenance of low Na^+/K^+ ratio. Exclusion refers to the restricted uptake of Na^+ ions and non restricted uptake of K^+ ions for

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tolerant rice varieties. For dilution effect, usually the tolerant varieties grow faster than non tolerant varieties under salt condition. It was experimentally found that lower shoot Na^+ content in a variety is not due to any better control of Na^+ transport by its roots but is directly attributable to the dilution effect of its rapid vegetative growth (Munns *et al.*, 2006). Compartmentalization occurs when the rice plant is exposed to salt conditions and the older leaves die due to high amount of Na^+ accumulation while younger ones remain green and growing. This physiological behavior of rice is called compartmentalization which is a useful feature of *gramineae* to maintain low Na^+ / K^+ ratio. It is now established that salt tolerant varieties maintain a lower Na^+/K^+ ratio compared to non-tolerant varieties (Senguttuvel *et al.*, 2010). Low Na^+/K^+ ratio is positively correlated with a high level of salt tolerance and can be taken into consideration for screening rice lines (Mishra *et al.*, 1996). Yeo *et al.* (1990) demonstrated genetic diversity for sodium uptake and commented that salt tolerance persists within modern rice cultivars and observed additive effects with high heritability in Na^+ content in the shoot at seedling stage. Mahmood *et al.* (2002) reported that non additive gene effects were more pronounced for Na^+ and Na^+/K^+ ratio. Mahmood *et al.* (2004) found that both additive and dominance effects were important shoot Na^+ and K^+ concentration. Mishra (1991) reported similar results under saline-sodic environments.

All accessions used in the screening for salt tolerance identification germinated in alkaline soils except IR 61247-3B-8-2-1. The reason for this non-germination was probably related to the seed quality. The germination rate varied from 0% to 100%. It has been reported that salt affects rice plants to varying degrees from germination to maturity; however the effect may vary depending on the stage of plant development (Aslam *et al.*, 1994; Castro and Sabado, 1977). Rice is tolerant during germination, very sensitive during early seedling stages, tolerant during vegetative stages,

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again becomes sensitive during pollination and fertilization, and then becomes increasingly tolerant towards maturity (Anonymous, 1967; Singh *et al.*, 2009). That is why the germination was not selected as a salt tolerant parameter in this study.

The different rice accessions evaluated in this study showed varying growth characteristics at the seedling stage. Good vigor was earlier found to be associated with the ability of the rice to tolerate soil salts (Yeo and Flowers, 1986), and was strongly correlated with survival under salt (Yeo *et al.*, 1990). This is because high vigor is a reflection of faster plant growth and provides dilution of salt concentration in plant tissues and therefore gives the plant the ability to survive in salt-affected areas (Yeo and Flowers, 1984). In fact, breeding for vigorous plants has been argued to be agronomically the most effective approach for increasing yield in salt soils (Richards, 1992). Tester and Davenport (2003) noted however, that such an approach must have its limits and a strategy whereby plants are selected for their ability to maintain yields in salt soils must also be employed. Since vigor allows the plant to be tolerant, accessions with low scores (high vigor), such as Sahel 210, CSR11, Damodar India (IRGC 17038) and CSR10, can be expected to thrive under alkaline conditions compared with accessions with high scores (low vigor), such as IR29, ROHYB 6 and IR 65192-4B-11-3, which are predicted to perform poorly in alkaline environments. These predictions were further substantiated by the strong correlations of vigor with the spikelet fertility, the grain weight.

Screening of rice accessions based on traits like spikelet fertility is being used by breeders in salt affected soils. Spikelet fertility is defined as the ratio of filled grains to total number of grains in the panicle. A high percentage of spikelet fertility relates to a high level of salt tolerance in rice (Deepa *et al.*, 2011). Percentage of spikelet fertility in different accessions was different. Spikelet fertility was one of the more affected traits under alkaline soil conditions than any other

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trait, indicating that it will be a useful criterion for selection of the materials under salt stress conditions. Sterility is normal in rice and high yields in rice are obtained with normal spikelet sterility of as much as 10 to 15% (Mishra *et al.*, 2006) and spikelet fertility is controlled by dominance gene action (Deepa *et al.*, 2008). These studies reported increasing spikelet fertility under high salt environments and reported that high percentage of spikelet sterility relates to a low level of salt tolerance. Rao *et al.* (2008) reported that higher floret fertility contributed to higher seed set and grain yield in tolerant genotypes while higher spikelet sterility led to poor seed set and lower grain yield in sensitive genotypes. Thus spikelet fertility is useful for identifying salt tolerant rice genotypes.

The weight of 100 grains constitutes an indicator for yield characterization and a good criterion for salt tolerant genotype selection. The accessions grown in alkaline soil conditions differed in weight of 100 grains. The accessions identified with good spikelet fertility also had good weight of 100 grains, thus they were tolerant to alkalinity stress. Reduction in crop yield related to salt stress has been reported for rice (Mahmood *et al.*, 2009). According to Munns (2002), salt stress decreases growth in most plants and these plants are not able to produce their maximum biomass. This stress at grain filling stage can cause a decrease in the photosynthates mobilization to grains and thereby decrease grain weight (Sadeghipour, 2008).

The significant and negative association of spikelet fertility percent, grain weight indicated that vigor (SES) could be used to predict the salt tolerance at seedling stage and a precursor for salt tolerance at reproductive stage for the spikelet fertility percent, the weight of 100 grains and the grain weight. Studies have reported similar results on spikelet fertility and yield of rice under salt conditions (Zeng *et al.*, 2002).

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Based on Na^+ and K^+ content and the Na^+/K^+ ratio in flag leaves associated with the vigor, the spikelet fertility percent, the weight of 100 grains and the total grain weight, the morphological and physiological analysis relationships between different rice accessions revealed four accessions (Sahel 210, Damodar India (IRGC 17038), CSR 10 and CSR11) in the same group as the tolerant check (Pokkali).

Twenty six (26) rice genotypes were fingerprinted with 10 SSR markers and all markers were polymorphic. The average number of allele was comparable to the result of Pervaiz *et al.* (2009) using different Asian rice germplasm and observed an average of 4.5 alleles per locus (range of 2 to 13). Similarly, the average number of alleles per locus of this study was higher than that reported earlier by Islam *et al.* (2008), who reported an average of 5 alleles per locus using different rice germplasm of Bangladesh. These differences in alleles per locus might be due to the genotypes used and selection of SSR markers. Markers that have the ability to detect high number of discernible alleles are the suitable markers for molecular characterization and genetic diversity analysis (Islam *et al.*, 2008). The result of this study suggests that these markers could be used as tools to assess the genetic diversity of rice germplasm of various traits linked to alkalinity.

The size differences between the smallest and the largest alleles for a given SSR locus varied from 3 to 48 bp. Maximum variation in allele size was observed for RM markers with CTT repeat motifs (RM493, 48 bp), AG repeat motifs (RM551, 46 bp); TC repeat motifs (RM572, 34 bp); CT repeat motifs (RM250, 20 bp; RM208, 14 bp). The results are comparable to published SSR diversity data in rice, which have reported high variation in allelic size for markers with GA, AT, ATT and CTT repeat motifs (Islam *et al.*, 2012). SSR loci with greater size difference between the alleles would be relatively better for diversity analysis.

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Polymorphic information content (PIC) value ranged from 0.47 to 0.90 with an average of 0.74. The PIC values observed in this analysis were consisted with the previous report involved in microsatellite marker analysis in rice: Islam *et al*. (2008) reported a PIC value of 0.55 using 21 rice genotypes, Joshi and Behrea (2006) reported 0.54 using 38 rice genotypes, and Pervaiz *et al.* (2009) reported 0.60 using 35 rice genotypes. PIC value of this study was higher than the earlier reported by Xu *et al.* (2004); Brondani *et al.* (2006) and Giarrocco *et al.* (2005) observed an average PIC value of 0.66, 0.73 and 0.69 for the world collection, traditional Brazilian rice varieties and Argentine rice germplasm, respectively. Findings of these PIC value might be due to inclusion of more diverse set of rice germplasm. The PIC value of a marker reflects marker allele diversity and frequency among the cultivars. Higher the PIC value of a marker indicates higher probability of detecting the number of alleles among cultivars. RM208 had the highest PIC value (0.90), followed by RM551 (0.88) and RM142 (0.47) following by RM537 (0.52) had the lowest PIC. Therefore, SSR marker, RM208 and RM551 was found to be superior for the analysis of genetic diversity in future. This result describes the usefulness of these markers for characterizing rice genotypes.

Among the 10 polymorphic markers used with morpho-physiological characters only RM208 was significantly (P-value = 0.01) associated with the potassium content in the flag leaves at 37% of correlation coefficient. Although there were no significant (P-value = 0.06 and 0.23) association between the markers RM208 and RM572 and the ratio Na^+/K^+, the expression percentage of these markers was, respectively, 22% and 14% of correlation coefficients (Table 4.10.). Analyzing for salt tolerance especially in leaves is appropriate because most Na^+ that is delivered to shoots remains there (Munns and Tester, 2008). Salt tolerance depends on Na^+ and K^+ uptake, ion balance compartimentalization (Yeo and Flowers, 1986).

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Although they were not significantly associated, the markers RM572 (17% of spikelet fertility), RM493 (13% of grain weight) and RM142 (13% of spikelet fertility, 14% of 100 grain weight and 11% of grain weight) were good indicators for salt tolerance rice identification.

Alignment of rice genotypes in closely associated clusters was related to tolerance level with morpho-physiological characters. It was evident that genotypes with higher levels of salt tolerance had lower Na^+ /K^+ ratio, higher level of spikelet fertility percent and good weight of 100 and total grain weights and clustered into the same group as Pokkali; the tolerant check (Figure 4.3.). Some of the accessions from IRRI and AfricaRice showed tolerance and the rest of them were sensitive or moderately tolerant in alkaline conditions. This may be due to the source and genetic backgrounds of the materials. Adaptation of rice to salt soils is different among genotypes with diverse genetic backgrounds (Zeng *et al.*, 2004). The rice accessions at the extreme clusters can be used in breeding program to develop new tolerant genotypes to alkaline soils.

4.5 Conclusion

The morphological and physiological analysis relationships between different rice accessions revealed four accessions (Sahel 210, Damodar India (IRGC 17038), CSR 10 and CSR11) in the same group of the tolerant check (Pokkali).

Among the 10 polymorphic markers used with morpho-physiological characters, only RM208 was significantly (P-value = 0.01) associated with the potassium content in the flag leaves at 37% of correlation coefficient. The markers RM572 (17% of spikelet fertility), RM493 (13% of grain weight) and RM142 (13% of spikelet fertility, 14% of 100 grain weight and 11% of grain weight) were detected as good indicators for alkalinity tolerance rice identification.

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Phenotypic and genotypic association for alkaline tolerance identification revealed that genotypes with higher level of salt tolerance have lower Na^+ /K^+ ratio, higher level of spikelet fertility percent and good weight of 100 and total grain weights and clustering into the same group as Pokkali; the tolerant check. The accessions clustering in the same group as Pokkali were Sahel 210, Damodar India (IRGC 17038), CSR 10 and CSR11.

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CHAPTER V

5. EVALUATION OF F_2 RICE POPULATIONS FOR ALKALINITY TOLERANCE AT SEEDLING STAGE

5.1 Introduction

The quantification of alkalinity tolerance poses serious difficulties. In the field, the stress may be experimentally uncontrollable because of climatic effects upon rainfall, soil preparation per seed bed and water tables. Field heterogeneity for salinity or alkalinity is notoriously high (Condom, 2000; Marlet *et al.*, 1998) and is likely to confound any planting plan designed for field experiments. Salt uptake and sensitivity is modulated by environmental conditions that may affect each variety differently (Sophia, 2010). Any parameter that affects the transpiration rate (such as light intensity, temperature and humidity) can dramatically change a plant's susceptibility to alkalinity. Abiotic stress tolerance, especially alkalinity stress is complex because of variation in sensitivity at various stages in the crop life cycle. Rice is comparatively tolerant to salt stress during germination, active tillering (vegetative growth), and during the later stages of maturity. It is most sensitive during seedling establishment and reproductive stages (Lafitte *et al.*, 2004; Singh *et al.*, 2009). Screening of rice germplasm at seedling stage is readily acceptable as it is based on a simple criterion of selection; whereas rapid screening becomes difficult at vegetative and reproductive stages (Gregorio, 1997; Gregorio *et al.*, 1997; Titov *et al.*, 2009). Screening under controlled condition has the benefit of reduced environment effects and the hydroponic system is free from complex variations associated with soil related stress factors (Titov *et al.*, 2009). The conventional methods of plant selection for salt tolerance are not easy because of the large effects of the environment and low narrow sense heritability of salt tolerance (Gregorio, 1997; Titov *et al.*, 2009). This hinders the development of an accurate, rapid

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and reliable screening technique. Screening at an early growth stage (2 to 4 week old seedlings) is more convenient than at flowering as it is quick, seedlings take up less space, tolerant seedlings may be recovered for seed production and seedling tests are more efficient in terms of time and costs. Seedling screening offers the possibility of pre-selection of breeding lines and progeny, and cultivars before field evaluation.

The genotypic variability of rice genotypes at the germination and seedling stages at different levels of salinity were evaluated by (Maiti *et al.*, 2008; Singh *et al.*, 2009) and found a considerable amount of genotypic variability under control and saline conditions at the seedling stage with respect to the variables shoot height and root length. The high heritability observed for these variables offers good scope for genetic improvement for salinity tolerance both at the germination and seedling stages. The present study was focused in alkalinity stress instead of salinity stress to evaluate the F_2 progenies of different cross combinations of which the objectives were to:

- produce F_1 and F_2 seeds;
- validate the hydroponic alkaline solution (high pH) protocol used to measure salt tolerance;
- evaluate phenotypic performance of F_2 rice populations for alkalinity tolerance at seedling stage.

5.2 Materials and Methods

5.2.1 Parental lines

Pedigree, origin, seed sources, type of genotype, traits selected for breeding program and salt tolerance of the selected accessions and varieties are shown in Table 5.1.

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Table 5.1: Pedigree characterization of rice genotypes tolerant and sensitive to salt used in the crosses for progeny salt tolerance in 2011

Genotype	Pedigree	Origin	Source of seed	Traits selected for breeding program	Salt tolerance
Damodar India (IRGC 17038)	TKM6/IR8	India	IRRI	Seedling vigor, low Na^+/K^+	Tolerant
Sahel 210		Cuba	Africa Rice	Seedling vigor, low Na^+/K^+ ratio and good spikelet fertility	Tolerant
CSR 10	M40-431-24-114/Jaya	India	IRRI	Seedling vigor, low Na^+/K^+ ratio and good spikelet fertility	Tolerant
CSR 11	M40-431-24-114/BAS.370	India	IRRI	Seedling vigor, low Na^+/K^+ ratio and good spikelet fertility	Tolerant
BG 90-2	Peta*3/TNI/Remadja	Sri Lanka	IER	Yield and swelling after cooking	Sensible
Kogoni 91-1	Gambiaka Kokum / IR 36	Mali	IER	Taste, aroma and grain quality	Sensible
Adny 11		Sierra Leone	IER	Yield and grain quality	Sensible

Several studies on the genetics of salt tolerance have been carried out. Gregorio and Senadhira (1993) reported the genetics of salinity tolerance in varieties Nona Borka, Pokkali, and SR 26B in culture solution. The Na+/K+ ratio was used as a criterion for selecting parents with high general and specific combining abilities. Their study revealed reciprocal differences and suggested that tolerant plants should be used as females. Jones (1986) also studied the genetics of salt tolerance in two mangrove varieties, viz. Pokkali and Pa Merr 108A in Sierra leone. The study indicated additive genetic variation, maternal effects, and transgressive segregation in parents and the progenies. Additive and non-additive gene action governed sodicity tolerance (Geetha *et al.*, 2006; Sophia, 2010). Geetha *et al.* (2006) and Sophia (2010) also identified the best combiners for grain yield and a significant reciprocal variance indicated the influence of maternal inheritance. Marked environmental influences and genotypic effect for alkalinity tolerance has already been established. Many efforts have been made elsewhere to improve

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cultivated rice varieties for salt-tolerance and studies on the associated genetics. Such studies have never been done in varieties grown under Malian alkaline environments. Therefore, the present study was undertaken to evaluate the phenotypic performance of tolerant F_2 rice progenies at seedling stage under alkaline hydroponic solution. The tolerant genotypes were used as females and sensitive ones as males. Four salt tolerant accessions were crossed with three sensitive varieties (Table 5.2).

Table 5.2: Parental accessions and varieties used in different cross combinations

Cross combinations
Sahel 210 x Kogoni 91-1
Sahel 210 x BG 90-2
Sahel 210 x Adny 11
CSR10 x BG 90-2
Damodar India x BG 90-2
Damodar India x Kogoni 91-1
Damodar India x Adny11
CSR11 x Kogoni 91-1
CSR11 x BG 90-2

5.2.2. Methodology

5.2.2.1. Production of F_1 and F_2 seeds

The crosses were made from July to December 2011. Seeds of all seven rice genotypes were sown in pots. Seeds were sown at 3 different dates with 7 days interval between successive sowings. Twenty one day old seedlings were transplanted into the hybridization block and crosses were made following the crossing procedures described by Jennings et *al.* (1979). Female parents were emasculated at an early blooming stage of the florets in the early morning. Florets on selected panicles that were expected to be fertilized on the following day were clipped of to a third to half of their original length, thus, the upper part of the lemma and palea in a slanting position just above the anthers. All the six anthers of each floret were sucked out

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carefully with fine pointed pipette attached to a vacuum pump (vacuum emasculator) without any damage to the stigma. The emasculated panicles were covered with glassine bags to prevent any out-crossing. The following morning before pollination, emasculated florets were re-examined carefully to ensure that no anthers were left in any floret. For pollination, the panicles of the respective male parents were collected in the morning before pollen shedding. The panicles were kept in a small container with little water to keep the panicles fresh. When pollen shedding began, the male panicles were shaken by hand over the female emasculated panicles and the date of pollination was recorded. Naked F_1 seeds were harvested about 25 – 30 days after pollination without glumes. Seeds from each female were harvested and bagged separately and parents of the cross were recorded. The number of matured seeds harvested per cross combination was recorded.

The F_1 seeds from each cross combination were sown to generate the F_2 seeds in non alkaline field (Appendix 5.1**)**. During F_2 generation, the parents and F_1 of each cross combination were generated in the same field. Each F_1 and their parents were sown in three lines. The parent female was on the left, the male on the right and F_1 in the middle. The distance of each line was 2 meters with 10 pots per parent, whiles the pot number of F_1 was varied depending on the seed number harvested. The distance between each pot was 0.2 m and between lines 0.5 m. A total of 9 F_1 were used. The F_1 plants that did not flower 160 days after sowing were covered with black box from 4:00 PM to 9:00 AM to stimulate flowering. The cover time varied from cross to cross.

5.2.2.2 Protocol optimization for alkalinity tolerance in rice

5.2.2.2.1 Plant material and salt treatments

The experiment was conducted in a screenhouse at the Sahel Regional Station of Africa Rice in Saint Louis Senegal between 15 and 30 November 2012 to validate the protocol developed by

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Singh *et al.* (2002) (Table 5.3.). Two international tolerant (FL478) and sensitive (IR29) checks were used for this study. Seeds of the two cultivars were surface sterilized with 0.1% bleach for 10 min and then washed several times with sterile distilled water. One hundred and fifty seeds of each genotype were sown in Petri dishes containing filter paper (Plate 5.1). All the Petri dishes were placed in steam room at 45ºC for 72 hours to facilitate pre germination, because of the low temperature (min and max) and relative humidity during this period (Figure 5.1.). Germinated seeds were transferred onto Styrofoam seedling float seedling that fit in a square plastic tray of 12 liters capacity size containing distilled water for 2 days. This was to allow the repair of any damage to the radicle resulting from the transfer of young seedling onto the Styrofoam.

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Plate 5.1: Germinated seeds in Petri dish containing filter paper at Saint Louis (Senegal) in 2012

The Styrofoam seedling float is a fabrication of a rectangular Styrofoam of size 28 x 32 x 1.25 cm having 100 holes (10 x 10) with nylon net bottom and 2.5 cm thick frame pasted on top (Appendix 5.2). Each Styrofoam seedling float received two rice genotypes evenly distributed in 50 holes each. There were three replicates for each treatment. After 2 days periods, the distilled water was replaced with a culture solution developed by Singh *et al.* (2002). The experimental treatments were applied by varying the pH of the solution by using the Tris (sigma) buffer. Tris treatment with 2.5 mM and 4.5 mM were used for pH 8.6 and pH 9.0 respectively. The hydroponic solutions were changed every 4 to 5 days because of the degradation of the Tris within time (Figure 5.1).

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Table 5.3: Modified Singh *et al.* 2002 culture solution

Elements	Salts	Modified Yoshida (Singh *et al.* 2002)
		Element per liter
Macronutrients (mg/l)		Culture solution
N	NaH_4NO_3	39.99
P	KH_2PO_4	8.26
K	K_2SO_4	39.1
Ca	$CaCl_2 2H_2O$	27.36
Mg	$MgSO_4\ 7H_2O$	39.44
Na	Na_2SO_4	8.96
Micronutrient (µM)		Foliar application
Mn	$MnCl_2\ 4H_2O$	9.4
Mo	$(NH_4)6Mo_7O_{24}$ $4H_2O$	0.075
Zn	$ZnSO_4\ 7H_2O$	0.15
Zn	$ZnSO_4$	0.01
B	H_3BO_3	18.8
Cu	$CuSO_4\ 5H_2O$	0.15
Fe	$FeSO_4$	0.02
Fe (g/l) (Culture solution)	FeEDDHA	12.79

5.2.2.2.2 Methodology, data collection and statistical analysis

A split plot design with three replications was used. Two Tris treatments and normal treatment were main-plots and genotypes were sub-plots. Tris treatments of 2.5 mM and 4.5 mM, respectively, at pH 8.6 and pH 9.0 were used in comparison to pH 5.0 (normal for rice growing). The stresses treatments (pH 8.6 and pH 9.0) were applied once per week during two weeks.

Data collection was done 14 days after the maximum desired stress level was achieved. Scoring for salt tolerance on the basis of salt injury at seedling stage was done following the IRRI (2002)

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Standard Evaluation System. The length of shoot and root were recorded two weeks after salt treatments.

All the data were tested for normality and the parameters were submitted for analysis of variance (ANOVA) with two levels of classification (alkalinity and genotype). They were subject to analysis of variance using the Genstat software release edition 4. Analysis of variance (ANOVA) was performed to determine statistically significant differences among genotypes, alkalinity levels, and their interactions. Simple linear correlation coefficients were calculated to establish relationships between physiological attributes of genotypes under alkalinity.

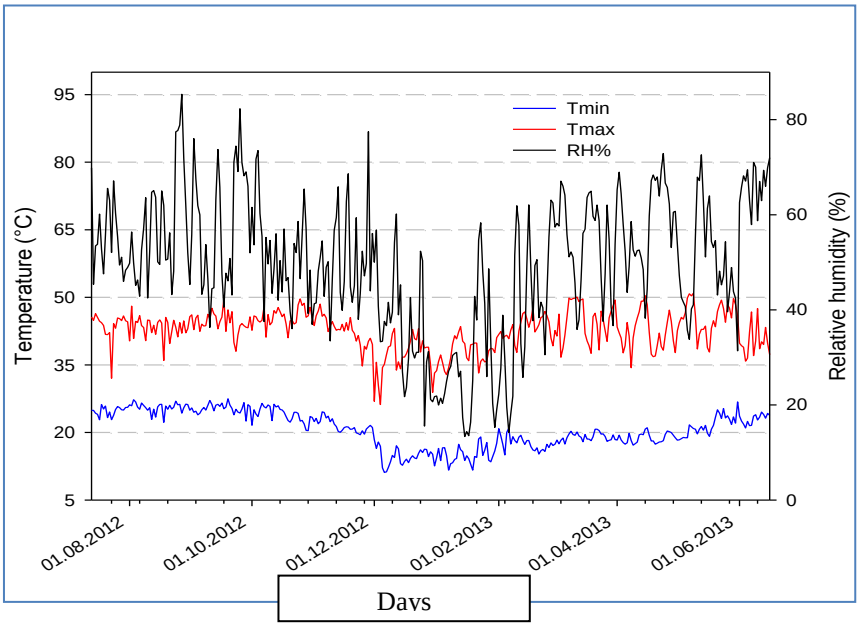

Figure 5.1: variation of temperature (min and max) and relative humidity (%) between 01/08/12 and 01/06/2013 in the screenhouse of AfricaRice Saint Louis (Senegal).

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5.2.2.3 Screening for alkalinity tolerance in F_2 rice populations at seedling stage

5.2.2.3.1 Plant material and salt treatments

The experiment was conducted in a screen house at the Sahel regional station of Africa Rice in Saint Louis Senegal, between December 2012 and 03 January 2013. One thousand-eight hundred (1,800) F_2 rice populations were evaluated using the modified Yoshida solution protocol. Seeds obtained from the F_2 populations of different cross combinations between alkaline tolerant accessions and farmer preferred varieties considered as sensitive were screened. Seeds of each cross combination were surface sterilized with 0.1% bleach for 10 min and then washed several times with sterile distilled water. Two hundred (200) F_2 seed per cross combination were pre germinated in Petri dishes containing filter paper. Each cross combination was divided into four Petri dishes, in which 50 seeds per genotype were placed. All the Petri dishes were placed in steam room at 45ºC for 72 hours to facilitate the germination. Germinated seeds were transferred to Styrofoam seedling floats for screening in modified Singh *et al.* 2002 culture solution (Table 5.3) as described above. The Tris treatment with 4.5 mM was applied as stress treatment. The pH at 9.0 was maintained daily. To avoid biodegradation of the Tris the hydroponic solution was changed every 4 to 5 days.

5.2.2.3.2 Methodology, data collection and statistical analysis

Data collection was done 15 days after the maximum desired stress level was achieved. The desired stress level is the level of salt stress that can discriminate tolerant and sensitive rice genotypes. Scoring for salt tolerance on the basis of salt injury at seedling stage was done following the IRRI Standard Evaluation System (2002). The length of shoot and root in cm were recorded after 15 days of salt treatments. Mean values and frequencies of parents, checks and F_2 progenies expressed in percentages were calculated using excel software. The survival rate in

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percentage of F_2 progenies 25 days after transplantation (DAT) was calculated. The data were subject to the linear regression analysis across F_2 progenies using the Gensta software release edition 4.

5.3 Results

5.3.1 Production of F_1 and F_2 seeds

The number of F_1 seeds produced from the different cross combinations varied from 3 for the cross between Damodar India and Kogoni 91-1 to 108 for the cross between Damodar India and BG 90-2 (Table 5.4). Twenty seeds from those cross combinations with more than twenty recorded at F_1 generation were sown to produce F_2 progenies seed's. For those where the seed number recorded was less than 10 were all sown to produce F_2 seeds (Table 5.4).

Table 5.4: Number of F_1 and weight of F_2 seeds produced per cross

Cross	Number of F_1	Weight (g) of F_2
CSR11 x Kogoni 91-1	30	150
CSR11 x BG 90-2	54	228
Damodar India x Kogoni 91-1	3	70
Damodar India x Adny 11	5	89
Damodar India x BG 90-2	108	270
Sahel 210 x Kogoni 91-1	55	166
Sahel 210 x Adny 11	37	145
Sahel 210 X BG 90-2	6	95
CSR10 x BG 90-2	9	128

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5.3.2 Optimization for rapid alkalinity screening of *Oryza sativa* L. at seedling stage in Modified Yoshida's solution

5.3.2.1 SES score, shoot height and root length at three levels of pH

Mean squares from the ANOVA for SES (Standard Evaluation System) score, shoot height and root length of FL478 (Salt tolerant) and IR29 (salt sensitive) are presented in Table 5.5. The genotypes reacted differently to different levels of pH (5.0, 8.5 and 9.0) for SES scoring. Significant effects (P<0.001) for SES scoring and the root length were obtained with the two genotypes. For salt injury based on SES scoring, the interaction among the genotypes and pHs' was significant (P<0.001).

Table 5.5: Mean squares of treatments on salt injury (SES score), shoot and root length

Source of variation	d.f.	SES score	Shoot height	Root length
Genotypes	1	58.9389***	7.96	95.12***
pH	2	48.65***	4237.11	969.86
Genotypes x pH	2	17.3389***	41.82	1.38
Residual	4	0.683	44.03	11.88
Total	9			

***= P<0.001

5.3.2.2 Comparison of the effects of different pH levels on SES score

The effect of different pH levels on SES score of the two genotypes was significant (P<0.001) (Table 5.6a). The pH effect at 8.5 and 9.0 was more expressed in IR29 than FL478. At pH 9.0 the score of FL478 was 3.7 (tolerant according to SES score) whereas the one of IR29 was 5.9 (sensitive according to SES score). Thus at pH 9.0 it was possible to differentiate tolerance and sensitive genotype following the SES score developed by IRRI in 2002 (Figure 5.2). The overall mean for SES score for the two genotypes was 3.92 with 0.17%, 0.25% and 0.28% LSD at 5% for genotypes, pH levels and the interaction genotypes x pH levels respectively. The %CV

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observed was 3.7, 2.9 and 21.5 for genotypes, pH levels and the interaction genotypes x pH levels respectively.

Table 5.6a: pH effects on SES score

Genotypes	pH levels			Mean
	5.0	8.5	9.0	
FL478	3.0	3.3	3.7	3.34
IR29	3.0	4.6	5.9	4.49
Mean	3.0	3.95	4.8	3.92
P-value		0.001		
LSD (5%) Genotypes		0.17		
LSD (5%) pH		0.25		
LSD (5%) Genotypes x pH		0.28		
Genotypes (% CV)		3.7		
pH (% CV)		2.9		
Genotypes x pH (% CV)		21.5		

The effect of pH at different levels on shoot height was not significant (P>0.05) (Table 5.6b). The pH effect observed on shoot height was more is the pH level; more is the reduction of shoot height for both the two genotypes (Figure 5.2). However there was significant difference between genotypes for each pH level. At pH 9.0 shoot height of FL478 was higher than IR29. The overall mean for shoot height for the two genotypes was 22.28 with 1.08%, 16.01% and 15.97% LSD at 5% for genotypes, pH levels and the interaction genotypes x pH levels respectively. The CV% observed was 4.2, 31.7 and 14.7 for genotypes, pH levels and the interaction genotypes x pH levels respectively.

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Table 5.6b: pH effects on shoot height (cm)

Genotypes	pH levels			Mean
	5.0	8.5	9.0	
FL478	30.62	19.63	15.96	22.07
IR29	32.59	20.26	14.61	22.49
Mean	31.60	19.95	15.29	22.28
P-value		0.058		
LSD (5%) Genotypes		1.08		
LSD (5%) pH		16.01		
LSD (5%) Genotypes x pH		15.97		
Genotypes (CV%)		4.2		
pH (CV%)		31.7		
Genotypes x pH (CV%)		14.7		

The pH effect at different levels on root length (cm) was not significant (P>0.05) (Table 5.6c). Root length was more affected by the pH level with both two genotypes (tolerant and sensitive). However there was difference between genotypes for each pH level. At pH 9.0 root length of FL478 was lower than IR29. The overall mean for root length for the two genotypes was 9.85 cm with 0.38%, 7.28% and 7.27% LSD at 5% for genotypes, pH levels and the interaction genotypes x pH levels respectively. The CV observed was 3.3, 32.6 and 20.6 for genotypes, pH levels and the interaction genotypes x pH levels respectively.

Table 5.6c: pH effects on root length (cm)

Genotypes	pH levels			Mean
	5.0	8.5	9.0	
FL478	13.20	8.69	5.48	9.12
IR29	15.00	10.01	6.72	10.58
Mean	14.10	9.35	6.10	9.85
P-value		0.337		
LSD (5%) Genotypes		0.38		
LSD (5%) pH		7.28		
LSD (5%) Genotypes x pH		7.27		
% CV Genotypes		3.3		
% CV pH		32.6		
% CV Genotypes x pH		20.6		

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5.3.2.3 Correlation among traits across FL478 and IR29

A simple correlation analyses was carried out across FL478 (tolerant) and IR29 (sensitive) at seedling stage. The two varieties reacted differently to alkalinity stress. The salt injury (SES score) was highly significant (P< 0.001) and negatively associated with shoot height and root length. Root length was highly significantly (P<0.001) and positively associated with Shoot height (Table 5.7).

Table 5.7: Correlation among traits studied in rice entries (FL478 and IR29)

	Three levels of pH	
	SES score	Root length (cm)
Shoot height (cm)	-0.43 ***	0.93***
Root length (cm)	-034 ***	

*** = P<0.001

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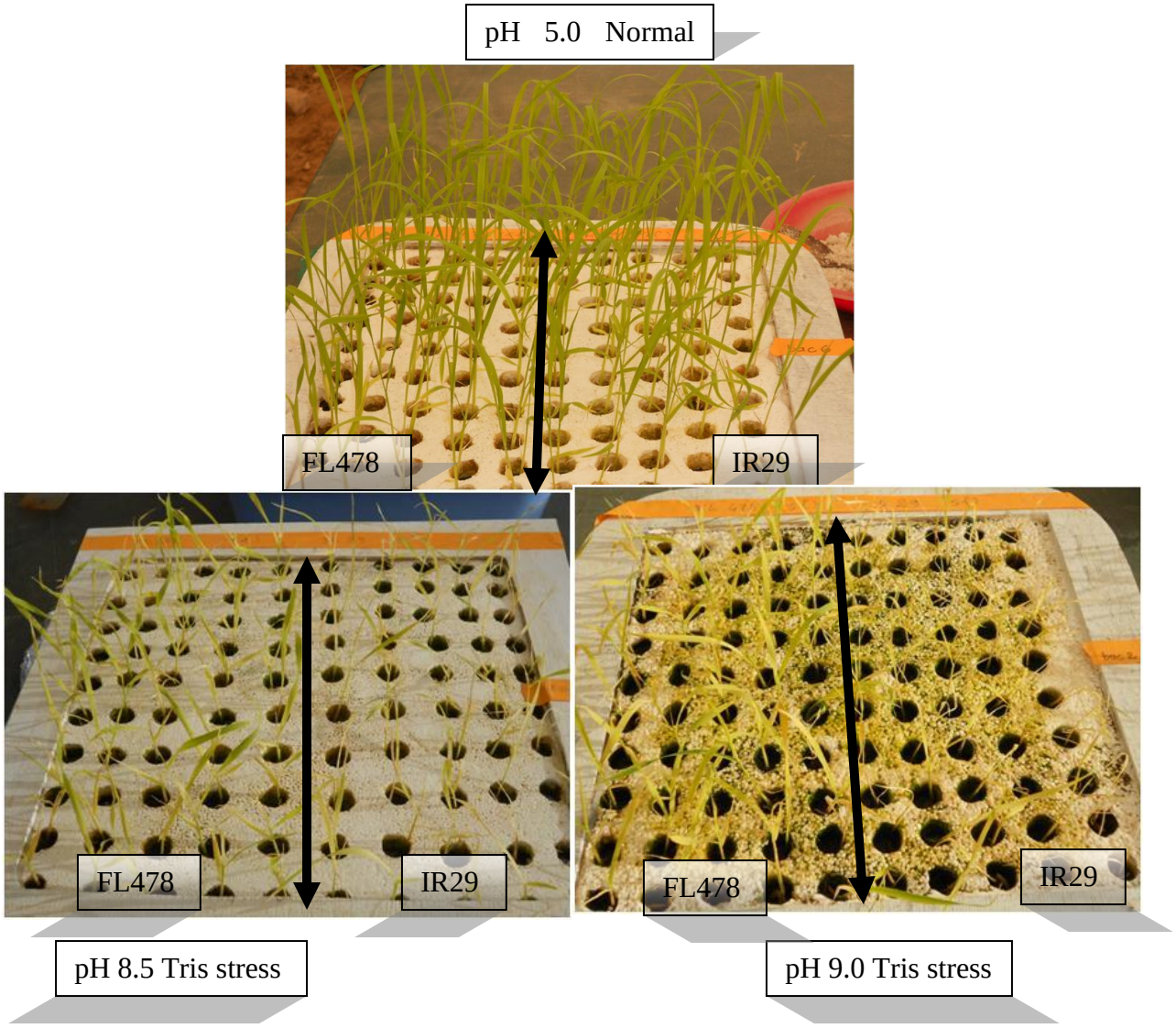

Figure 5.2: Plant injury symptoms of genotypes under alkaline and non-alkaline nutrient solution.

5.3.3 Screening for alkalinity tolerance in F_2 rice populations at seedlings stage

5.3.3.1 Alkalinity stress symptoms on parents and checks at seedling stage

Four tolerant accessions, three farmers preferred varieties and two checks (tolerant and susceptible) were screened under alkaline hydroponic solution. Pokkali (tolerant check), which is a traditional non-dwarf landrace, had the lowest SES score, the longest shoot and root length and

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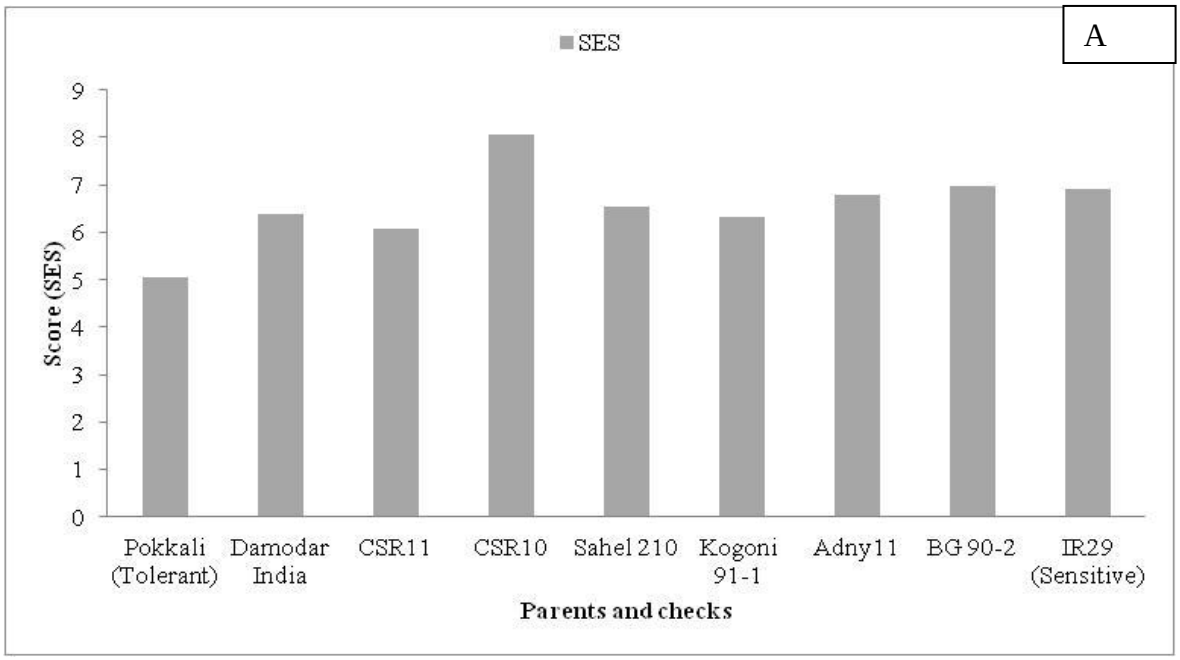
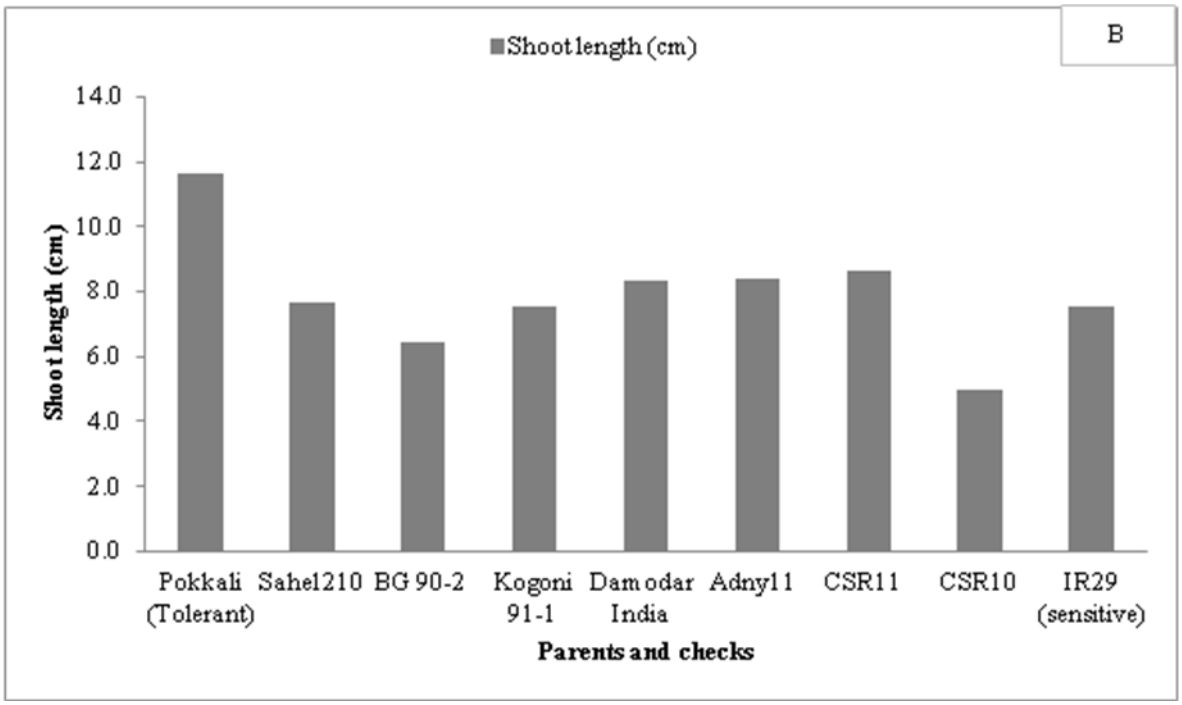
therefore was the most alkaline tolerant variety (Figure 5.3 A, B, C). CSR10 had the highest SES scores, the shortest shoots and roots hence the least salt tolerance. Damodar India (IRGC 17038), Sahel 210, CSR10 and CSR11 were identified as alkaline tolerant accessions under the degraded soil study in the previous chapter.





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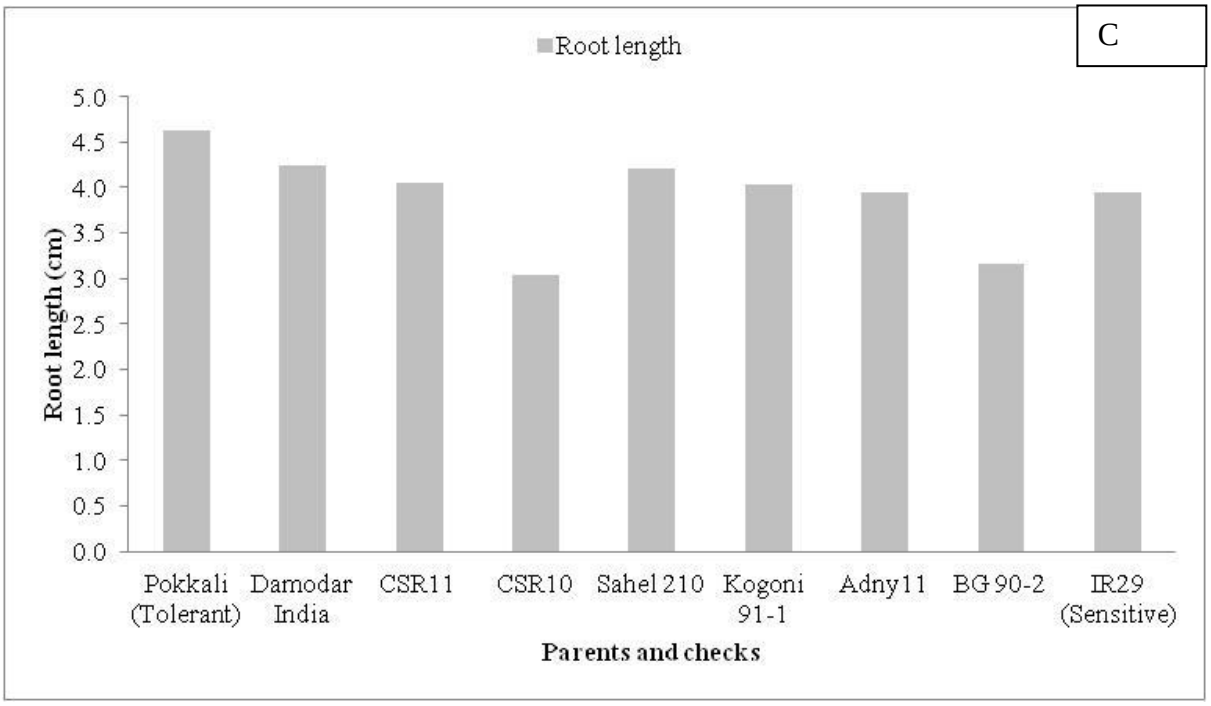

Figure 5.3: Alkalinity stress injuries on selected parents used in different cross combinations and checks (tolerant and sensible) 15 Days After Alkaline stress Application (DAASA). A: SES score, B: Shoot height (cm) and C: Root length (cm)

5.3.3.2 Frequency of phenotyped F_2 rice populations

Nine different cross combinations of F_2 rice populations were screened at fifteen Days After Alkalinity Stress Application (DAASA) (Table 5.8). Individual salt tolerant plants after 15 days of alkalinity treatment were phenotyped. The percent of F_2 rice populations phenotyped for each cross varied from 83.5% to 99.5% from the original individual populations of 200 each. Damodar India (IRGC 17038) x BG 90-2 and CSR11 x BG 90-2 had the highest percent of F_2 progeny phenotyped (99.5%), while the cross Damodar India (IRGC 17038) x Adny11 had the lowest (83.5%). Overall 1,652 F_2 progenies from the nine cross combinations were phenotyped in the screenhouse.

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Table 5.8: Tolerant F_2 populations from different cross-combinations 15 DAASA

No	Cross combinations	Total No of progenies	No of progenies phenotyped	Percent (%) of progenies phenotyped 15 DAASA
1	Sahel 210 x Kogoni 91-1	200	176	88
2	Sahel 210 x BG 90-2	200	175	87.5
3	Sahel 210 x Adny 11	200	171	85.5
4	CSR10 x BG 90-2	200	181	90.5
5	Damodar India x BG 90-2	200	199	99.5
6	Damodar India x Kogoni 91-1	200	198	99
7	Damodar India x Adny11	200	167	83.5
8	CSR11 x Kogoni 91-1	200	186	93
9	CSR11 x BG 90-2	200	199	99.5
Total		1,800	1,652	

DAASA: Days After Alkaline Stress Application

5.3.3.3 Alkalinity tolerance in F_2 populations means at the seedling stage using SES

Alkalinity tolerance in F_2 populations varied from one cross combination to another in SES scores (Fig.5.4). The F_2 populations of crosses Damodar India (IRGC 17038) x BG 90-2 and Damodar India (IRGC 17038) x Kogoni had the highest tolerance frequencies of 60.3% and 52.5%, respectively, whereas the F_2 population of Sahel 210 x Adny11 had the lowest tolerance frequency of 9.4%. Genotype with 1-5 SES score is tolerant and genotype with 6-9 SES score is sensitive. C10B = CSR10 x BG 90-2; SK = Sahel 210 x BG 90-2; DIB = Damodar India (IRGC 17038) x BG 90-2; DIK = Damodar India (IRGC 17038) x BG 90 – 2; DIA = Damodar India (IRGC 17038) x Adny11; C11K = CSR11 x Kogoni 91-1; C11B = CSR11 x BG 90-2 ; SA = Sahel 210 x Adny11 and SB = Sahel 210 x BG 90-2.

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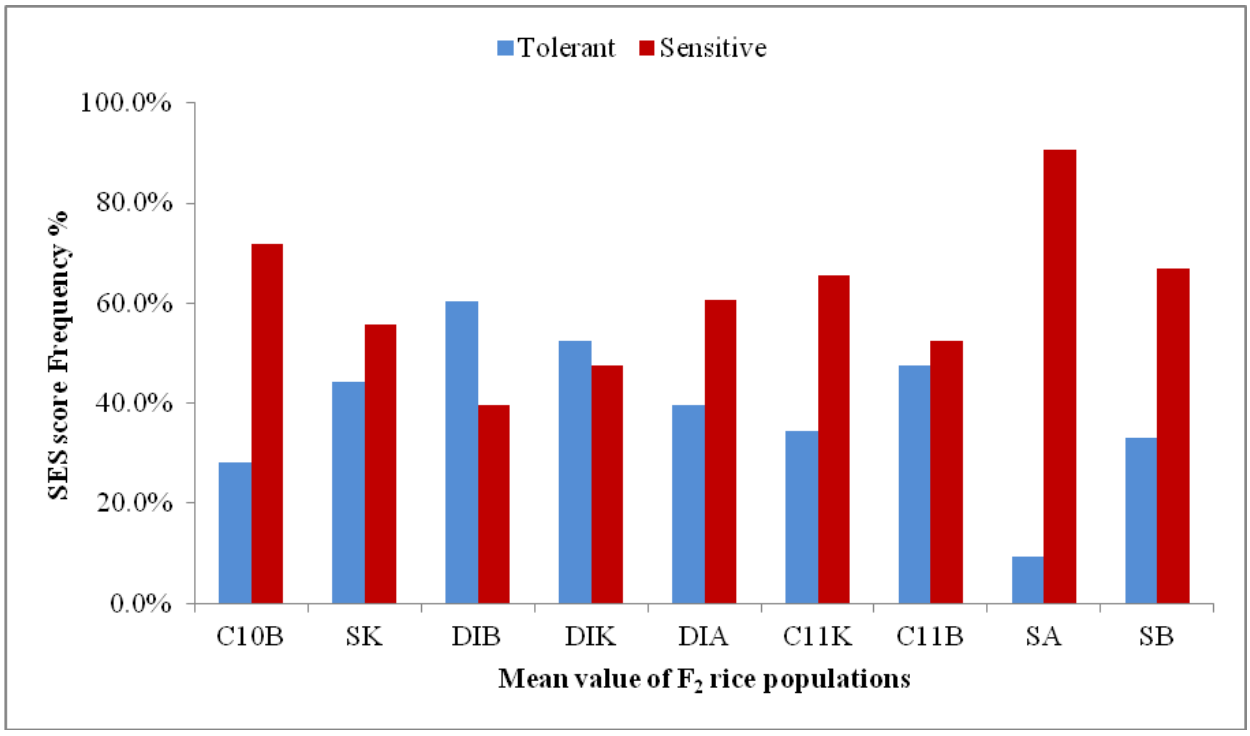

Tolerant = SES score of 1-5. Non tolerant = SES score of 1-9. C10B = CSR10 x BG 90-2; SK = Sahel 210 x BG 90-2; DIB = Damodar India x BG 90-2; DIK = Damodar India x BG 90 – 2; DIA = Damodar India x Adny11; C11K = CSR11 x Kogoni 91-1; C11B = CSR11 x BG 90-2; SA = Sahel 210 x Adny11 and SB = Sahel 210 x BG 90-2.

Figure 5.4: Frequency of F_2 populations of different cross combinations to alkalinity stress in the greenhouse of AfriaRice at Saint Louis (Senegal) in 2012

5.3.3.4 Alkalinity stress effects on shoot and root length of F_2 populations

The means of shoot and root length were calculated for both tolerant and sensitive F_2 populations means among the different cross combinations. Shoot and root length means varied from one F_2 population to the other (Fig 5.5A and 5.5B). The highest Shoot heights were recorded among tolerant F_2 populations for all cross combinations. In a similar manner mean root length of tolerant F_2 populations were higher among the different cross combinations compared to the non tolerant population except for the cross combinations Damodar India (IRGC 17038) x BG 90-2, Damodar India (IRGC 17038) x Kogoni 91-1, Sahel 210 x Adny11 and Sahel 210 x BG 90-2. Thus, alkalinity stress was observed to be more severe in non-tolerant F_2 populations than tolerant F_2 populations.

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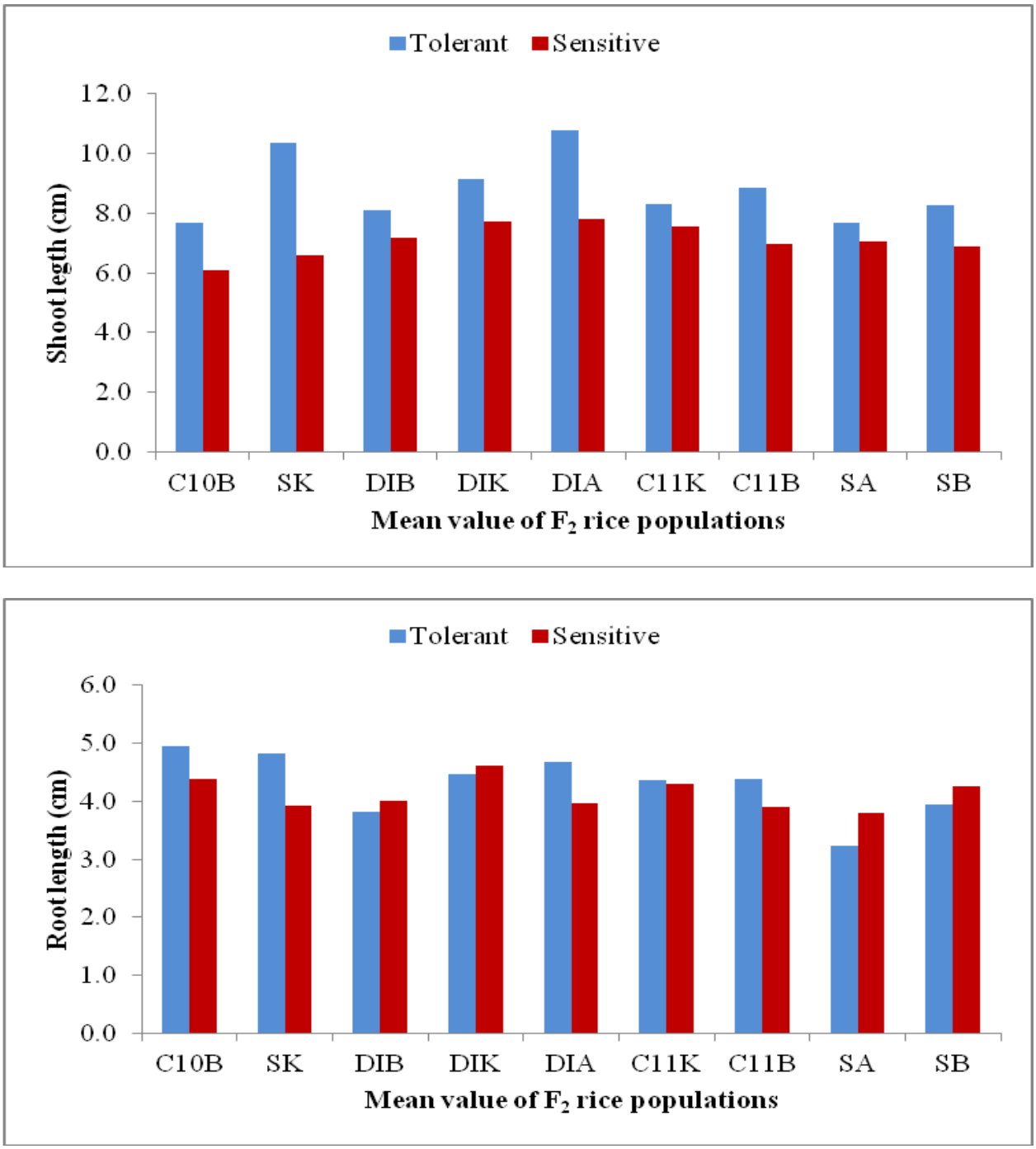

Tolerant = SES score of 1-5. Non tolerant = SES score of 1-9. C10B = CSR10 x BG 90-2; SK = Sahel 210 x BG 90-2; DIB = Damodar India x BG 90-2; DIK = Damodar India x BG 90 – 2; DIA = Damodar India x Adny11; C11K = CSR11 x Kogoni 91-1; C11B = CSR11 x BG 90-2; SA = Sahel 210 x Adny11 and SB = Sahel 210 x BG 90-2.

Figure 5.5: Tolerant and non tolerant F_2 population means of different cross combination to alkalinity stress in the greenhouse of AfriaRice at Saint Louis (Senegal) in 2012. A: alkalinity stress effect on Shoot height. B: alkalinity stress effect on root length.

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5.3.3.5 Correlation among traits within F_2 progenies per population

A simple correlation analysis of salt injury (SES scoring), shoot and root length was done for progeny of nine different cross combinations under alkaline hydroponic nutrient solutions (Table 5.9). The F_2 progenies reacted differently to alkalinity stress.

Within the F_2 progenies of CSR10 x BG 90-2, the SES scoring was significant ($P<0.001$) and negatively correlated with shoot height. Root length was significant ($P<0.01$) and positively correlated with shoot height.

For F_2 progenies of Sahel 210 x Kogoni 91-1 and Damodar India (IRGC 17038) x BG 90-2 the SES score was significantly ($P<0.001$) and negatively correlated with shoot height and root length. Shoot height was significantly ($P<0.001$) and positively correlated with root length.

Across the F_2 progenies of Damodar India (IRGC 17038) x Kogoni 91-1 and Damodar India (17038) x BG 90-2, shoot height was significantly ($P< 0.001$) and negatively correlated with SES score.

The SES score of CSR11 x BG 90-2 and Sahel 210 x BG 90-2 F_2 progenies was significantly ($P<0.001$) and negatively correlated with shoot height. Shoot height was significantly ($P<0.001$) and positively correlated with root length.

Root length was significantly ($P<0.01$) and positively correlated with shoot height for the F_2 population of CSR11 x Kogoni 91-1, while SES score was significantly ($P<0.05$) and negatively correlated with shoot height.

With the F_2 population of Sahel 210 x Adny11; shoot height was significantly and positively correlated with root length ($P<0.001$) and negatively correlated with SES score ($P<0.01$). SES score was significantly ($P<0.05$) and negatively correlated with root length.

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Table 5.9: Correlation between SES score and shoot height growth and root length

Cross combination at F_2 generation	Correlation	Root length	SES score
CSR10 x BG 90-2	SES score	-0.14	
	Shoot height	0.22**	-0.50***
Sahel 210 x Kogoni 91-1	SES score	-0.40***	
	Shoot height	0.36***	-0.72***
Damodar India (IRGC 17038) x BG 90-2	SES score	0.02	
	Shoot height	0.13	-0.35***
Damodar India (IRGC 17038) x Kogoni 91-1	SES score	0.06	
	Shoot height	0.02	-0.45***
Damodar India (IRGC 17038) x Adny11	SES score	-0.29***	
	Shoot height	0.30***	-0.56***
CSR11 x Kogoni 91-1	SES score	-0.07	
	Shoot height	0.20**	-0.19*
CSR11 x BG 90-2	SES score	-0.12	
	Shoot height	0.37***	-0.31***
Sahel 210 x Adny11	SES score	-0.16*	
	Shoot height	0.36***	-0.23**
Sahel 210 x BG 90-2	SES score	-0.04	
	Shoot height	0.30***	-0.51***

*, ** and *** significant at 0.05; 0.01 and 0.001 probability level respectively

5.3.3.6 Survival rate twenty five (25) days after transplantation (DAT)

Two hundred F_2 progeny from all nine different crosses were evaluated for survival twenty days after transplanting (Table 5.10). Survival rate varied from zero 0% to 3.5% for Sahel 210 x Adny11 and Damodar India (IRGC 17038). The cross between Sahel 210 x Kogoni 91-1 produced the highest percentage survival (4.0%) whiles the cross between Sahel 210 x Adny 11 produced no F_2 survivors. Overall, 34 F_2 lines from eight cross combinations were recovered and raised in pots. These plants in pots were arranged in the screenhouse of Africa Rice Centre Saint Louis (Senegal) for agronomical characterization and seed production. The agronomic characterization data are represented in Appendix 5.3.

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Table 5.10: Survival of F_2 progenies from different cross-combinations after 25 DAT

No	Cross combinations	No of progenies phenotyped	No of progenies that survived 25 DAT	Percent (%) survival after 25 DAT
1	Sahel 210 x Kogoni 91-1	176	7	4.0
2	Sahel 210 x BG 90-2	175	3	1.7
3	Sahel 210 x Adny 11	171	0	0.0
4	CSR10 x BG 90-2	181	4	2.2
5	Damodar India x BG 90-2	199	5	2.5
6	Damodar India x Kogoni 91-1	198	5	2.5
7	Damodar India x Adny11	167	6	3.6
8	CSR11 x Kogoni 91-1	186	3	1.6
9	CSR11 x BG 90-2	199	1	0.5
Total		1,652	34	

NB: Survival = lines with Standard Evaluation System (SES) score of 1-5.

5.4 Discussion

Sodicity or alkalinity is a significant problem for rice production in Mali and yet is a difficult condition to mimic in the laboratory. The addition of bicarbonate ions added to the culture solution commonly used for growing rice (Yoshida *et al.*, 1972) did not result in a stable pH. Consequently, the buffer (Tris) was used to adjust the pH. This buffer was not itself toxic to plants at neutral pH, but at high pH it can precipitate the micronutrients and make their uptake by rice root difficult. To avoid this constraint, these micronutrients were supplied in the form of foliar application on the leaves of genotypes. Thus the deleterious effects on the plants due to high pH (Tris concentration) were not due to the non-availability of micronutrients but to the macronutrients such as nitrogen, potassium, calcium and magnesium. The high pH leads to low micronutrient availability and decreases the availability of macronutrients such as potassium, calcium, magnesium and phosphorus (Tesfai *et al.*, 2002). The accumulation of sodium in rice from the roots to the shoots could result in direct toxicity and may lead to plant injury or reduced

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growth and eventually death in more sensitive plants (Sophia, 2010; Tesfai *et al.*, 2002). The genotypes reacted differently to the different pH levels. Salt tolerant seedlings were distinct from the sensitive seedlings grown in alkalinized condition. Seedlings grown in alkalinized condition showed different visual symptoms to that of salt injury. The symptoms were prominent on the first and second leaves and were characterized by leaf rolling, formation of new leaves, yellowish and whitish leaf tips, drying of leaves and also reduction in root growth, stunted shoot growth with thickened stems leading to a complete cessation of growth and dying of seedlings. There was a highly significant difference between genotypes FL478 (Salt tolerant) and IR29 (salt sensitive), pH levels (5.0, 8.5 and 9) and the interaction genotypes and pH levels for SES scoring. For root length, significant difference was observed between FL478 and IR29 and highly significant difference was seen between pH levels.

For Shoot height, highly significant differences were found between different levels of pH. The higher the pH level, the more severe the salt injury was expressed. For salt injury detection pH 9.0 which was harmful, can be useful for alkaline tolerance identification in rice at the seedling stage.

Salt stress in rice was associated with the increase of pH, with consequences as shoot height and root length reduction. The high pH leads to low micronutrient availability and decreases the availability of macronutrients such as calcium, magnesium and phosphorus (Tesfai *et al.*, 2002). The availability of sodium increases in culture solution and become in future toxic for shoot and root development (Singh *et al.*, 2002). The effect of high pH to leaves was attributed to the osmotic adjustment of roots in external high concentrations of salts (Lin *et al.*, 2004). In non-stress conditions, IR29 (sensitive) was taller than FL478 (Tolerant) while in alkaline condition shoot height and root length were significantly reduced showing high pH effect on seedling

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growth. Plant growth inhibition is a common response to salt stress and plant growth is one of the most important agricultural indices of salt stress tolerance (Abbas *et al.*, 2013; Al-Thabet *et al.*, 2004). However at shoot height level, the pH 9.0 differentiated sensitive and tolerant genotypes. At root length level sensitive genotype was longer than the tolerant one. It is reported that soil salinity suppresses shoot growth more than the root growth (Islam *et al.*, 2007; Ramoliya *et al.*, 2004). Thus pH 9.0 will be useful for shoot height and root length evaluation for alkalinity tolerance in rice at seedling stage to differentiate tolerant and sensitive genotypes.

Correlations between salt injury and shoot height and root length were significant and negatively associated. This implies that salt tolerant genotypes (with lower salt tolerance score) exhibited height shoots and long roots. Increasing Shoot height would allow more salt tolerance (Ramoliya *et al.*, 2004). Islam *et al.* (2007) showed the differences in Shoot height of rice varieties with different salinity levels. FL478 genotype at pH 9.0 showed higher shoots than IR29 and was salt tolerant.

In summary, the optimized protocol for alkaline hydroponic solution shows that the pH 9.0 induces more salt injuries in rice at seedling stage by reducing shoot height and root length; therefore differentiate tolerant and sensitive genotypes to alkalinity stress. This protocol was selected to evaluate the F_2 progenies from alkaline tolerant rice accessions crossed with alkaline sensitive farmer preferred rice varieties.

In Office du Niger zone, factors such as salt type, duration of exposure and concentration, temperature, humidity, solar radiation, soil and water pH contribute to soil degradation and make rice production difficult (Akbar, 1986). One of the constraints for rice production in alkalinized soil mentioned by farmers during participatory rural appraisal activities was the sensitivity of their cultivated rice at the seedling stage. Based on this, the F_2 rice populations from different

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cross combination were screened under alkaline hydroponic conditions for tolerant genotypes identification at seedling stage. These F_2 rice populations were from different crosses between tolerant accessions and famers preferred rice varieties.

According to IRRI (2006), F_2 progenies were divided into sensitive to alkalinity stress from 6 to 9 and tolerant from 1 to 5. The frequency of sensitive among the F_2 rice populations was higher than tolerant F_2 rice populations except for the cross, Damodar India x Adny 11. This might be occurred, because not all progenies obtained from crosses were screened under alkalinity stress which might also explain their non conformation to the Mendelian theory of segregation, thus the distortion observed among the progenies.

Defining salt tolerance, however, is quite difficult because of the complex nature of salt stress and the wide range of plant responses (Dionisio-Sese and Tobita, 2000). Salt tolerance of rice varies throughout its growing cycle. Rice is tolerant during germination but seedlings are sensitive until the age of at least 4 weeks.

At the pH 9; F_2 progenies shoot and root length in alkaline solution are affected. This result was confirmed by Jamil and Rha (2007) on transgenic lines of rice at the seedling stage. They have observed the reduction of shoot and root length in all the lines and found T-99 and T-112 to be tolerant as opposed to T-115 and T-121. Similar findings were reported by Anotai *et al.* (2009).

The correlation between SES score, root length and Shoot height was significant and negatively correlated. Shoot height was significant and positively correlated with root length in alkaline condition. This implies that alkaline tolerant F_2 rice populations (with lower SES score) exhibited higher plant height and root length. Titov *et al.* (2009) reported that seedling height of sensitive genotypes to salt stress showed higher percent reduction than tolerant genotypes. Peng *et al*. (1999) reported that increasing plant height would allow greater biomass production.

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Zhang *et al*. (2004) reported that increase of plant height was responsible for increase in biomass; so as to increase yield potential.

Damage to the rice plant resulting from salt stress after transplanting in normal condition, increases its sensitivity to salt stress (Dicko, 2005). The antiporter transports Na^+ from the cytoplasm into the vacuoles using the pH gradient (Fukuda *et al.*, 1998). Plants with high salt injury must maintain a higher K^+/Na^+ ratio in the cytoplasm than in the vacuoles and manage the osmotic balance of the cell with the environment by accumulating Na^+ in the vacuoles (Fukuda *et al.*, 1998). However in normal environment because of osmotic adjustment, the accumulated Na^+ moves from the vacuole to cytoplasm and could be one of the reasons for the reduction of surviving F_2 progenies of different cross combinations. Zeng and Shannon (2000) reported that the reductions in seedling survival rates and growth are major causes of the stand loss in salt-affected rice fields. The observed effects of salt stress on seedling growth are a function of both salt level and time of exposure. This indicated that tolerant seedlings were the one that could tolerate high levels of alkalinity for relatively longer time of exposure. The higher the pH and exposure time, the lesser the tolerance level of the survived seedlings (Singh *et al.*, 2002). Janaki Ramayya *et al.* (2009) reported similar results and showed that IR 28 had a high score of 8.9 whereas CSR 27 had a low score of 3.2 in the scale of 1-9 for alkalinity tolerance indicating that IR28 is sensitive and CSR27 tolerant. Sahel 210 x Kogoni 91-1 had more F_2 survived seedling twenty five (25 days) after transplanting whiles no progeny from Sahel 210 x Adny survived. The survived F_2 progenies can be developed from the crosses progenies of their set parents for useful segregants and alkaline tolerant homozygous lines associated with farmer preferred traits.

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5.5 Conclusion

Nine cross combinations were used and the number of seed produced varied from one cross to another. The F_1 of each cross was advanced to the F_2 generation in non alkaline conditions for seed production.

A protocol based on an alkaline hydroponic solution was adopted and validated. It was identified that pH 9.0 proved to be sufficiently high enough to separate tolerant and sensitive genotypes in alkaline culture solution at early seedling stage.

Based on this pH level, two hundred individuals (200) F_2 seeds from each cross combination were selected, pre-germinated and stressed a 14 days period in alkaline hydroponic solution. A total of 1,800 individual F_2 genotypes were screened out of which 1,652 were phenotyped. The frequency of F_2 sensitive populations was higher than tolerant progenies based on SES score. Shoot and root length of tolerant F_2 populations were longer than sensitive progenies. The number of surviving F_2 individuals at twenty five (25) days after transplantation was 34 and varied from one cross combination to another. The surviving F_2 progenies can provides useful segregants and alkaline tolerant homozygous lines associated with farmer preferred traits.

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CHAPTER VI

GENERAL DISCUSSION

Climatologists predict that Sub-Sahara Africa (SSA) countries will be severely affected by climate change in arid and semiarid regions where salt affected areas are still (Yu *et al.*, 2004) increasing. Accumulation of excess salts in the root zone due to high evaporation resulting in rice yield reduction and loss of soil productivity is a worldwide phenomenon.

The most serious salt affected soils are in the irrigated arid and semi-arid regions. In these regions, irrigation is essential to increase agricultural production to satisfy food requirements.

The release of improved salt tolerant varieties will directly benefit farmers by improving productivity in salt affected soils. Increased crop production will not only improve the economy and well being of the poor farmers/owners of land, but will increase the employment of local people during the cropping season in addition to improving the country's storehouses.

The objective of this study is to contribute to improved food security in Mali through breeding adaptable and alkaline stress tolerant rice varieties, which are appreciated by farmers. The study objectives are emphasized followed by the implications of the findings to breeding.

For PRA activities, the results indicated the enormity of the problem and emphasized the urgent need for developing varieties tolerant to salt at the seedling stage. Farmers are of the view feel that alkalinized soil was a major problem that needs serious attention, and the use of alkaline tolerant varieties would improve their rice production. Improving the alkaline tolerant cultivars by introgression of farmers' preferred rice varieties traits into the alkaline tolerant lines is the best and cheapest way to develop varieties that farmers prefer. Therefore, farmers will be motivated to plant a completely new variety. Farmer involvement in genotype acceptance, utilization of their knowledge and understanding their preferences for rice varieties may increase

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adoption of new varieties. However, this should be integrated with a strong official focus in breeding programmes to improve farmers' value and put into practice better improvement measures.

The performances of varieties Sahel 210, Damodar India (IRGC 17038), CSR 10 and CSR11 under alkaline stress environments indicated that these varieties are as morphologically and physiologically alkaline tolerant as Pokkali. However, differential performances of genotypes under alkaline environment were observed, whereby, genotypes such as Pokali performed extremely well under alkaline conditions in terms of SES score compared to the other alkali tolerant genotypes identified. In alkalinized soil, Pokkali was not good at reproductive stage compared to other accessions in the same cluster, indicating the influence of alkali environment on the reproductive performance of rice genotype, suggesting that genotypes selection should be done under specific growth stage in alkali stress condition.

All the genotypes screened for alkalinity tolerance and fingerprinted with SSR markers were different indicating the genetic diversity among the genotypes used in this study. The genetic diversity was identified by a total of 76 alleles indicating the genetic variability of different genotypes tested. The genetic diversity suggested that the use of molecular markers in conventional breeding would increase the efficiency of the varietal development of salt tolerance (Singh *et al.*, 2010; Singh *et al.*, 2011). Among the markers used for morphology and physiological characterization, RM208 was associated with the $\%K^+$ content in the flag leaves. The $\%K^+$ content in flag leaves is one of the major physiological traits providing salt tolerance in rice. Its presence in the flag leave can increase spikelet fertility and yield in alkaline conditions. Therefore, the use of molecular tools in conventional breeding can help breeder to identify salt tolerance or sensitivity at early generations. Phenotypic and genotypic association studies for

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alkaline tolerance in rice showed that Sahel 210, Damodar India (IRGC 17038), CSR 10 and CSR11 are similar to Pokkali (Tolerant check) and are tolerant to salt stress. These genotypes can be considered as useful donors for tolerance genes and can be useful in developing tolerance in locally preferred cultivars.

This study also evaluated the F_2 progenies from crosses of *Oryza sativa* L. *indica* rice varieties at seedling stage for alkalinity tolerance. Shoot and root lengths of tolerant F_2 progenies were greater than sensitive F_2 progenies indicating that tolerant genotypes can withstand alkaline solution for longer times than sensitive genotypes. The roots of tolerant F_2 progenies can extract water in soils with high salt concentrations and regulate high concentrations of salts within their shoots. The rate of survival for F_2 progenies after transplanting to normal soil conditions was greatly decreased indicating the influence of salt effects on the whole plant; therefore suggesting that genotypes selection should be done under specific salt conditions.

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CONCLUSIONS

It was evident that breeding for alkalinity tolerance has potential for increasing rice yields that match with "green revolution" in cereals. This work has revealed rice production constraints related to soil degradation through alkalinization. Farmer's knowledge about developed strategies to overcome soil alkalinization constraint was identified. Farmer's need about tolerant genotype development to alkalinity stress was also identified. The study confirmed the potential of alkaline tolerant accessions for irrigated rice system which may be, to a large extent, applicable to other salt prone rice irrigation systems in Mali and sub-Saharan Africa. It also confirmed that genetic variability, the foundation of breeding, exists in available rice accessions and varieties. Selections from these genotypes alone can lead to substantial yield gains. The potential of salt tolerant genotypes contribution to the well being of resource poor farmers in arid and semi arid environments remains high.

RECOMMENDATIONS

Alkaline tolerant rice varieties, particularly in marginal environments, could contribute to rice production increase in Office du Niger and specifically in irrigated ecologies of Mali where soil degradation through alkalinization constitutes the most important constraint. Specific area farmer contributions to variety development and adoption should be considered. Extension work should focus on adequate agricultural tools supply, land and irrigation water managements to mitigate alkalinity and salt problems. The surviving individuals F_2 progeny identified during the study need further investigations for QTLs identification linked to alkalinity tolerance and farmer preferred traits. More useful segregants with uniform alkaline tolerance and good agronomic characters should be developed from the surviving F_2 populations.

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APPENDICES

Appendix 3.1: Questionnaire Design for Individual Farmers

Breeding Rice for Alkalinity tolerance in Mali

1.0 **Demographic Data**

Name……..

Village……

Township……

Age……

Sex Male ( ) Female ( )

Marital Status Married ☐ Single ☐

2.0 **What are the** rice **varieties grown? Please tick all that apply.**

a) Kogoni 91-1 ( ) **b)** BG 90-2 **c)** Sambala Malo ( ) **d)** Nerica L2-IER ( )

e) Nerica L1-IER ( ) f) Nionoka ( ) g) Telimani ( ) h**)** Wassa ( )

i) Others (specify)……

3.0 **Where do farmers obtain their rice seeds from?**

	Sources of seed	**Varieties**	**Reason**
a.	Self or Farm saved seed		
b.	Market		
c.	Input shop		
d.	Farmer Associations		
e.	NGO's (Please specify)		
	……		
	……		

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4.0 **Which of the following rice production constraints do you face? Rank in order the level of importance in severity. Assign the number one (1) to the most important; number two (2) to the second most important in that order etc.**

	Rice production constraints	**Please tick all that apply**	**Ranking**
a.	Water management		
b.	Declining soil fertility		
c.	Diseases		
d.	Varietal qualities		
e.	Land shortage		
f.	Lack of access to improved seeds		
g.	Delay in land preparation		
i.	Weed control		
j.	High cost of seeds		
k.	High cost of fertilizers		
	Others (Please specify)		
l.	……..		
m.	……		
n.	……		

5.0 **What soil alkalinity problems do you have in your rice fields? Please tick all that apply.**

	Soil alkalinity problems in rice	**Tick all that apply**
a)	Soil degradation (white salts, black salts)	
b)	Continuous cropping (Double culture of rice)	
c)	Quantity of surface and ground water	
d)	Non drainage of irrigated water	
e)	Soil type (clay, sand, silt)	
f)	Fertilizer application	
g)	Nature of soil Ploughing	
Others (Please specify)		
h)		
i)		
j)		

5.1 **Give the top three (3) soil alkalinity problems which affect production severely?**
(1) ……
(2) ……..
(3) ……..
5.2 **What do you do on your farm to manage soil alkalinity problems? Tick all that apply.**
a) Setting in mud and flushing ( ) **b)** Dampen with water before ploughing ( )
c) Use of organic fertilizers ( ) d) Crop rotation ( ) e) Tolerant varieties ( )
f) If tolerant varieties please can you listed them? …….
g) Others (Please specify)……..…..

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6.0 **Problems due to alkalinity on the rice plant?**

	Parameters	**Impacts**	**Action/Approach to solve alkalinity problems**
1.	Germination		
2.	Establishment		
3.	Vigor		
4.	Tillering		
5.	Grain yield (grain filling)		
6.	Spikelet fertility		
7.	Grain quality: - **Size** - **Length** - **weight**		
	Others (please specify)		
8.			
9.			
10			

NB. This is a guide to some of the "***actions/approaches***" to solve alkalinity problems in the field which include:

a) Crop rotation **b)** Application of inorganic fertilizer **c)** Application of mineral fertilizer (gypsy)

d) Application of enough water e**)** Others (Please specify)

7.0 **Please indicate your preferences for selecting a rice variety for growing in your farm**

	Cowpea variety	**Indicate yes(Y) or No(N)**	**Criteria**
1)	Kogoni 91-1		
2)	BG 90-2		
3)	Nionoka		
4)	Sambala malo		
5)	Nerica L1-IER		
6)	Nerica L2-IER		
7)	Wassa		
8)	Telimani		
	Others (specify)		
9)			
10)			
11)			

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8.0 **What characteristics of rice do you prefer for its market value?**

a) Grain colour (specify)……..
b) Grain size (specify)……..….
c) Grain aroma (specify)……..……
d) Maturity (specify)…….…….
e) Yield ……..…….
f) Diseased/Not diseased ……
g) Pest infested/Not pest infested…….

9. **Indicate your rice preference for home consumption by rank order. 1 = the most preferred;**
2 = the second most preferred in that order.

	Rice preference (home consumption)	**Rank order**
a)	Easy to cook and easily stored	
b)	Grain swelling after cooking	
c)	Taste	
	Others	
d)		
e)		

10. **What traits of alkalinity should breeders incorporate in rice varieties to improve rice production?**

a)……..……
b)……
c)……...
d)……..……

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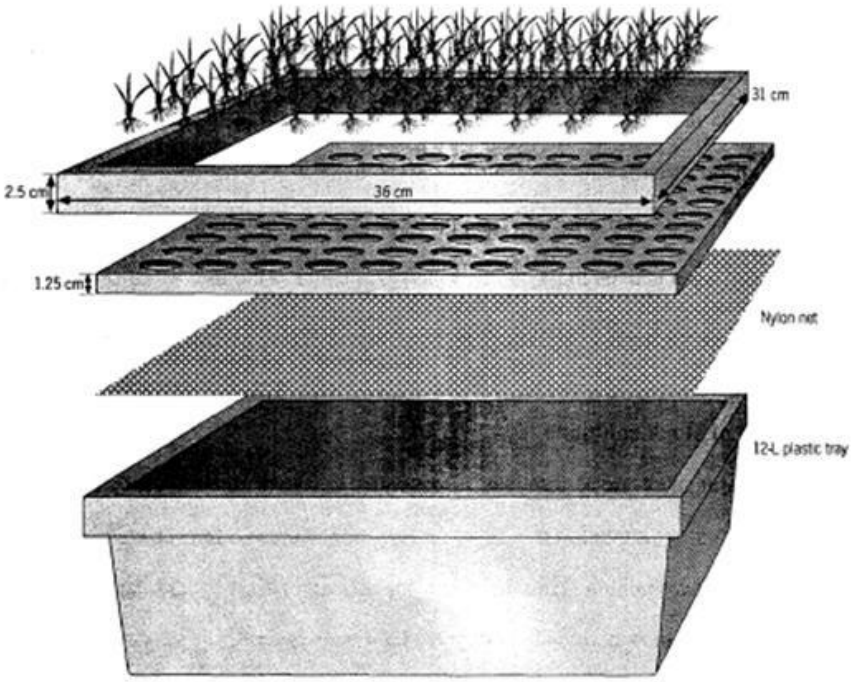
Appendix 5.1: F_1 Generation to F_2 in non alkaline field at Kogoni Station (Mali) in May 2012.



Appendix 5.2: Seedling float for screening at the seedling stage. Source: Gregorio (1997)

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Appendix 5.3: Agronomic characters of survived F_2 rice plants and their parents

Genotypes	Tiller Number	Sowing-start flowering (days)	Sowing- 50% flowering (days)	Height (cm)	Panicle number	Grain/panicle (Number)	Filled grains/panicle (Number)	Empties grains/panicle (Number)	Grain weight/plant (g)	Filled grain weight (g)
Parents										
Damodar India (IRGC 17038)	23	111	123	110	44	125	75	50	72.9	34.7
Kogoni 91-1	54	126	137	66	51	45	21	24	31.3	23.9
Sahel 210	33	126	137	79	36	112	43	69	44.5	36.8
CSR11	32	107	118	65	41	48	35	13	34.3	24.4
Adny 11	32	111	123	75	37	54	30	24	33.8	24.6
CSR10	60	100	111	59	43	82	42	40	25.3	18.7
BG 90-2	41	145	157	74	47	46	35	11	39.1	26.5
Progenies										
DIB 134	26	113	124	102	62	100	34	66	46.6	36
DIB 137	28	158	169	101	38	66	40	26	28.4	18.9
DIB 200	8	147	158	100	30	145	25	120	8.6	1.7
DIB 197	9	145	156	101	29	100	52	48	3.6	1.1
DIB 57	20	143	153	78	49	180	139	41	74.3	54.4
DIK 137	39	126	146	99	93	71	15	56	28.4	9.6
DIK 183	40	107	118	70	44	81	35	46	48.4	43.6
DIK 189	25	111	123	69	25	76	34	42	10.1	7.9
DIK 41	67	126	137	89	44	51	18	33	70.9	59.7
DIK 104	37	107	117	95	140	205	13	192	7.9	1.6
SK 195	43	146	157	72	50	69	55	14	20.1	15.2
SK 88	40	104	115	75	44	69	35	34	60.1	52.3
SK 35	47	114	126	67	46	110	43	67	22.7	18.1
SK 6	20	129	150	73	30	96	62	34	42.8	29.7

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Appendix 5.3 (Cont'd): Agronomic characters of survived F_2 rice plants in non alkaline soil

Genotypes	Tiller Number	Sowing-start flowering (days)	Sowing- 50% flowering (days)	Height (cm)	Panicle number	Grain/panicle (Number)	Filled grains/panicle (Number)	Empties grains/panicle (Number)	Grain weight/plant (g)	Filled grain weight (g)
SK 36	55	118	130	70	42	79	15	64	32.1	21.3
SK 39	39	112	124	69	23	65	38	27	23.8	6.7
SK 29	38	114	125	76	52	84	68	16	60.7	46.4
DIA 40	20	107	119	105	30	54	29	25	38.6	38.6
DIA 197	42	137	148	114	37	41	8	33	13.6	3.4
DIA 160	35	100	110	64	76	83	6	77	15.7	0.3
DIA 183	41	93	106	87	117	59	15	44	49.1	14.5
DIA 191	25	111	122	76	37	114	74	40	28.6	21.4
DIA 71	38	109	119	109	47	99	20	79	41.2	20.5
C11K 38	58	112	123	70	67	58	6	52	74.3	0.6
C11K 181	19	126	137	54	20	74	0	74	28.4	0
C11K 229	21	135	146	71	40	50	16	34	36.7	27.1
CSR11	32	107	118	65	41	48	35	13	34.3	24.4
C11B 147	77	157	168	50	52	69	34	35	9.4	3.1
C10B 197	27	145	157	67	50	72	30	42	20.7	17.2
C10B 189	33	113	124	84	33	73	49	24	44.5	14.8
C10B 182	30	113	124	62	29	30	19	11	22.9	19.9
C10B 181	33	113	124	68	70	48	22	26	20.5	15.1
SB 161	44	155	166	71	23	50	40	10	12.4	9.3
SB 164	35	155	166	63	24	124	66	58	11.4	6.4
SB 174	47	135	175	89	59	124	71	53	72.9	54.9
CSR11	32	107	118	65	41	48	35	13	34.3	24.4
CSR10	60	100	111	59	43	82	42	40	25.3	18.7