GENETIC CHARACTERISATION OF TOLERANCE TO FERROUS IRON TOXICITY IN WEST AFRICAN RICE CULTIVARS

By

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DECLARATION

I hereby declare that except for references to work 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

Iron toxicity is one of the main constraints of lowlands rice production in West Africa. The adoption of tolerant varieties constitutes a sustainable approach to address this constraint. The objectives of this study were to: (i) assess the lowland rice production constraints, farmers' preferred varietal traits and their perceptions of iron toxicity in the Casamance zone (Senegal) through a Participatory Rural Appraisal (PRA) approach; (ii) assess the genetic diversity among rice germplasm from West Africa including landraces from the Casamance area; (iii) identify sources of tolerance to iron toxicity in the rice genotypes; and (iv) identify candidate genes associated with tolerance to iron toxicity. The PRA was conducted in six districts of the Casamance region with data collected through focus group discussion and semi-structured questionnaire. The results indicated that salinity (30%) and iron toxicity (26%) were the main constraints and caused significant yield losses. Most of the farmers observed iron toxicity in their fields during the last five years (95%). Farmers were aware that iron toxicity was associated with the soil characteristics and flooding conditions of their lands. Grain yield (27%) and earliness (20%) were the most preferred traits in addition to tolerance to salinity and iron toxicity. The genetic diversity study with 12 Simple Sequence Repeat (SSR) markers revealed that the entire collection of 480 genotypes including the landraces from Casamance and the collection from AfricaRice which was composed mainly of accessions from the West African countries (57%), presented an intermediate genetic diversity (GD) of 0.63. Mean of the polymorphic information content (PIC) was 0.59. The collection from AfricaRice were more diverse (GD = 0.67) than the landraces (GD = 0.50) collected from 19 villages across six districts of the Casamance zones. Three main genotypic groups where identified corresponding to the *Oryza glaberrima* and the *Oryza sativa* rice species and subspecies. Two groups of *indica* genotypes were identified where one was mainly composed of landraces from Casamance as confirmed by the population structure analysis with 148,934 Single Nucleotide Polymorphic (SNP) resulting from imputation with 0.5% minor allele frequency (MAF). Based on the results from the genetic diversity study, a subset of 282 rice genotypes composed of both rice species were selected for the screening for tolerance to iron toxicity and the genome wide association study (GWAS). The evaluations were conducted in hydroponic solutions with 1500 mg L^{-1} Fe concentration for ten days and two hot spot fields in Djibelor (Senegal) and Ndeman (The Gambia). Consistency in the genetic variation in response to iron toxicity was also assessed across these different experimental conditions. Differences in the levels of tolerance were observed among the genotypes in the hydroponic screening as well as in the field evaluations. In addition to the classical agro morphological parameters, secondary traits such as chlorophyll content (CC) (SPAD value) and leaf bronzing (LB) were assessed in these experiments. Leaf bronzing had a mean score of 4 in hydroponic screening and varied between 3 and 4 in hot spot fields at Ndeman and Djibelor, respectively. Significant variation was observed among the genotypes for most of the traits recorded in the different experiments. The environment had significant effect on most of the traits. Shoot dry weight and root dry weight were reduced by 59 and 53% respectively in hydroponic conditions. Grain yield and biomass production were reduced by 56% in the field evaluation at Djibelor where the iron toxicity was more severe compared with the non-stress field experiment also implemented at Djibelor. Some stability was found in the genotypic response under the different experiments and consistency was observed in the tolerance of some genotypes. These genotypes comprised accessions from West Africa: BEN11-172, WONKIFON 17, previously identified tolerant variety: IR75887-1-3-WAB1 (ARICA 6) and landraces from Casamance: EHAUB and BANDIOUL MANO. These genotypes presented low iron content (2229 to 7518 mg kg^{-1}) in shoots and high roots iron retention (45769 to 176930 mg kg^{-1}) capacity. The identification of candidate genes associated with tolerance to iron toxicity through GWAS using the 148,934 SNPs was performed mainly on the *indica* subpopulation which was more representative in terms of diversity and population size (186 genotypes). A total of nine significant SNPs with $-\log_{10}$ P>4 were identified using five traits assessed in hydroponic conditions. For the field conditions, 60 significant SNPs with threshold of $-\log_{10}$ P>4 were detected for eight traits. The new candidate loci identified in hydroponic for SPAD value (CC) were also confirmed in the field conditions. These loci included LOC_Os08g09220 and LOC_Os08g09250, described to be putatively involved in the biosynthesis of OsFBX262- F-box domain and glyoxalase family proteins, reported to be variously regulated when rice seedlings are exposed to oxidative stresses. The locus LOC_Os07g05800 associated with harvest index and the previously reported LOC_Os07g02350, identified for leaf bronzing were reported as putatively involved in the biosynthesis of glutathione S-transferase and casein kinase II subunit alpha-2, respectively. These proteins were suggested as relevant in the tolerance of rice to iron toxicity. Further investigations of these candidate genes including transcriptomic analysis and functional characterization are necessary.

DEDICATION

To my lovely mother, brother and sisters

To my husband

To the blessed memory of my father

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AfricaRice	Africa Rice Center
ARICA	Advance Rice for Africa
CTAB	Cetyl Trimethyl Ammonium Bromide
CIRAD	Centre de Coopération International en Recherche Agronomique pour le Développement
DAPSA	Direction d'Analyse et de Prévision des Statistiques Agricoles
DAT	Day After Transplanting
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra acetic Acid
FAO	Food and Agriculture Organization
GD	Genetic Distance
GWAS	Genome Wide Association Study
IITA	International Institute of Tropical Agriculture
ISRA	Institut Sénégalais de Recherches Agricoles
PIC	Polymorphic Information Content
QTL	Quantitative Trait Loci
RFLP	Restriction Fragment Length Polymorphism
SNP	Single Nucleotide Polymorphic
SSR	Simple Sequence Repeat
SSA	Sub-Saharan Africa

CHAPTER ONE

1.0 GENERAL INTRODUCTION

Rice is among the most important source of food energy in sub-Saharan Africa (Seck *et al.*, 2010). It is grown in various climatic zones and ecologies around the world. Irrigated rice represents about 55% of the lands used for rice cultivation and around 75% of the total rice production in the world (Khush, 1997). Rice is a self-pollinated crop with two cultivated diploid species, *Oryza sativa* L. and *Oryza glaberrima* Steud (Khush, 1997). In research activities, rice is considered as a model crop due to its small genome size of 415 to 463 Mb (Arumuganathan & Earle, 1991; Chen *et al.*, 2002).

In Africa, rice represents 25% of the total cereal consumed and is ranked second after maize in consumers' preferences with local production which covers only 60% of the continent's needs (Mendez del Villar & Bauer, 2013). In West Africa, the consumption of rice increases each year due to demographical expansion and consumer preferences (AfricaRice, 2007; Mendez del Villar & Bauer, 2013). West and East Africa are the most important rice producers in sub-Saharan Africa (SSA). West Africa contributed 65% of the production of paddy rice in SSA from the period of 2001 to 2005 (AfricaRice, 2007). This production is however considered to be low compared to the increase in harvested areas. According to the Food and Agricultural Organization (FAO), the average yield is nearly 2 t ha^{-1} compared to the rest of the world which is about 4 t ha^{-1} (FAOSTAT, 2017).

As in many West African countries, Senegal has high consumption rate of rice which is estimated at 92 kg year^{-1} per capita (Demont & Rizzotto, 2012). From 2010 to 2015, rice ranked second after pearl millet in terms of mean production and harvested area and represented about 32% of the total cereal produced (ANSD, 2017). However, the national production represents only about 20% of the country's needs (Rizzotto & Demont, 2010). Production is indeed characterized by very low yield, especially the rainfed system which is estimated at about 2 t ha^{-1} (ANSD, 2017). Rainfed production system is practiced in the South-West, the Middle, the East and the South-East part of the country, especially in Fatick, Tambacounda, Kedougou and mainly the Casamance agro ecological zone.

Rainfed rice producing system, including the upland and lowland ecologies, represented 63% of the total rice cultivated areas in SSA but for only 37% in the world (AfricaRice, 2007). Rice produced in the lowland ecologies contributed to about 49% of the total production of West Africa in 2009 (Diagne *et al.*, 2013). However, rice productivity in these lowland areas is mainly limited by several soil biophysical constraints in addition to the use of inappropriate agronomical practices specifically the small-scale farmers. In SSA, farmers perceived generally soil constraints as the most important limiting factor for rice production (Diagne *et al.*, 2013). Among these biophysical constraints, iron toxicity is one the more widespread oxidative stress. High levels of reduced iron, mainly associated with acid soils, is a recurrent problem of lowland ecologies particularly in West Africa where it limits considerably rice productivity (Cherif *et al.*, 2009; Audebert & Fofana, 2009).

In Senegal, the lowland ecologies represent about 25% of the rice producing areas (Diagne *et al.*, 2013). These lowland ecologies are mainly located in the Casamance zone and among the limiting factors, oxidative stresses such as salinity and iron toxicity are the most important economically (PAPSEN, 2013; PADERCA, 2014; Sambou *et al.*, 2014). Iron toxicity was first reported in mangrove areas which have previously been differentiated as "tannes" (Prade *et al.*, 1990). Combined with high acidity, the Lower and Middle agro ecological zones of Casamance are more affected due to the nature of the soil and the interflow of water from upper slopes.

However, few studies have been reported on the assessment of the incidence of this constraint and its impact on rice productivity in Casamance (Toure, 1981). Very limited knowledge has also been reported on local farmers' mitigation strategies.

Iron toxicity not only decreases rice production but also renders a vast majority of lands in West Africa unproductive. In West and Central Africa (WCA), 60% of the lowland ecologies are prone to develop iron toxicity (WARDA, 2002). These lowland ecologies predisposed to develop this constraint are mainly located in the humid forest areas (WARDA, 2002; Becker & Asch, 2005). Rice plants experience iron toxicity when there is high concentration of soluble iron (Fe^{2+}) in the soil solution. Iron toxicity causes physiological disorders to the plants and is often combined with other nutritional deficiencies (Engel *et al.*, 2012). The effect of iron toxicity on rice plants result in a high reduction of plant growth and grain yield production and also the formation of necrotic brown spots on the leaves, which is the main visual symptom (Sahrawat, 2005; Elec *et al.*, 2013). The decrease in grain yield can reach up to 100% in the case of susceptible varieties (AfricaRice, 2006). Ways of mitigating the constraint include the development of appropriate soil management practices and the use of tolerant rice varieties.

Identifying sources of tolerance to iron toxicity, specifically in the existing West African rice cultivars, is necessary in breeding activities for improving local germplasm and introduced cultivated high yielding varieties. Despite the challenges related to the screening, few donors of iron toxicity tolerance have been identified in diverse rice germplasm. These include: the rice diversity panel composed of accessions from different regions of rice cultivation in the world (Matthus *et al.*, 2015) and some African rice germplasm such as the *Oryza glaberrima* collection of AfricaRice (Sikirou *et al.*, 2016), the upland NERICA lines (Kang *et al.*, 2011) and other sets of varieties and breeding lines (Dramé *et al.*, 2010; Engel *et al.*, 2012; Onaga *et al.*, 2012, 2013).

However, there has been no extensive evaluation of a large collection of local rice germplasm from West Africa. Regarding the fact that iron toxicity is widespread in this region and farmers' grown varieties are usually well adapted to their local conditions, cultivars and landraces from West Africa may provide sources of tolerance to iron toxicity (Dramé *et al*., 2010). Besides, having a broad range of donors is important to guard against any loss in potency and to respond to the diversity of iron toxic environments. Traditional rice germplasm constitutes generally a huge source of genetic diversity. Their characterisation and evaluation aiming at proper utilisation in breeding programme could have significant impact on the improvement of rice productivity (Sanni *et al*., 2008; Sow *et al*., 2013).

Rice genotypes vary significantly in tolerance to iron toxicity (Fageria & Rabelo, 1987) therefore, providing an opportunity for exploiting the diversity for breeding for tolerance. This variation depends, however, mainly on the growing conditions and soil properties (Asch & Becker, 2005). Breeding for iron toxicity tolerance is therefore challenging because of large genotype by environment (GxE) interaction which limits significant achievement from conventional breeding. Thus, to enhance the tolerance of rice to Fe toxicity, an understanding of the genetic basis would be useful. The genetic mechanism controlling the tolerance of rice to iron toxicity is, however, largely unknown. Despite the numbers of QTL identified through linkage mapping in several studies, most of them are not well characterized or validated for use in breeding programmes in improving susceptible rice varieties (Sikirou *et al*., 2015). Beside the classical approach of gene mapping, Genome Wide Association Study (GWAS) has proved to be more efficient in exploring the genetic basis of complex traits (Famoso *et al*., 2011; El-soda *et al*., 2014). GWAS has been used to identify candidate genes associated with tolerance to iron toxicity in diverse collections of rice germplasm using high density marker platforms (Matthus *et* *al*., 2015; Zhang *et al*., 2017). Some of these genes were even reported to be good candidates for further studies including functional characterisation. The application of association mapping could effectively help to understand the genetic mechanism of iron toxicity tolerance in rice which will facilitate more efficient breeding strategies for the genetic improvement of elite varieties. However, these association mapping studies were mainly conducted on worldwide rice collections including accessions from different countries. Accessions and traditional rice varieties may present high genetic diversity which can help in improving cultivated modern varieties for tolerance or resistance to several biotic and abiotic stresses as well as for agronomic traits of economic importance (Sanni *et al*., 2013). Valuable candidate genes involved in the tolerance to iron toxicity could be identified in the local germplasm for the development of improved tolerance to Fe toxicity in elite varieties.

Newly developed varieties will however be adopted only if they meet farmers' criteria and preferences. Taking into consideration the contribution of local farmers' knowledge, in breeding activities, will facilitate the adoption of new varieties which could sustainably increase crop productivity (Kimani *et al*., 2011). Participatory Rural Appraisal (PRA) is a widely adopted approach for investigating and enhancing indigenous involvement in research programme for a reliable and accessible development strategy (Chambers, 1994; Goma *et al*., 2001; Uddin *et al*., 2013). In rice farming, PRA has been an effective method for assessing constraints and farmers' needs in terms of adapted and performing technologies (Asante *et al*., 2013; Sow *et al*., 2015; Goita *et al*., 2015). Findings from PRA approach can serve as guide in defining research objectives in breeding programmes and also to prioritize traits for actions to meet end users' needs.

The general objective of this research therefore was to contribute to the improvement of rice productivity in the lowland ecologies affected by iron toxicity in Senegal which could also benefit the other West African countries.

The specific objectives were to:

i. assess the lowland rice production constraints, farmers' preferred varietal traits and perceptions of iron toxicity in the Casamance area of Senegal;

ii. assess the genetic diversity among the rice germplasm mainly composed of accessions from West Africa and landraces from the Casamance area;

iii. identify sources of tolerance to iron toxicity among the rice genotypes; and

iv. identify candidate genes associated with tolerance to iron toxicity.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and evolution of cultivated rice species

Rice belongs to the genus *Oryza* containing about 21 wilds and two cultivated species, *Oryza sativa* and *Oryza glaberrima* respectively from Asian and African origin. The cultivated species were derived from a common ancestor (Figure 2.1) (Khush, 1997) and are AA genome with 12 chromosomes (Park *et al*., 2003; Sweeney & McCouch, 2007). *Oryza sativa* which is the widely cultivated specie is composed of two main subspecies namely the *indica* and the *japonica* subsp. These two subspecies are morphologically and genetically distinct (Sweeney & McCouch, 2007). The *japonica* subspecies are characterized by short grain size and are mainly cultivated in dry land conditions of temperate and sub-temperate regions, whereas the *indica* subspecies present long grain size and are widely cultivated in warm and humid regions (Zhao *et al*., 2011). Different groups were identified within each of these two major subspecies based on morphological and molecular characterization (Garris *et al*., 2005; Sweeney & McCouch, 2007). These groups include *indica*, *aus*, *aromatic*, temperate *japonica* and tropical *japonica*. Relationships exist however between the groups of *indica* and *aus* as well as for the *aromatic*, temperate *japonica* and tropical *japonica* which are also closely related (Garris *et al*., 2005). The Asian rice *Oryza sativa*, was introduced in Africa in the fifteenth and seventeenth centuries (Chang, 1984; Sow *et al*., 2013).

The domestication of the African rice, *Oryza glaberrima*, was done about 3500 years ago (Porteres, 1950) before the *O. sativa* was introduced in Africa (Semagn *et al*., 2007; Agnoun *et al*., 2012). The domestication was reported to have taken place in three main centres including

Mali, Guinea and Sene-Gambia. The primary centre was however suggested to be the Niger River delta (Porteres, 1970). It continues to be cultivated in some areas in West Africa mainly for their adaptability to local conditions and for cultural reasons (Linares, 2002; Agnoun *et al.*, 2012).

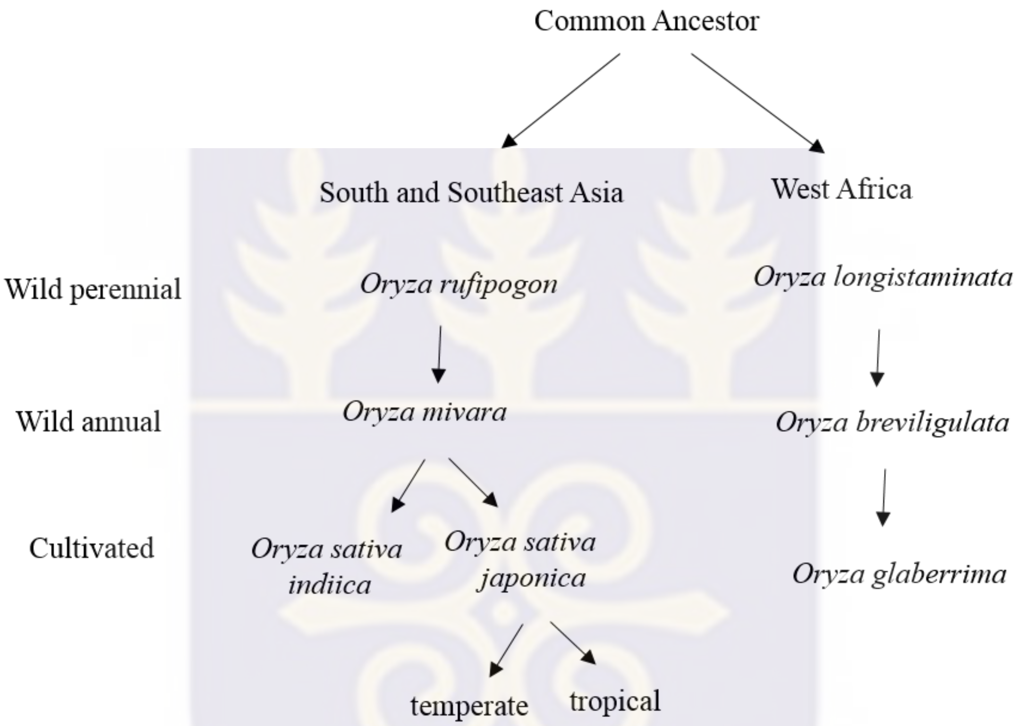


Figure 2.1: Evolutionary pathway of the cultivated rice species (Khush, 1997)

2.2 Rice production and main constraints in West Africa

Paddy rice production in West Africa is increasing faster each year compared to the mean increase in production throughout the continent. In spite of the observed land increase for rice production, West Africa imports up to 40% of its rice needs (Mendez del Villar & Bauer, 2013).

From 2008 to 2013, rice production in Senegal has increased significantly (400,000 to 600,000 tons) compared to the period from 2001 to 2007 where the production was only about 200,000 tons (FAOSTAT, 2017). Rice production in Senegal remains relatively low compared to most of the other West African countries. In 2013 and 2014, Senegal was ranked seventh in terms of rice production with only 3% of the total production in the West Africa sub region, behind Nigeria (32%), Mali (15%), Guinea (14%), Cote d'Ivoire (13%), Sierra Leone (9%) and Ghana (4%) (FAOSTAT, 2017). In Senegal, rice is mainly produced in irrigated, lowland and upland ecologies.

The rainfed system, expected to cover about 40% of the national production, is well practiced in the Casamance area. In this agro ecological zone, the main cereal crops produced in the period 2010 to 2016 were rice (47%), maize (23%), pearl millet (21%) and sorghum (9%), with harvested areas of 34% for rice and 32% for pearl millet (ANSD, 2017). This production system is mainly practiced in the lowland ecologies. However, in addition to the rudimentary agronomic practices, the productivity is mainly limited by soil problems. Among these limiting factors, salinity and iron toxicity are the most recurrent.

As many countries in West Africa, the unproductive lands due to high levels of iron concentration in Senegal, particularly in the Casamance zone, is expected to increase. Considering the fact that most of the lowland soils are acid sulphate types which are predisposed to develop iron toxicity. However, except for using tolerant varieties, none of the recommended agronomic cropping techniques which include liming, drainage and washing away soluble iron are accessible to local farmers.

2.3 Constraint of iron toxicity and its economic significance in West Africa

Described for the first time by Ponnamperuma *et al.* (1955), the iron toxicity problem in rice productivity has been reported in many wetland soils of the tropical and subtropical regions. It occurs in different lowland soil such as acid sulphate and acid clay soils (Becker & Asch, 2005). High concentrations of iron in the soil solution can be due to either or both ground water interflows from adjacent upper slopes and from *in-situ* ferrous release when the parental rock is rich in iron (WARDA, 1988). The occurrence of iron toxicity is influenced by high acidity, low cation exchange capacity, relatively high organic matter content and high concentrations of reducible iron (Sahrawat, 2005; Sahrawat, 2010). The reduction of ferric iron is also influenced by the redox potential of the soil solution (Fageria *et al*., 2008; AfricaRice, 2006). The low soil pH is also an important parameter as it promotes oxido-reduction reactions and the availability of ferrous iron in the soil solution. In flooded anaerobic condition pH less than 6.5 was observed to be associated with the occurrence of iron toxicity (Fageria *et al.*, 2008). The lower concentration of reduced iron (Fe^{2+}) in the soil solution that affect rice productivity in lowland ecologies is 20 mg L^{-1}. This concentration can be above 2500 mg L^{-1} depending on the rice variety and the environment (Becker & Asch, 2005).

Iron toxicity is widespread in Asia, South America, West and Central Africa (Sahrawat, 2010; Onaga *et al.*, 2013), and in some countries of East Africa such as Uganda, Rwanda, Burundi and Madagascar (Becker & Asch, 2005). In West Africa, iron toxicity is more prevalent in the humid forest lowland zones (Sahrawat, 2000; Becker & Asch, 2005). A survey conducted in Ivory Coast, Ghana and Guinea (Cherif *et al.*, 2009) showed that about 10% of the cultivated lands were abandoned due to high levels of iron. Iron toxicity hot spots were further identified in Ghana and Guinea by AfricaRice using remote-sensing data (Dramé *et al.*, 2010). As many countries in SSA (Dramé *et al.*, 2010), there is however few recent data on the extent of iron toxicity in West Africa.

Iron toxicity causes important yield losses which have been estimated between 12 and 100% (Cherif *et al.*, 2009; Sahrawat, 2010). Audebert & Fofana (2009), using crop simulation and data from field experimentations conducted in Cote d'Ivoire and Guinea from 1994 to 1998, it has been shown that, in the presence of toxic levels of iron, rice yields can be reduced by 16% to 78%. The low performance under iron toxicity conditions depends on the growing environments and the susceptibility of the variety used. Becker & Asch (2005) have classified the iron toxicity environments into three groups mainly based on soil characteristics, the period of occurrence and the importance of yield losses (Table 2.1).

Table 2.1: Characteristics of the main iron-toxic environments

	Cluster 1	**Cluster 2**	**Cluster 3**
Land form	Costal planes, river delta	Marshes, highlands swamps	Inland valley swamps
Soil	Young acid sulfate soils	clayey Ulti and Histosols	Sandy valley bottom soils
Soil iron (mg Fe^{2+} L^{-1})	500-2500	300-900	20-600
Cropping season	Dry and wet season	mainly dry season	mainly wet season
Spatial distribution	Whole field	Isolated patches (org. mater, H2S)	Valley fringe (zone of upwelling)
Maximum yield losses (%)	40-100	15-50	30-70
Country examples	Vietnam (MD), Liberia, Senegal, Thailand	Philippine, Indonesia (Java), Burundi, Madagascar	Guinea, Madagascar, Cote d'Ivoire, Srilanka

Sourced from Becker & Asch (2005)

2.4 Iron uptake and main toxicity symptoms

Two mechanisms of Fe uptake exist in rice; strategy I with the reduced form (Fe^{2+}) as in non-graminaceous crops and strategy II (Fe^{3+}) involves the use of chelating compounds as happens in graminaceous plants (Jeong & Guerinot, 2009; Bashir *et al.*, 2010; Kobayashi *et al.*, 2014). In submerged conditions, the rice plant absorbs ferrous iron Fe^{2+} using mainly the OsIRT1 and OsIRT2 transporter which are, however, mainly reported in the case of low iron concentration (Ishimaru *et al.*, 2006; Fukuda *et al.*, 2012). Uptake of a large amount of Fe causes serious injury to the rice plant, therefore uptake is tightly controlled by various mechanisms. High intracellular levels of iron induce the synthesis of hydroxyl radicals that are responsible for many biological and physiological injuries (Asch *et al.*, 2005; Fukuda *et al.*, 2012). Rice plants can however limit the Fe uptake based on the capacity of oxidation and exclusion of iron through the rhizosphere. Though, most of the iron transporters regulation activities were mainly investigated under low Fe concentration yet their role in the case of toxicity is not well understood.

In severe cases, iron toxicity causes damage of plant cells or serious physiological and nutritional disorders (Asch *et al.*, 2005). The symptoms of iron toxicity can manifest themselves at any stage of the plant’s development. However, they are more recurrent during the maximum tillering and heading stages (Prade & Ottow, 1993; Sahrawat, 2010). Significant yield losses was reported when the tissue iron content is above 500 mg Fe kg^{-1} of dry leaf (AfricaRice, 2006). The main symptom associated with iron toxicity is “leaf bronzing” which appears first on the lower leaves as tiny brown spots starting from the leaf tip and spreading toward the base (Audebert & Fofana, 2009). In the most severe cases, the whole leaf turns orange-yellow to brown and dies. It was reported that the brown spots appearing with Fe toxicity result from the oxidative stresses due to the formation of reactive oxygen species catalysed by high concentration of iron in the leaves (Engel *et al.*, 2012). Overall plant growth and tillering ability are also dramatically affected by iron toxicity. Thus, the plant has stunted growth and limited tillering. The development and the function of the root system are highly reduced (AfricaRice, 2006).

2.5 Mitigation strategies

A wide range of management techniques have been developed to alleviate the negative effects of iron toxicity on rice performance. The more sustainable approach to cope with iron toxicity is a combination of tolerant varieties with appropriate agronomic practices (Sahrawat, 2010). These agronomic practices include performing drainage, sowing on ridges, liming and application of optimum plant nutrients (Ramirez *et al.*, 2002; Becker & Asch, 2005; Dramé *et al.*, 2010). In the rhizosphere, iron affect other plant nutrients (Cherif *et al.*, 2009) resulting in a general nutritional deficiencies and high decrease in plant performance. The effects of iron toxicity are reported to be further increased by P, K and Zn deficiencies (Yoshida, 1981; AfricaRice, 2006). Application of plant nutrients was therefore suggested as a way of mitigating the effects of iron toxicity (Prade & Ottow, 1993). However, some studies have reported that iron toxicity in rice plants did not cause several nutritional deficiencies and only the toxic effects of Fe was critical (Sahrawat, 2000). Management of iron toxicity should also include the control of influx of Fe^{2+} by reducing the interflow of water (Becker & Asch, 2005).

2.6 Mechanisms of Fe tolerance

Two types of Fe-toxicity tolerant varieties have mainly been described. The “includers” can tolerate high levels of intracellular iron and the “excluders” which limit the uptake and/or translocation by adopting mechanism of physiological avoidance (Becker & Asch, 2005). For the “excluders” corresponding to a physiological avoidance, the ferrous iron Fe^{2+} is oxidized into

Fe^{3+} in the rhizosphere which makes iron less available to the rice plants (Sahrawat, 2010). This oxidation occurs through anaerobic microbial or chemical processes in the presence of molecular oxygen released in the rhizosphere. This Fe oxidation also induces the formation of iron plaque which constitutes a physical barrier to further entries into the plant. It has been reported that the oxidation power of the roots depends on the developmental stage of the plants. It is higher at the maximum tillering and decreases around flowering stage (Becker & Asch, 2005). High Fe exclusion can however cause low grain Fe content (Ruengphayak *et al.*, 2015). The "includers" types can retain the iron in the root, stem and leaf tissues which reduce its level in the leaves. This reduces iron levels in the leaves for optimal photosynthetic processes. However, under high transpiration conditions or reduction of the retention capacity of the stems, more transportation of Fe to the leaves is observed (Becker & Asch, 2005). Excessive iron concentration in the leaves causes the formation of reactive oxygen species and hydroxyl radicals (Dufey *et al.*, 2012; Wu *et al.*, 2014).

2.7 Genetic improvement of rice for tolerance to iron toxicity

2.7.1 Screening methods

Hydroponic, pot screening and hot spot field conditions are the main screening methods used to evaluate the tolerance of rice to iron toxicity (Sikirou *et al.*, 2015). Hydroponic method of screening is a type of culture solution containing plant nutrients in different concentration with optimal pH and toxic iron concentration which depends on the protocol used (Wang *et al.*, 2008; Elec *et al.*, 2013). In this approach, plants are generally evaluated at early developmental stage with data recorded mainly on growth parameters, physiological and biophysical variables such as leaf bronzing, tissues iron concentration and chlorophyll content (Fageria & Rabelo, 1987; Dufey *et al.*, 2012). Large panels of rice germplasm have hydroponically been assessed to investigate the genetic variation associated with tolerance to iron toxicity (Dufey *et al.*, 2012; Matthus *et al.*, 2015; Zhang *et al.*, 2017). Furthermore, the hydroponic screening techniques have highly been improved with respect to the precipitation of iron which could decrease the level of toxicity in the culture solution (Shimizu *et al.*, 2005; Wang *et al.*, 2008; Elec *et al.*, 2013).

Hot spot field screening represents the natural conditions and occurrence of iron toxicity. It has been used to select tolerant varieties in different countries including West African countries, Sri Lanka, the Philippines and Malaysia (AfricaRice, 2006). In West Africa, important results have also been achieved for the selection of tolerant varieties since 1970s and 1980s (WARDA, 1988 ; AfricaRice, 2006; Sikirou *et al.*, 2015). AfricaRice has identified through field evaluations new varieties through multi location trials with local partners (AfricaRice, 2017). These rice varieties include WAS 21-B-B-20-4-3-3 (identified for release in Ghana), WAT 1046-B-43-2-2-2 (released in Burkina Faso and identified for release in Guinea). The pot screening is generally conducted using hot spot soil or washed sand soil supplemented with iron (AfricaRice, 2006; Dufey *et al.*, 2012; Sikirou *et al.*, 2015).

2.7.2 Genetic variation under different iron toxicity environments

Some studies have reported differences in the genetic variation of rice plants under diverse iron toxicity environments (Asch *et al.*, 2005). The inconsistencies, observed across field evaluations or between different screening methods (Wang *et al.*, 2008), could mainly be due to the complex nature of the tolerance mechanisms (Sikirou *et al.*, 2015). In screening for tolerance, some optimized techniques were reported in order to reproduce similar ranking of tolerant genotypes. For the hydroponic screening approach, these techniques include mainly the use of EDTA as chelator with optimal pH and the agar nutrient in the culture solution (Wang *et al.*, 2008; Elec *et al.*, 2013). Significant correlations were found among data recorded across the fields and the improved hydroponic when compared to the conventional method (Wang *et al.*, 2008; Elec *et al.*, 2013). Various studies have also reported similarities in the performance of some traditional rice cultivars under different hot spot fields conditions (Audebert & Fofana, 2009; Sahrawat., 2010). Some of them were even suggested as potential sources of tolerance (Sahrawat., 2010).

2.7.3 Conventional breeding

Conventional breeding programme for tolerance to iron toxicity has been carried out by many national and international research institutes in West Africa. In the 1970s and 1980s tolerant rice varieties were identified by the West Africa Rice Development Association (WARDA) and the International Institute of Tropical Agriculture (IITA) through conventional breeding (WARDA, 1988). Those varieties included the tolerant genotype widely used as check, Suakoko 8 which was released in some West African countries, and some WITA lines (Sikirou *et al.*, 2015). Among these identified tolerant varieties, mainly through multi environment field trials, only a few are currently grown. This is mainly due to their relatively low yielding capacity specifically the traditional ones and the evolution of the constraint. High achievement of the conventional breeding is limited by the genotype by environment interactions (GxE) (Dramé *et al.*, 2010; Sikirou *et al.*, 2015).

Inheritance of related traits of tolerance to iron toxicity was investigated in some rice genotypes such as Suakoko 8 and Gissi 27 (Abifarin, 1986). Results from that study revealed that tolerance in Suakoko 8 was associated with dominant gene, whereas for Gissi 27 it was recessive. The inheritance of tolerance was also investigated by Owusu Nipah *et al.* (1999) using two F_1 mapping population developed from crosses between tolerant and susceptible rice varieties (CK 4 x ITA 330 and CK 73 x Bouake 189) evaluated under field conditions. Dominant gene actions were suggested for leaf bronzing and a heritability of 74% and 25% were found across the two populations for some related traits of tolerance.

2.7.4 Molecular breeding

2.7.4.1 Linkage mapping

The use of molecular marker combined with conventional breeding could help make significant achievements in the development of adapted crop varieties (Moose & Mumm, 2008). Molecular markers allowed the identification of candidate genes associated with phenotypic variations. Candidate gene is the region of the genome of known putative function which is associated with variation of a trait of interest. These genes can be functional or involved in the functions of regulatory metabolism which are associated with the traits (Pflieger *et al.*, 2001). Identified candidate genes need further studies including their validation and the characterization of their functional involvement with regards to the trait.

QTLs linkage mapping to identify candidate genes associated with tolerance to iron toxicity have been performed in different independent studies using genetic linkage maps mainly developed from different crosses (Wu *et al.*, 1997, 1998 ; Wan *et al.*, 2005; Shimizu., 2009; Dufey *et al.*, 2012, 2015). Traits including leaf bronzing, chlorophyll content, stomatal resistance and yield parameters were targeted in these studies using molecular markers which included mainly the Restricted Fragment Length Polymorphic (RFLP) and the Simple Sequence Repeat (SSR). To be useful for breeding programmes, the validation of these QTLs in different environments is necessary (Dufey *et al.*, 2012). However, none of these QTLs conferring tolerance to high Fe conditions have been reported to being used in marker assisted selection (Sikirou *et al.*, 2015). An integrated physical map was performed by Dufey *et al.* (2015) using data from 14 previous independent QTL linkage mapping studies. Regions of interest were identified on different chromosomes where the mining has yielded some candidate genes.

2.7.4.2 Association mapping

Linkage mapping is a useful technique in detecting regions of the genome that affect the expression of complex traits. However, the resolution is low (Darvasi & Soller, 1994) because of the limited number of recombination events that occurred during the development of the populations. Compared to the classical method of gene mapping, advantages of the genome wide association techniques include the use of diverse genetic material where several recombination events could happen. Furthermore, high throughput DNA genotyping is generally used in the GWAS. Association mapping is indeed considered as an efficient approach to investigate the genetic architecture underlying variation of complex traits in plants (Zhao *et al.*, 2011). It offers the possibility to have a higher mapping resolution and greater allele number (Yu & Buckler, 2006; Famoso *et al*., 2011). Many studies using this technique have been published in many model crops such as maize, barley, sorghum and rice (Holeyachi *et al.*, 2013). In rice, association mapping studies were performed to identify candidate genes associated with tolerance to many oxidative stresses such as aluminium toxicity, boron toxicity, zinc and iron toxicity (Famoso *et al*., 2011; Matthus *et al*., 2015; Neto *et al*., 2017; Zhang *et al*., 2017). For iron toxicity, candidate genes for shoot Fe concentration and leaf bronzing score were identified using 326 genotypes of the rice diversity panel (Matthus *et al*., 2015).

Information referring to linkage disequilibrium (LD) is however necessary prior to the application of association mapping in any plant species. It is commonly defined as a non-random association of alleles in a given population. It is influenced by population structure, mutation, selection, genetic drift and genetic linkage (Garris *et al.*, 2003). For association mapping, LD preserved by linkage is more advantageous than the ones by mutation, relatedness or selection (Stich *et al.*, 2006). Furthermore, information on LD extent assisted to figure out the number of markers needed for a given genetic material. The extent of LD, which depends on the panel of germplasm used, was reported to fall between 50-500 kb (Garris *et al.*, 2003; McNally *et al.*, 2009; Famoso *et al.*, 2011) in rice. The resolution of GWAS also depends on LD decay. In rice, this resolution is suggested to be high when LD decay is rapid (Matthus *et al.*, 2015).

2.8 Rice breeding programme for tolerance to iron toxicity in Senegal

For mitigating the constraint of Fe toxicity, application of agricultural lime, wood ash and ground shells were suggested to have significant effect for improving soil properties in the lowland ecologies of Casamance (Toure, 1981). In breeding activities, few studies have been reported for the identification of tolerant varieties. Some breeding lines (267 entries) sourced from Rokupr station (Sierra Leone) were evaluated in lowland hot spot field in Casamance in 1994 (AfricaRice, 2006). Due to high iron concentration in the soil (up to 1456 mg kg^{-1}), none of the introduced lines survived. Recently, some promising varieties from AfricaRice are being evaluated in the Casamance areas through participatory varietal selection (PVS). Few reports exist however on the evaluation of existing rice germplasm for tolerance to iron toxicity.

2.9 Participatory research approaches for the adoption of new rice varieties

Assessing farmers' need and local challenges prior to any action could ensure large adoption of newly developed technologies (WARDA, 1991). Farmers' demand depends on their cropping systems and this is mainly specific to each agro ecological zone (Efisue *et al.*, 2008). With respect to crop varieties, this concerns resistance to local constraints, processing facilities and particularly high yielding and early maturing as most of the farm families traditionally practice subsistence agriculture. Involving farmers allow to assess their knowledge and know about their preferences to build an interactive and adaptive breeding programme (Mapfumo *et al*., 2013; Singh *et al*., 2014).

Participatory research involving indigenous users is a common approach adopted in research programmes to better address local demands. In plant breeding, participatory research can comprise different aspects such as Participatory Varietal Selection (PVS), Participatory Plant Breeding (PPB), Rapid Rural Appraisal (RRA) and Participatory Rural Appraisal (PRA). These approaches are mainly differentiated based on how extent farmers are involved in the development of the varieties. RRA and PRA are two related approaches of investigations and interactions with rural communities which were developed in the 1980s and 1990s respectively (Chambers, 1994). PRA was however reported to give more credit to farmers' needs and criteria for planning and prioritizing research actions (Chambers, 1994).

In rice cropping system, PRA and PVS are commonly conducted for assessing farmers' need and selecting adapted varieties under favourable (Asante *et al*., 2013; Sow *et al*., 2015) and unfavourable conditions of oxidative stresses such as salinity and alkalinity constraints (Singh *et al*., 2014; Goita *et al*., 2015). Participatory actions are also conducted to enhance farmers' access to newly developed technologies in rice farming (Buah *et al*., 2011).

CHAPTER THREE

3.0 LOWLAND RICE PRODUCTION CONSTRAINTS, FARMERS' PREFERRED TRAITS AND PERCEPTIONS OF IRON TOXICITY IN THE CASAMANCE AREA OF SENEGAL

3.1 Introduction

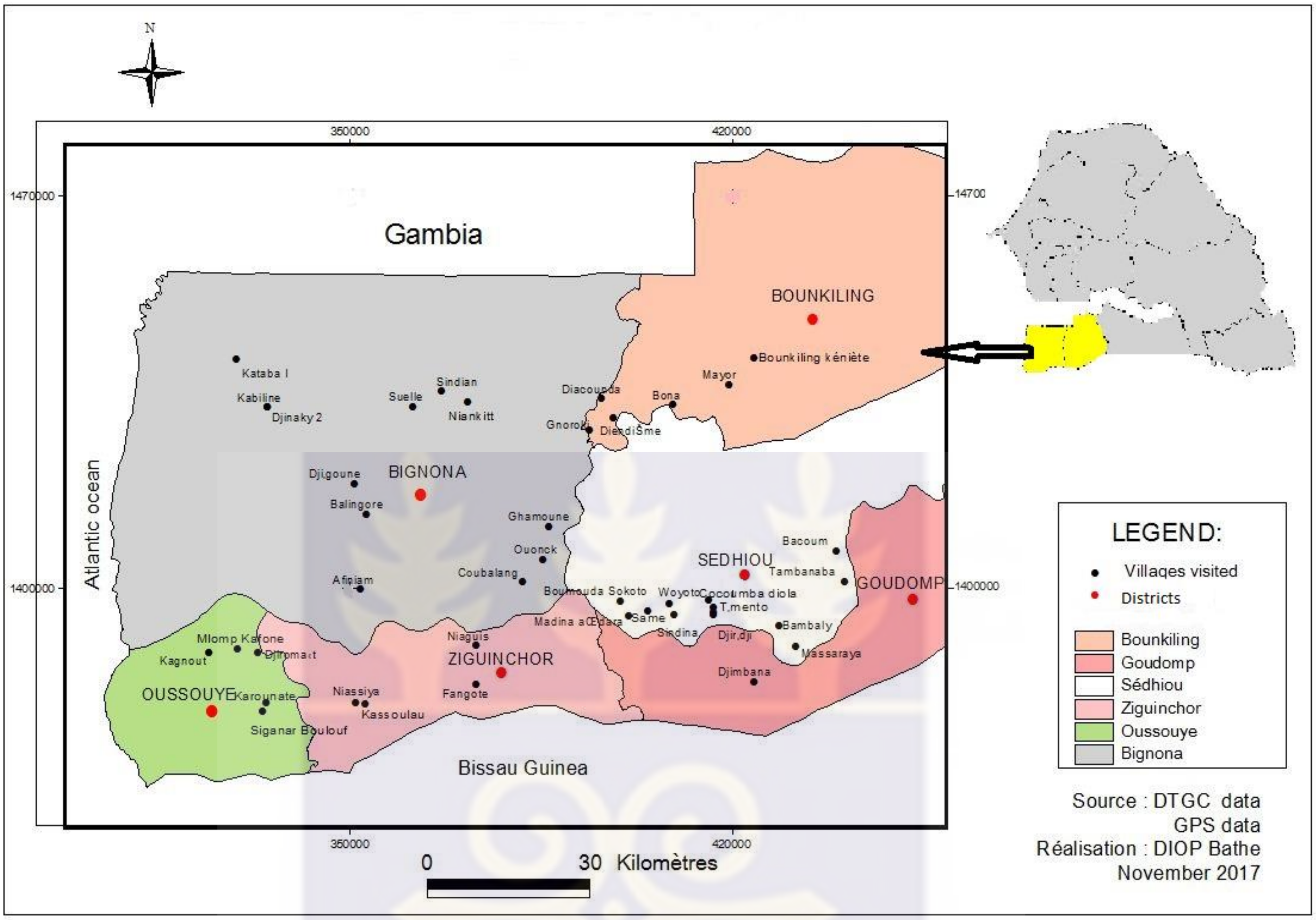
The Casamance agro ecological zone of Senegal experiences low production levels of rice mainly because of the traditional production methods employed and consequences from the decrease of the rainfall observed in 1970s and 1980s (AfricaRice, 2006; PAPSEN, 2013; Sambou *et al.*, 2014). The average grain yield in the last seven years was 2 t ha^{-1} according to the agricultural extension services. Biophysical constraints such as salinity and high level of acidity associated with iron toxicity are among the most important limiting factors. These problems occur mainly in the Lower and Middle Casamance zones and cause important yield losses. Regarding these recurrent soil constraints, it was noticed that farmers mainly do not have access to the developed new technologies (PADERCA, 2014). For improving rice productivity and adaptability to local conditions, farmers' needs must be assessed and taken into account in research programmes for achieving significant contribution to the production and food self-sufficiency in this area. These needs refer to adapted technologies including improved varieties which meet their main preferred traits. Farmers' varietal preferred traits for rice are mainly associated with the ability of the variety to be processed manually from harvesting to milling in addition to tolerance to local constraints (Efisue *et al.*, 2008). Farmers' preferences are mainly specific to local farming practices and considering these criteria can result to a wide adoption of newly developed varieties (WARDA, 1991). However, for more than 30 years, few studies have been reported to evaluate farmers' knowledge and preferences, in relation to the evolution of the agro ecological and climate conditions of Casamance. In the 1980s, rice farming systems in Casamance, particularly in the Lower part, have been characterized based on criteria including gender division of the work, methods of seeding and the main local equipment used (ISRA, 1985). These finding led to the division of the Lower Casamance into five agro ecological zones and research activities were prioritized based on specific local farming practices. Since then, few studies have been carried out in some villages by projects and NGOs to assess rice cropping techniques and results from these evaluations are mainly used as baseline information for their activities. Therefore, a wide investigation is needed to have a general view on the production system particularly in the lowland ecologies. Findings from this study will constitute an update and will provide key information which has to be considered in agricultural research, specifically in plant breeding programme targeting the agro ecological conditions of Casamance. This study was therefore designed to:

1. assess the lowland rice production constraints in Casamance;
2. identify farmers' preferred varietal traits; and
3. assess farmers' perceptions of iron toxicity and mitigation strategies.

3.2 Materials and methods

3.2.1 Description of the study areas

A participatory rural appraisal (PRA) was conducted during the cropping season of 2015 in the Casamance region. The Casamance agro-ecological zone is represented by the administrative regions of Ziguinchor, Sedhiou and Kolda. Based on the importance of lowland ecologies used for rainfed rice production and the possibility of occurrence of iron toxicity, the Lower and Middle Casamance agro-ecological zones were selected for this activity. These regions are delimited by The Gambia in the North, Guinea Bissau in the South, the Atlantic Ocean in the West and the region of Kolda in the East. One rainy season, extending from June to October, exists with an annual mean rainfall which varies between 900 and 1200 mm (Ndong, 1995; ANSD/SRSD-Kolda, 2015; ANSD/SRSD-Sedhiou, 2015). The Lower Casamance which corresponds to the region of Ziguinchor includes the districts of Oussouye, Ziguinchor and Bignona. The Middle Casamance refers to the region of Sedhiou, with the districts of Bounkiling, Sedhiou and Goudomp. The visited villages across these six districts are shown in Figure 3.1. Summary of the baseline information about the selected districts is given in Table 3.1.



Source : DTGC data
GPS data
Réalisation : DIOP Bathe
November 2017

Figure 3.1: Map of the visited districts in the Lower and Middle Casamance zone

Table 3.1: Baseline information and geographical characteristics of the selected districts

				Mean production of paddy rice and harvested areas from 2010 to 2016			
Regions	Mean Rainfall (mm)	Main soil type	Suitable areas for rainfed rice (ha)	Production (Tonnes)	Harvested area (ha)	Selected District	Altitude (m)
Lower Casamance	1200 to 1300	halomorphic, hydromorphic	116 000	38224	23762	Ziguinchor	8
						Oussouye	22
						Bignona	24
Middle Casamance	1000 to 1200	Hydromorphic, halomorphic and Ferraltic	56 111	56318	31710	Sedhiou	45
						Bounkiling	20
						Goudomp	16

3.2.2 Sampling method

The work was carried out in a collaborative effort between socio-economist, agronomist and plant breeder from ISRA. Agricultural extension services, non-governmental organization (NGO) and Project of Development working in the targeted districts were also involved. The agricultural extension services included the "*Direction Régionale du Développement Rural* (DRDR)" of Sedhiou and the one of Ziguinchor. Local authorities were contacted for more information about the selected villages. The districts, villages and farmers respondents were selected following a purposive sampling method. The different criteria used in the sampling procedure were mainly based on the:

- importance of lowland rice production;
- occurrence of iron toxicity;
- representativeness of the region in terms of agro-ecological conditions and farming systems;
- accessibility in terms of availability of roads; and

3.2.3 Data collection

A semi-structured questionnaire (Appendix 3.1) was administered to 147 farmers. Focus group discussions and field visits were conducted to collect data on farming system, farmers' preferred traits of varieties and the main constraints of lowland rice production. In addition to these data, the severity and farmers' perceptions of iron toxicity were determined. A total of 28 valleys were visited across the Lower and Middle Casamance agro ecological zones.

Eight focus group discussions were conducted in Ziguinchor, Bignona, Bounkiling, Sedhiou and Goudomp. Fifteen farmers, mainly females, participated in the focus group discussions. They were individually invited to rank the main points already discussed concerning the origin and occurrence of iron toxicity in their fields. *In-situ* pH measurements were also performed in farmers' fields to determine the levels of acidity which is highly associated with the occurrence of iron toxicity (Fageria *et al*., 2008; Sahrawat, 2010). The geographical coordinates of the different villages were determined using a Global Positioning System (GPS).

3.2.4 Data analysis

The collected data were analysed using SPSS 22.0 statistical software (IBM Corporation, New York, USA, 2011). For each variable collected across the different districts, descriptive statistics including cross tabulations were performed. Chi-square test were computed to determine significance of association of categories.

3.3 Results

3.3.1 Farmers' characteristics

In the six districts, 76% of the 147 households interviewed were females and 24% were males (Table 3.2). In the Bignona and Sedhiou districts 43 and 53 farmers were interviewed, respectively. The respondents were between 35 and 49 years old for 35% of the household and between 50 and 64 years old for 37%. The Jola ethnic group, which represented 54% of the respondents, was more concentrated in the Lower Casamance, while the Mandingo ethnic group (26%) was found mainly in the Middle Casamance region. Most of the respondents did not have formal education, they were essentially illiterate (36%) or initiated to Quran (27%), particularly in the districts of Sedhiou, Bounkiling and Goudomp. Few the respondents had completed primary (19%) and secondary (14%) education. Farming activities, including rainfed rice cultivation and offseason gardening, were the main sources of income for 60% of the respondents. The other activities (40%) comprised micro-trading of forest products and sea food.

Table 3.2: Farmers' characteristics in the surveyed districts

Variables	Districts						Total	*P* value
	Ziguinchor	Oussouye	Bignona	Sedhiou	Bounkiling	Goudomp		
Sex								
Male	6 (60)	6 (75)	20 (46.5)	3 (5.7)	0	0	35 (23.8)	0.000
Female	4 (40)	2 (25)	23 (53.5)	50 (94.3)	26 (100)	7 (100)	112 (76.2)	
Age (year)								
18-24	0	0	1 (2.3)	2 (3.8)	0	0	3 (2)	0.078
25-34	0	0	7 (16.3)	9 (17)	4 (15.4)	0	20 (13.6)	
35-49	1 (10)	4 (50)	15 (34.9)	16 (30.2)	9 (34.6)	6 (85.7)	51 (34.7)	
50-64	6 (60)	4 (50)	11 (25.6)	20 (37.7)	13 (50)	1 (14.3)	55 (37.4)	
65+	3 (30)	0	9 (20.9)	6 (11.3)	0	0	18 (12.2)	
Ethnic								
Jola	8 (80)	7 (87.5)	41 (95.3)	7 (13.2)	17 (65.4)	0	80 (54.4)	0.000
Mandingo	2 (20)	0	1 (2.3)	22 (41.5)	7 (26.9)	7 (100)	39 (26.5)	
Manjack	0	0	0	9 (17)	1 (3.8)	0	10 (6.8)	
Balante	0	0	0	7 (13.2)	0	0	7 (4.8)	
Bainouk	0	0	0	4 (7.5)	0	0	4 (2.7)	
Pular	0	0	1 (2.3)	3 (5.7)	0	0	4 (2.7)	
Serere	0	1 (12.5)	0	0	0	0	1 (0.7)	
Wolof	0	0	0	1 (1.9)	1 (3.8)	0	2 (1.4)	
Education level								
Illiterate	1 (10)	2 (25)	11 (25.6)	22 (41.5)	14 (53.8)	3 (42.9)	53 (36.1)	0.001
Alphabetized	1 (10)	0	2 (4.7)	1 (1.9)	1 (3.8)	0	5 (3.4)	
Quran education	1 (10)	0	9 (20.9)	22 (41.5)	5 (19.2)	3 (42.9)	40 (27.2)	
Primary education	3 (30)	3 (37.5)	12 (27.9)	4 (7.5)	5 (19.2)	1 (14.3)	28 (19)	
Secondary education	4 (40)	2 (25)	9 (20.9)	4 (7.5)	1 (3.8)	0	20 (13.6)	
High education	0	1 (12.5)	0	0	0	0	1 (0.7)	
Source of income								
Farming	6 (60)	5 (62.5)	27 (62.8)	29 (54.7)	18 (69.2)	4 (57.1)	89 (60.5)	0.887
Others	4 (40)	3 (37.5)	16 (37.2)	24 (45.3)	8 (30.8)	3 (42.9)	58 (39.5)	

The percentage of respondents are indicated in parentheses

3.3.2 Cropping system

3.3.2.1 Ecologies for rice cultivation and soil preparation

The traditional lowland areas were mostly exploited for rice cultivation (95%) in all the surveyed districts (Table 3.3). Ploughing and land preparation were specific to agro-ecological zone and were generally performed manually (79%) using traditional equipment which are mainly gender specific (Figure 3.2). In the Lower Casamance, farmers, specifically the men, used a traditional equipment named “Kadiandou” to plough the land by making small ridges, after flooding. In the Middle Casamance, ploughing and land preparation in the lowland areas for rice cultivation were mainly performed by women, using equipment named “daba and Bara”, on flat and non-flooded fields in Sedhiou (66%), Bounkiling (57%) and Goudomp (85%). The mechanical ploughing (21%) was not well adopted as only a few farmers could afford these equipment in the districts of Bignona (18%), Sedhiou (20%), Bounkiling (42%) and Goudomp (21%). These equipment were mainly represented by the rotary tiller.

(**a**) Kadiandou: a traditional equipment used for soil preparation in Jola ethnic group

(**b**) Bara: a traditional equipment used for soil preparation by the women

Figure 3.2: Gender specific traditional equipment used for soil preparations in the Casamance zone

Table 3.3: Ecologies used for rainfed rice production in the six districts and soil preparations

Variable	District						Total	*P* value
	Ziguinchor	Oussouye	Bignona	Sedhiou	Bounkiling	Goudomp		
Ecologies								
Lowland	8 (80)	7 (87.5)	39 (90.7)	51 (98)	26 (100)	7 (100)	138 (95)	0
Mangrove	1 (10)	1 (12.5)	1 (2.3)	0	0	0	3 (2)	
Upland	1 (10)	0	3 (7)	2 (2)	0	0	6 (3)	
Ploughing								
Mechanical	0	0	8 (18.6)	11 (20.8)	11 (42.3)	1 (14.3)	31 (21.1)	0
Manual, ridges	8 (80)	8 (100)	32 (74.4)	7 (13.1)	0	0	55 (37.4)	
Manual, flat	2 (20)	0	3 (7)	35 (66.1)	15 (57.7)	6 (85.7)	61 (41.5)	

Numbers in parentheses correspond to the relative proportions in household responses.

3.3.2.2 Type of rice varieties and field management

Rice was the main food crop across the six districts surveyed. Its cultivation in the rainy season represented about 80% of the households' activities. Strong evidence of association existed between farmers' responses and the cropping techniques referring to the varieties used (*p* value = 0.004), the origin of seed (*p* value = 0.000), method of sowing (*p* value = 0.000) and the use of fertilizer (*p* value = 0.000) (Table 3.4). The respondents grew three to four types of varieties of short to medium duration. The traditional rice varieties were widely grown by farmers (55%), even though a progressive adoption of improved varieties was observed. About 37% of the respondents grew both improved and traditional varieties in their fields and 8% grew only the improved ones. The origin of the seeds was generally from the harvest of the previous season (87%). Transplanting of 30 to 40 days seedlings in flooded land usually one to two weeks after ploughing was particularly observed in the Lower Casamance with the districts of Ziguinchor (70%), Oussouye (100%) and Bignona (84%). The direct seeding technique on flat ploughed lands was generally observed in the Middle Casamance with the districts of Sedhiou (53%), Bounkiling (35%) and Goudomp (72%). The use of mineral fertilizer (NPK and urea) remained limited across the districts. Most of the respondents (51%) did not apply mineral fertilizer in their fields. The respondents who used fertilizer did not have a clear understanding of the combination, the amount and the period of application, as recommended by the extension services. Many farmers (42%) mixed both urea (topdressing fertilizer) and NPK (basal fertilizer) in one use and applied them at once during the growing period, mainly 20 days after sowing. This was the case in the districts of Sedhiou (55%), Bounkiling (54%) and Goudomp (86%). Some farmers applied only urea (3%) or NPK only (4%) once. Beside the non-use or the incorrect use of mineral fertilizer, most of the households did not perform weeding. Only a few of them did it once, mainly 15 to 20 days after transplanting or direct sowing. Based on farmers' estimation of their production and harvested areas, mean grain yield varying significantly (*P* value <0.001) between 589 and 810 kg ha^{-1} were estimated across the six districts. The average harvested areas did not vary significantly over the districts with a mean of 0.34 ha (Table 3.5). Rice was mainly the only crop grown in the surveyed lowland ecologies, with a period of cultivation followed by a fallow, in general.

Table 3.4: Farmers' cropping techniques across the six districts

Variable	District						Total	*P* value
	Ziguinchor	Oussouye	Bignona	Sedhiou	Bounkiling	Goudomp		
Type of varieties used								
Traditional	4 (40)	6 (75)	16 (37.2)	39 (73.6)	12 (46.2)	4 (57.1)	81 (55.1)	0.004
Improved	2 (20)	0	8 (18.6)	0	1 (3.8)	0	11 (7.5)	
Traditional and improved	4 (40)	2 (25)	19 (44.2)	14 (26.4)	13 (50)	3 (42.9)	55 (37.4)	
Origin of seeds								
Harvest from last year	7 (70)	8 (100)	30 (69.8)	50 (94.3)	26 (100)	7 (100)	128 (87)	0
From Neighbors	1 (10)	0	1 (2.3)	3 (5.7)	0	0	5 (3.4)	
From NGOs, Research Institutes from seed producers	2 (20)	0	12 (27.9)	0	0	0	14 (9.5)	
Sowing								
Direct sowing	3 (30)	0	7 (16.2)	28 (52.9)	9 (34.6)	5 (71.5)	52 (35.4)	0
Transplanting	7 (70)	8 (100)	36 (83.7)	25 (47.2)	17 (65.4)	2 (28.6)	95 (64.7)	
Fertilizer								
No mineral fertilizer	8 (80)	6 (75)	30 (69.8)	21 (39.6)	10 (38.5)	0	75 (51)	0
Urea alone	0	2 (25)	0	1 (1.9)	1 (3.8)	0	4 (2.7)	
NPK alone	0	0	2 (4.7)	2 (3.8)	1 (3.8)	1 (14.3)	6 (4.1)	
NPK and Urea	2 (20)	0	11 (25.6)	29 (54.7)	14 (53.9)	6 (85.7)	62 (42.2)	

In parentheses the corresponding proportions of respondents

Table 3.5: Estimated average yield and harvested areas in farmers' field across the six districts

	Districts						Mean	F pr.
	Bignona	Bounkiling	Goudomp	Oussouye	Sedhiou	Ziguinchor		
yield (kg ha^{-1})	810	589	752	663	804	628	746	<.001
Harvested areas (ha)	0.35	0.32	0.29	0.38	0.36	0.31	0.34	0.26

3.3.2.3 Main constraints in lowland rice production

As presented in Table 3.6, salinity (30%) and iron toxicity (26%) were the main constraints of lowland rice production in most of the valleys across the six districts. These constraints were followed by the weeds which represented a mean percentage of 13% in farmers' responses. Constraints associated with insect attack (8%) and low soil fertility (7%) were also identified.

Table 3.6: Main constraints of lowland rice production across the six districts

Variable	District						Total %	*P* value
	Ziguinchor	Oussouye	Bignona	Sedhiou	Bounkiling	Goudomp		
Iron toxicity	80	100	86	100	100	100	26	0.003
Salinity	90	126	83	114	120	114	30	0.058
Weeds	50	88	33	57	54	0	13	0.221
Low soil fertility	0	38	21	28	27	43	7	0.14
Insect attack	10	50	16	25	38	29	8	0.52
Silting of valleys	0	13	28	30	15	29	5	0.187
Lack of mechanization	0	0	14	13	19	29	3	0.567
Access to seed quality	20	0	16	6	0	29	3	0.306
Bird attack	30	13	9	6	4	0	3	0.166

Values in the table are percentages of respondents in each district

3.3.2.4 Farmers preferred traits for lowland rice varieties

Grain yield (27%) and earliness (20%) had the highest score in farmers' most preferred traits across the six districts (Table 3.7). Plant height (moderately to tall type) and grain quality (referring mainly to the milled ability) were represented by 12% and 13% of the responses, respectively. Tolerance to salinity and iron toxicity represented respectively an average percentage of 6% and 10% of farmers' preferences. Farmers in the Lower Casamance region showed more interest on tillering ability and plant height for adopting new varieties.

Table 3.7: Farmers preferred traits for lowland rice varieties across the six districts

Variable	District						Total %	*P* value
	Ziguinchor	Oussouye	Bignona	Sedhiou	Bounkiling	Goudomp		
Grain yield	80	100	100	100	96	100	27	0.034
Tillering ability	70	63	40	8	0	0	8	0.002
Pant height	70	100	58	19	15	0	12	0.019
Grain filling	30	0	28	17	8	0	4	0.73
Earliness	80	75	65	72	62	71	20	0.045
Grain quality	40	25	53	60	46	57	13	0.072
Tolerant to iron toxicity	30	25	33	38	46	43	10	0.064
Tolerant to salinity	20	25	28	8	15	14	6	0.304

Values in the table are percentages of respondents in each district

3.3.3 Iron toxicity constraint

3.3.3.1 Incidence of iron toxicity in the surveyed districts

Most of the respondents (99%) knew about iron toxicity and reported observing it in their fields during the last five years (95%) (Table 3.8). The percentage of farmers who abandoned parts of their land because of iron toxicity in the district of Sedhiou, Bounkiling and Goudomp varied between 85% and 100%. The percentage of farmers forced to abandon part of their fields varied between 50 and 77% in Ziguinchor. Table 3.9 shows the *in-situ* pH measurement of the water solution revealed relatively high acidity in some of the farmers' lands, selected randomly, during the field visits. Most of the fields where the pH was about 3 were completely abandoned and this was particularly observed in the valleys of Afiniam, Diacounda, Same and Niaguis.

Table 3.8: Incidence of iron toxicity in the surveyed districts in Casamance

		District						Total	*P* value
		Ziguinchor	Oussouye	Bignona	Sedhiou	Bounkiling	Goudomp		
Iron toxicity in last five years	Yes	9 (90)	8 (100)	43 (100)	47 (89)	25 (96)	7 (100)	139 (95)	0.191
	No	1 (10)	0	0	6 (11)	1 (4)	0	8 (5)	
Land abandoned	Yes	7 (70)	4 (50)	7 (77)	49 (93)	22 (85)	7 (100)	122 (83)	0.02
	No	3 (30)	4 (50)	10 (23)	4 (7.5)	4 (15)	0	25 (17)	
Know iron toxicity	Yes	10 (100)	8 (100)	42 (98)	53 (100)	26 (100)	7 (100)	146 (99)	0.786
	No	0	0	1 (2.3)	0	0	0	1 (0.7)	

In parentheses the corresponding proportions of respondents

Table 3.9: *In-situ* pH values in the randomly selected farmers' field in the surveyed districts

		Geographic coordinates		
District	Valley	Longitude	Latitude	pH value
Bignona	Afiniam	351862	1399674	3
Sedhiou	Diacounda	395928	1433712	3
Sedhiou	Same	404312	1395821	3.3
Ziguinchor	Niaguis	372930	1389661	3.5
Bignona	Djegoune	350852	1418495	4.1
Bounkiling	Mayor	419252	1436251	4.4
Sedhiou	Bambali	428423	1393284	5.1
Bounkiling	Nioroki	393716	1428132	5.2
Sedhiou	Suelle	361450	1432310	5.3
Bounkiling	Diendiem	398048	1430292	5.3

3.3.3.2 Severity and management of iron toxicity in farmers' field

For most of the respondents across the different districts, the symptoms of iron toxicity appeared mainly at the vegetative stage before flowering (88%) and included leaf bronzing (50%) and high mortality (33%) (Table 3.10). These symptoms were mainly observed three to six weeks after transplanting. Different local techniques for alleviating the effects of iron toxicity were used (Figure 3.3). These techniques consisted mainly of applying residues from previous harvest, including rice straw (22%), burying in the soil leaves of mango trees (32%), compost manure (15%) and ash (8%) from burned household wastes. Some of the farmers (16%) did not implement preventive approaches to cope with iron toxicity.

Table 3.10: Main symptoms of iron toxicity and period of incidence in farmer's field across the districts

	District							
Period of Incidence	Ziguinchor	Oussouye	Bignona	Sedhiou	Bounkiling	Goudomp	Total	*P* value
Vegetative stage	9 (90)	6 (75)	42 (97.7)	45 (84.9)	21 (80.8)	7 (100)	130 (88.4)	0.08
After flowering	1 (10)	0	0	5 (9.4)	4 (15.4)	0	10 (6.8)	
During all cycle	0	2 (25)	1 (2.3)	3 (5.7)	1 (3.8)	0	7 (4.8)	
Symptom								
Stunted plants	2 (20)	2 (25)	1 (2.3)	4 (7.5)	3 (11.5)	2 (28.6)	14 (10)	0.04
Leaf bronzing	4 (40)	4 (50)	14 (32.6)	36 (67.9)	12 (46.2)	3 (42.9)	73 (50)	
Weakness	2(20	1 (12.5)	5 (11.7)	1 (1.9)	2 (7.7)	0	11 (7)	
Mortality	2 (20)	1 (12.5)	23 (53.5)	12 (22.6)	9 (35)	2 (28.6)	49 (33)	

In parentheses the corresponding proportions of respondents across the surveyed districts

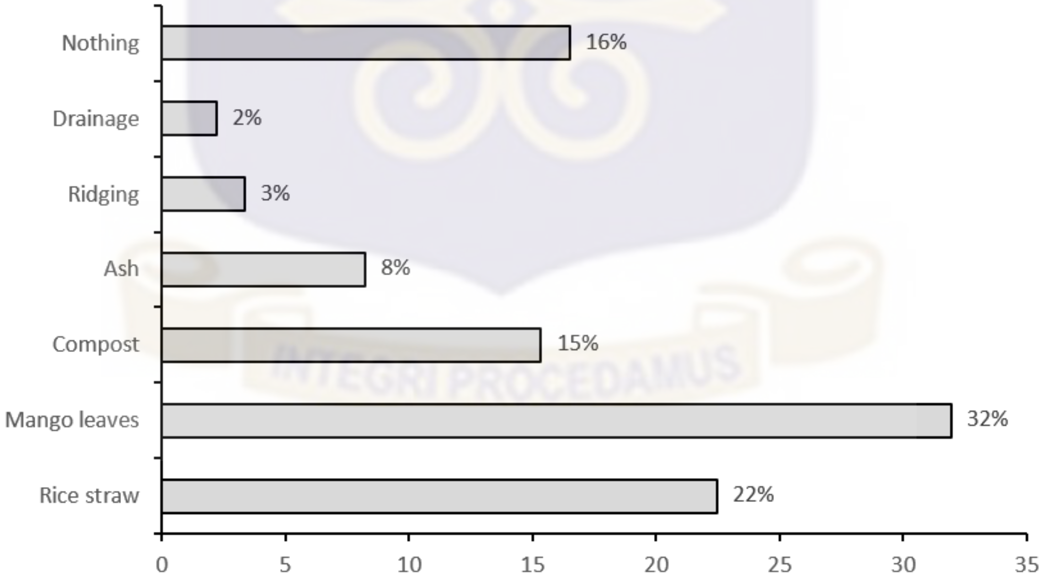


Figure 3.3: Local practices to cope with iron toxicity

3.3.3.3 Farmers' perception of iron toxicity

Iron toxicity named "weleuyi" in Jola and "kumo" in Mandingo, referring respectively to the red colour and the acidic taste of the water, was perceived by farmers as a phenomenon occurring only in lowland fields. They recognized this constraint by the appearance of a thin film on top of the water, in addition to its reddish colour, but also its effect on the rice plant. The respondents tried to give a reason why iron toxicity occurred in their field, even though few of them (6%) did not have idea about it or linked this constraint to the occurrence of salinity (5%) (Figure 3.4). Based on their experiences, they were conscious of the relationship between the flooding and the occurrence of iron toxicity. Most of the farmers in the focus group discussions associated the problem of iron toxicity with the submerged condition of the soil (48%) or linked it to soil problems (41%). This perception of iron toxicity motivated farmers in the Lower Casamance and some villages in the Middle part to adopt the method of transplanting rice seedlings on the ridges to create anaerobic condition.

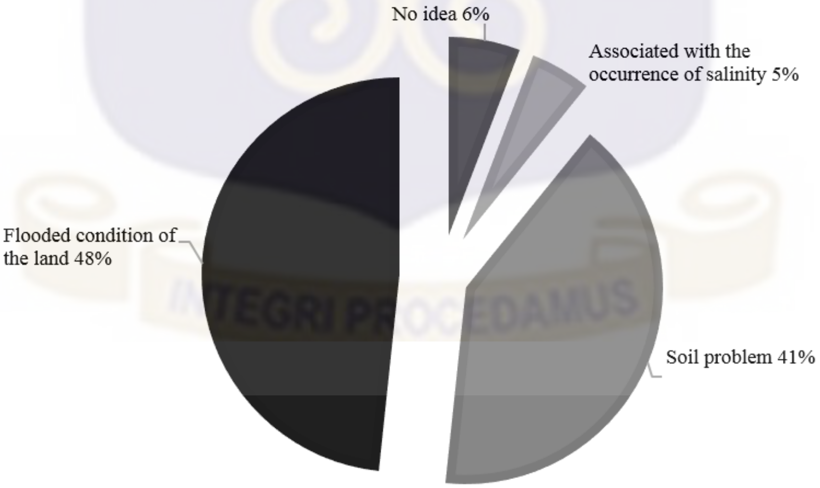


Figure 3.4: Farmers' perceptions of iron toxicity in the surveyed districts

3.4 Discussion

Salinity and iron toxicity were the two constraints identified by farmers as the most important limiting factors for the lowland rice production in the Lower and Middle Casamance. Farmers' perception of the main biophysical constraints in lowland ecosystems agreed with PADERCA (2014) who classified these problems as the most important economically. The constraints of salinity and iron toxicity are mainly associated with soil characteristics (Prade *et al*., 1990) which are related to the fact that these inland valleys were mostly derived from river marine deposits (AfricaRice, 2006). Different techniques of rehabilitating the affected lands have been proposed including leaching and washing away the soluble toxins and application of agricultural lime (Toure, 1981; PADERCA, 2014). Results from this study showed that farmers were aware of the important yield losses due to iron toxicity. This has motivated them to develop traditional techniques to cope with iron toxicity. These techniques included the use of compost, residues from harvesting, ash and tolerant varieties which were mostly local types. These constraints perceived by farmers as the most important should be considered in the rice breeding programme.

The main perceptions of the prevalence of iron toxicity were defined by farmers as a phenomenon resulting from flooding and soil characteristics. Farmers interviewed in the South Benin also had approximately the same perception of iron toxicity (Dansou-Jean *et al*., 2015). The incidence of iron toxicity, in relation to soil characteristics and anaerobic flooded condition of the land, was scientifically demonstrated by several studies conducted in the same agro ecological zones of Casamance (Toure, 1981; Prade *et al*., 1990). Other studies around the world also had the same conclusions (Becker & Asch., 2005; AfricaRice, 2006; Audebert. & Fofana., 2009; Sikirou *et al*., 2015). Indeed, soil such as the acid-sulphate types, which are well represented in Casamance, are naturally predisposed to develop iron toxicity (Becker & Asch., 2005). Furthermore, microbial reduction of ferric iron (Fe^{3+}) into its soluble form (Fe^{2+}) is highly influenced by the redox potential (Fageria *et al.*, 2008) and promoted by the flooded anaerobic conditions. Local farmers' techniques to overcome iron toxicity, particularly the drainage technique and the use of ash, were also studied and their beneficial effects confirmed (Toure, 1981). Ridging and the other methods for alleviating the negative effects of iron toxicity, in addition to the use of tolerant varieties, agreed with most of the recommendations formulated by research (AfricaRice, 2006). This study shows the pertinence of farmers' experiences and knowledge about this constraint. Involving local farmers in the development of technologies to mediate iron toxicity will be of great importance.

The other constraints identified by farmers, were basically the same across the two agro ecological zones. They included weeds, low soil fertility, insect attack and silting of the valleys which were particularly observed in the district of Sedhiou as identified earlier (PAPSEN, 2013).

When it comes to preferred traits, farmers in the Lower and Middle Casamance zone gave more credit to grain yield and earliness. These traits should be the main focus of the breeding programme targeting these areas. The interest in grain yield and early maturity for rice varieties observed in this work show the importance that local farmers' give to rice as food stuff and subsistence crop. Furthermore, as for the lowland ecologies in this southern part of Senegal, farmers in the groundnut basin also established the same criteria for adopting new upland rice varieties (Kanfany *et al.*, 2016). Grain yield of rice varieties was also perceived as key trait in Ghana (Asante *et al.*, 2013), Niger (Sow *et al.*, 2015) and Mali (Efisue *et al.*, 2008; Goita *et al.*, 2015). Farmers in Casamance preferred earliness also because of the variability of the rainfall, resulting in short periods of drought which occur frequently in early vegetative and late reproductive stages specifically in the wetland ecologies. Early maturing varieties can escape drought even though there could be a decline in grain yield (Shahriar *et al*., 2014; Dixit *et al*., 2014). The preference in other agronomic traits such as tillering ability and plant height, which is justified by the fact that harvesting are performed manually, was relatively specific to agro ecological zones. Tolerance to soil biophysical constraints such as salinity and iron toxicity and grain quality, which was mostly related to milling ability, were also identified as important by the local farmers.

More females than males were involved in this study. This gender disparity was due to the fact that the organization of the farming activities is subject to gender unbalance for socio cultural reasons. In the Lower Casamance, where the Jola ethnic group is concentrated, as established by Posner *et al*. (1988), even though both men and women are involved in the activities, the men are more specialized in soil preparation, while the women are concerned with activities as transplanting, harvesting and post-harvest processing. ISRA (1985) subdivided the Lower Casamance into five major zones, based on the farming system, using three main criteria, including gender unbalance (of the farming), the importance of transplanting practices and the agricultural equipment used. In the Middle Casamance however, with the Mandingo which represented the main ethnic group, as stated by Dieng & Ehemba (2013), the gender division followed the topographic sequence. Men's farming activities are predominant in the upland areas, where they cultivated mainly pearl millet, maize and cash crops such as groundout, while women were responsible for rice cultivation, and this is mainly done in the lowland ecologies. This finding is also in agreement with earlier observations reported by ISRA (1985). These results demonstrate that men in the surveyed areas were not fully involved in rice cultivation.

This situation is comparable to what happen in Mali (Efisue *et al*., 2008) but different in Niger where few women were involved in the cultivation of rice (Sow *et al*., 2015).

Rice farming, specifically during the rainy season, is the main activity of the surveyed households, as it is the case for many small scale farmers in West Africa (Seck *et al*., 2013). The over exploitation of lowland ecologies for rice cultivation observed in this study, was mainly related to the possibility of available water during short periods of drought when compared to the upland ecosystem. Furthermore, the mangrove ecosystem is practically unproductive due to the intense salinization and acidification, unless special agronomic practices and soil management are adopted (Toure, 1981). Rice cultivation in the study areas, like for many cropping systems in Senegal, was characterized by its traditional aspect in terms of agronomic practices, including the use of local, low yielding cultivars and landraces, as established earlier by Gergely & Baris (2009). This farming technique, essentially characterized by non-use of inputs, was combined with fallowing as mainly the only rotation system observed which could result in soil deficiencies in nitrogen and phosphorus (WARDA, 1991). This situation reflects a very weak annual rice production system which cannot even satisfy local demand. The access to new techniques of production for these vulnerable farmers of the Lower and Middle Casamance zones needs definitely to be enhanced for a sustainable productivity. These needs for small scale farmers to benefit from research findings are common to many West African countries. To end this situation, initiatives for assisting farm families in West Africa were undertaken by some Governments, in collaboration with NGOs and research organizations (Rice Today, 2013). This was the case in Senegal with the National Programme for Rice Self-sufficiency initiated by the Government.

3.5 Conclusions

The lowland rice production in Casamance is limited by two major constraints, salinity and iron toxicity. Farmers were conscious that the occurrence of iron toxicity is related to the soil characteristics and the flooded conditions of their fields. Farmers' perceptions of iron toxicity and local approaches of mitigating these constraints agree with most of the reported studies. Farmers believed that grain yield and earliness were the main criteria for adopting new lowland rice varieties, even though plant height and tolerance to iron toxicity were also considered as important. Harvested paddy rice from these localities could be more important if actions from research, development services and political decision are pooled together to assist vulnerable farmers to enhance their knowledge and farming systems.

CHAPTER FOUR

4.0 GENETIC DEVERSITY OF THE COLLECTION OF RICE GERMPLASM INCLUDING ACCESSIONS AND LANDRACES FROM WEST AFRICA

4.1 Introduction

Genetic characterization of diverse rice germplasm including the existing accessions from West Africa could facilitate their conservation and particularly their use in breeding programmes targeting different constraints to production including biotic and abiotic stresses. Genetic diversity in rice is a tremendous asset which allows rice cultivation in diverse environments. It is also of great importance for rice improvement through breeding, as it translates into genetic variation for traits of interest to breeders. The genetic diversity among rice accessions and landraces from West Africa has been addressed by several authors using different collections of germplasm. These collections included a panel of African rice *O. glaberrima* characterized by Semon *et al*. (2005), rice accessions from Niger by Sow *et al*. (2013) and from Cote d'Ivoire by Sanni *et al*. (2008). However, not much work has been reported on the genetic characterization of a wider collection of West African accessions and landraces sourced across different countries of the sub-region. According to Sanni *et al*. (2012) an efficient exploration of the existing local germplasm may end up with the identification of useful genetic diversity for varietal improvement to increase rice production and help achieve food self-sufficiency in Africa. Useful genetic diversity could comprise of resistance or tolerance to several biotic and abiotic stresses (Semagn *et al*., 2007) including mineral stress such as iron toxicity (Dramé *et al*., 2010). The AfricaRice genebank contains about 20,000 accessions and collections from about 85 countries (Sanni *et al*., 2013). Characterizing the one from West Africa may have an important implication in breeding activities targeting local conditions. The Casamance region located in the Southern part of Senegal also has a long history of rice cultivation. Indeed, it is the main area in Senegal where *Oryza glaberrima* and *Oryza sativa* are both cultivated (Linares, 2002). Landraces from this region could also be of interest. The main objective of this study was to evaluate the genetic diversity of the collection of rice germplasm in order to classify the rice collection in useful genetic patterns for their application in further breeding programmes.

4.2 Materials and methods

4.2.1 Germplasm collection

The genetic material was composed of a diverse collection of 480 rice germplasm. This collection included accessions adapted to lowland ecologies from different countries of West Africa (276 accessions) sourced from AfricaRice genebank, landraces from Casamance (151 landraces) and a set of cultivars also from AfricaRice (45 genotypes) adapted to local conditions. Eight accessions of Azucena from IRRI genebank were also included. Rice germplasm from the southern part of Senegal corresponding to the Casamance region was collected in 2013 in the districts of Ziguinchor, Oussouye, Bignona Goudomp, Sedhiou and Bounkiling. The collection was done in 19 villages across the six districts (Table 4.1). In each village, the accessions were collected based on their agronomic characteristics and tolerance to iron toxicity as perceived by farmers. For each sample, one single panicle was collected from farmers.

For the genetic characterization purposes, six varieties were used as reference genotypes. These varieties were RAM 163 (*Oryza glaberrima*), NERICA 6 (interspecific variety from cross *O. sativa* and *O. glaberrima*), IR 64 (*O. sativa*, adapted to irrigated condition), Suakoko 8 and Bouake 189 (traditional *indica* rice) and Moroberekan (tropical *japonica* subsp).

Table 4.1 : Villages of collection of the rice landraces from Casamance (Senegal)

Districts	Village	Latitude	Longitude	Altitude (m)
Bignona	Dianki	12°51’49.2’’ N	16°27’45.0’’ O	6
Bignona	Thiobon	12°51’34.9’’ N	16°33’12.9’’ O	11
Bignona	Kabiline 1	12°58’36.9’’ N	16°34’56.5’’ O	5
Bignona	Kabiline 2	12°57’43.4’’ N	16°30’58.4’’ O	2
Bignona	Thionk-Essyl	12°48’21.2’’ N	16°30’51.2’’ O	8
Bignona	Karthiack	12°51’29.9’’ N	16°30’56.4’’ O	7
Bignona	Badiana	12°54’46.9’’ N	16°25’55.7’’ O	6
Bignona	Kawane	12°59’27.6’’ N	16°32’38.7’’ O	4
Bounkiling	Diacounda	-	-	-
Bounkiling	Nioroki	-	-	-
Goudomp	Birkama	-	-	-
Goudomp	Kaour	12°34'6.59"N	15°54'18.60"O	9
Oussouye	Mlomp	12°49’22.1’’ N	16°31’14.7’’ O	9
Oussouye	Djembering	12°28'44.40’’N	16°47'17.18’’O	8
Oussouye	Etoune	12°27’40.2’’ N	16°46’38.2’’ O	7
Sedhiou	Medina Souane	12°42'02.24"N	15°51'13.47"O	7
Ziguinchor	Kamobeul	12°30’19.7’’ N	16°25’48.9’’ O	10
Ziguinchor	Medina	12°30’04.7’’ N	16°23’18.6’’ O	10
Ziguinchor	Bandial	12°34’02.2’’ N	16°28’37.0’’ O	10

4.2.2 DNA extraction and genotyping

Genomic DNA of each genotype was extracted from leaves of two week-old plants according to the protocol of Doyle & Doyle (1990). The DNA extracted was diluted in a ratio of 1:3 with TE 1× buffer and used as working solution for the different PCR reactions. The PCR reactions were carried out in a total volume of 10 µl containing 10× PCR buffer, 1 mM of dNTPs, 25 mM $MgCl_2$, 1µM of each of the forward and reverse SSR primer, 1U of Taq polymerase and 1.5 µl of the DNA working solution. PCR programme was set up in a GSTORM thermocycler machine as follows: pre-denaturation (94° for 5min), followed by 35 cycles including denaturation (94° for 30s), annealing (40° for 1min) and extension (72° for 1mn) and a final extension of 72° for 5min. The PCR products and the DNA ladder were loaded in 8% non-denaturing polyacrylamide gel and separated by electrophoresis (PAGE) using an electric field of 100V. TAE 1× was used as electrophoresis running buffer. A concentration of 3% ethidium bromide was used to stain the gel for 10 minutes and it was then visualized under UV trans-illuminator. A total of 15 random SSR markers covering the 12 rice chromosomes were used for the genetic diversity study. Allele scoring was done visually based on the presence (1) and absence (0) of each DNA fragment.

4.2.3 Data analysis

Statistical analysis including number of alleles, frequency of major allele, polymorphism information content (PIC) and genetic diversity (GD) were performed using PowerMarker software V3.25 (Liu & Muse, 2005). Population structure was determined using STRUCTURE V2 software (Pritchard *et al.*, 2000) and genotypes assigned to populations based on arbitrary ancestry percentage of min 70%. A permutation test of 100000 (burn in period) and 200000 repetitions were computed and the probability of subpopulation number (K) was calculated following the procedure of Evanno *et al.* (2005). Cluster analysis using DARwin v5.0.156 programme (Perrier & Jacquemoud-Collet, 2006) was performed using the SSR molecular data transformed into binary code of 1 (presence) and 0 (absence) of allele. Based on the quality of the PCR products, 12 amplified out of the 15 SSR markers were selected for the analysis. Dice (1945) index of similarity was used to estimate genetic distance between the genotypes. The method of Neighbour Joining proposed by Saitou & Nei (1987) was used to construct the Phylogenic tree from the generated genetic distance. The PIC, which measures the allelic diversity in a locus was computed as follows

$$PIC = 1 - \sum_{i=1}^{n} pi^2$$

Where: i = the ith allele of the jth marker; n = the number of alleles at the jth marker; p = allele frequency

The Dice indices of similarity which consider only the presence of marker among individuals was computed as follows:

$$S_{(i,j)} = 1 - \frac{2a}{2a + (b + c)}$$

Where: a= number of markers in common between two individuals; b = number of markers present in individual i and absent in individual j; c= number of markers absent in individual i and present in individual j; S= index of similarity

4.3 Results

4.3.1 Allelic diversity

The genetic diversity among the total 480 rice genotypes and information referring to the markers are given in Table 4.2. A total of 56 alleles were generated with an average of 4.67 allele per marker. The number of alleles varied from 3 for RM 277 to 6 for RM 310. The PIC value varied from 0.39 (RM 277) to 0.78 (RM 310) with a mean of 0.59 per locus. Mean gene diversity of 0.63 was estimated considering the total collection. Results from the genetic diversity analysis performed on the separated subsets of the collection from AfricaRice and the landraces from Casamance are presented in Appendix 4.1 and 4.2. The collection from AfricaRice presented a mean genetic diversity of 0.67, whereas, the landraces had 0.50.

Table 4.2: Genetic diversity among the total rice genotypes and characteristics of the SSR markers used

Marker	Chr	Repeat type and length	Annealing Temp (°C)	PCR product size range	Major Allele Frequency	Allele No	GD	PIC
RM 105	9	(CCT) 6	55	131-140	0.51	4	0.66	0.61
RM 206	11	(CT) 21	55	128-202	0.44	5	0.72	0.68
RM 212	1	(CT) 24	55	112-134	0.67	5	0.52	0.49
RM 216	10	(CT) 18	55	127-143	0.71	4	0.46	0.43
RM 218	3	(TC)24 (ACT)5(GT)11	55	120-148	0.43	5	0.72	0.68
RM 241	4	(CT) 31	55	102-142	0.46	5	0.67	0.61
RM 248	7	(CT) 25	55	80-104	0.39	5	0.74	0.70
RM 249	5	(AG)5A2 (AG) 14	55	154-174	0.58	5	0.59	0.55
RM 253	6	(GA) 25	55	125-143	0.68	4	0.49	0.45
RM 277	12	(GA) 11	55	118-124	0.73	3	0.43	0.39
RM 279	2	(GA)16	55	148-174	0.32	5	0.75	0.70
RM 310	8	(GT) 19	55	85-120	0.26	6	0.81	0.78
Mean	-	-	-	-	**0.51**	**4.67**	**0.6306**	**0.59**

Chr chromosome, Temp temperature, PCR polymerase chain reaction, No number, GD genetic diversity, PIC polymorphic information content

4.3.2 Population structure

Cluster analysis using the Neighbour Joining method, showed three groups based on the Dice similarity index (Figure 4.1). The group Grp1 contained most of the *O. glaberrima* rice accessions in addition to the reference *glaberrima* accession RAM 163. The group Grp2 which contained most of the *sativa* genotypes was divided into two sub groups Grp2-1 and Grp2-2. The collection of accessions from West Africa sourced from AfricaRice genebank and the other reference genotypes including IR64, Suakoko 8, moroberecan and Bouake 189 belonged mainly to Grp2-2. The landraces from the Casamance were mainly in Grp2-1. The group Grp3 include most of the intermediate genotypes derived from interspecific crosses.

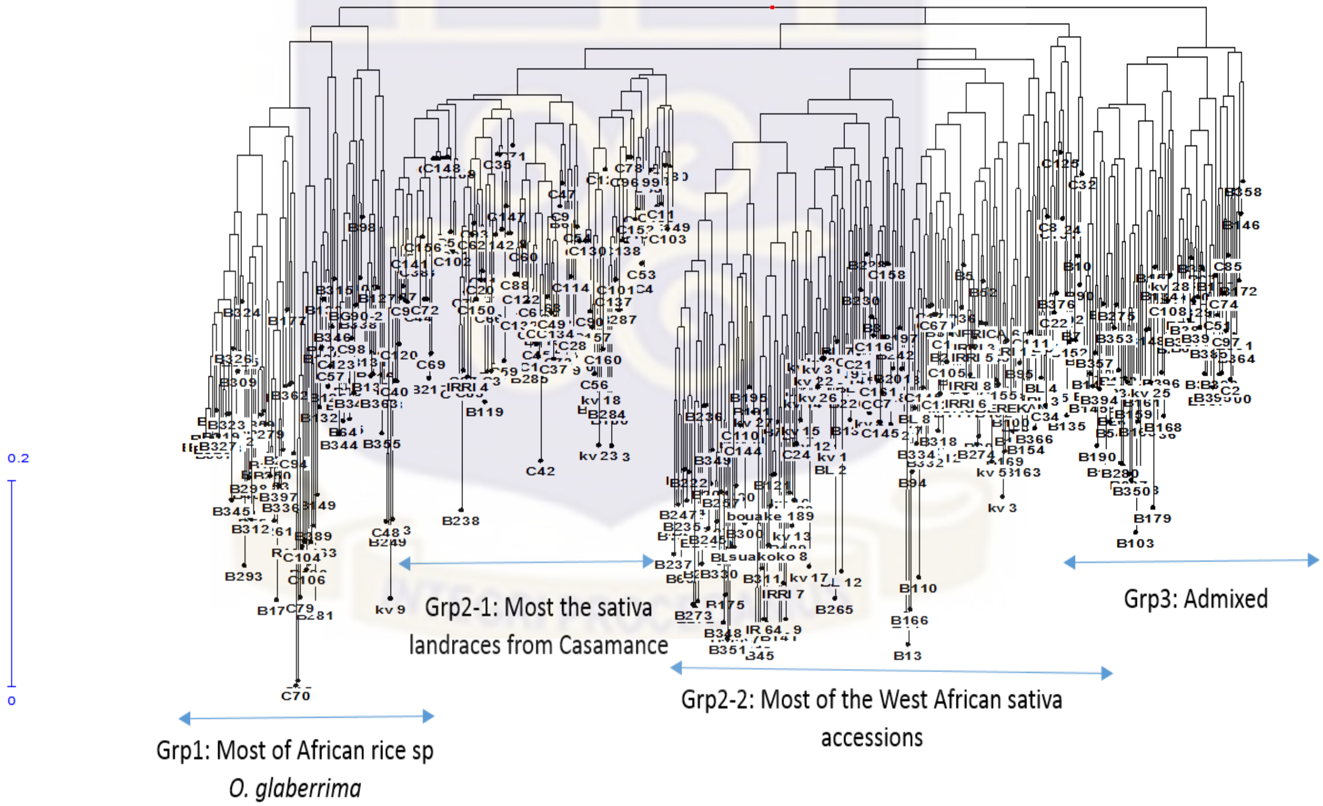


Figure 4.1: Dendrogram based on Neighbour Joining of the 480 rice genotypes

Population structure analysis performed with the STRUCTURE software also revealed an optimum number of sub populations of three (K=3) (Figure 4.2). Group 1 which represented 37% of the collection, was composed mostly of the *sativa* subspecies *indica* and *japonica* from the West African collection. Group 2 contained most of the *glaberrima* accessions and represented 9.8% of the collection. The landraces from Casamance were mainly in group 3 which represented 26.8% of the entire collection. Since the different groups were identified using the arbitrary ancestry percentage of minimum 70%, some admixed cultivars between *O. glaberrima* and *O. sativa* were uncovered. These cultivars represented 26.6% of the total collection.

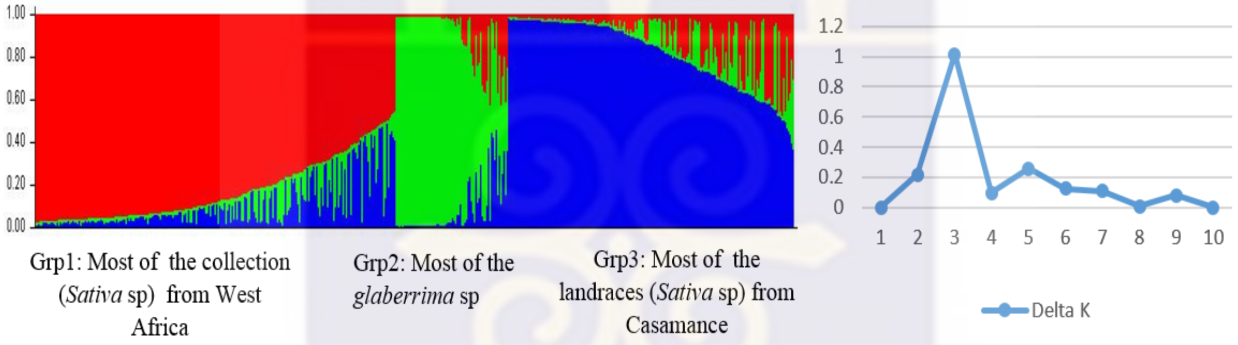


Figure 4.2: Population structure of the collection of 480 rice genotypes with 12 SSRs markers

4.3.3 Diversity distribution

The percentage of genotypes in each group based on their geographical distribution was calculated using statistical results from the structure analysis. Most of the accessions from Benin (56%), Burkina Faso (48%), Cote d'Ivoire (52%), Guinea (61%), Liberia (64%), Mali (72%), Sierra Leone (61%) and Senegal (63%) were in Grp1 which included most of the *sativa* subspecies *indica* and *japonica*. The improved varieties from AfricaRice (76%) were in the Grp1. Most of the accessions from Niger (57%) and the ones from Togo (81%) were in the *glaberrima* group which composed the Grp2. The eight Azucena accessions were in Grp3 dominated by the landraces from Casamance (74%). Only 4% of the landraces from Casamance belonged to the *glaberrima* group. The admixed or intermediate genotype included 67% and 37% of the accessions respectively from Guinea Bissau and Benin. Some of the landraces from Casamance (19%) were identified as admixed.

4.4 Discussion

An average allele number per SSR marker of 4.67 was obtained. This result was similar to what was reported by Onaga *et al.* (2013) who observed a mean of 4.61 in a collection of 30 rice accessions from Uganda, West Africa and IRRI. Higher mean allele per locus (9.89) was however found by Sow *et al.*, (2013) when studying 264 accessions from Niger. The mean allele number found in this study was also lower than the one observed from a wider collection of 234 rice accessions which was 11.8 per locus (Garris *et al.*, 2005). The mean amplified allele number in this study was however higher than the average of 3.28 per locus reported by Anandan *et al.* (2016) in a rice collection of 96 genotypes from India genebank. The lower allele number per locus detected in this study compared with the others could be explained by the difference in the SSR markers and the germplasm used. An averaged mean PIC (0.59) was observed in the entire collection used in this study. This was smaller than what was reported in other studies (Garris *et al.*, 2005; Sow *et al.*, 2013; Onaga *et al.,* 2013). The estimated mean genetic diversity (0.63) of the collection was also smaller than what was reported in these studies except for Anandan *et al.* (2016) who found a smaller value for both GD and PIC for the Indian rice accessions. The PIC value of SSR markers was reported to depend on the population used and the distribution of the markers (Anandan *et al.*, 2016). The number of amplified alleles may also influence the PIC since strong relationship was found between them (Onaga *et al.*, 2013). The landraces included in the collection could lower the PIC and GD of the collection since they were expected to be less diverse. Higher diversity was obtained when the analysis was performed only on the 272 accessions from West Africa. The average GD of these accessions was 0.67, similar to many studies including the worldwide collection of rice accessions assessed by Garris *et al.* (2005) (0.7) and the collection from Niger (0.69) (Sow *et al.*, 2013). Further, these accessions presented an average PIC (0.63) also similar to the one obtained by Garris *et al*. (2005) and Ni *et al*. (2002) who characterized a collection of 40 *indica*, tropical *japonica*, temperate *japonica* and wild relative accessions with 111 SSR markers. The analysis performed on the landraces from Casamance alone confirmed the fact that they represented a relatively low genetic diversity (0.50) compared to the West African collection (0.67). This can be explained by the fact that most of the farmers shared seeds and the same landrace may be named differently across the Districts. The genetic distribution observed in the entire collection showed the efficiency of the SSR markers as useful tool for studying and discriminating the genetic diversity existing in rice crop (McCouch *et al*., 2002). The dendrogram as well as the population structure analysis classified the germplasm collection into three groups. These groups were represented by the *Oryza glaberrima* specie, the *O. sativa* which divided into two subgroups (Grp2-1 and Grp2-2) and the group of admixture mainly from interspecific crosses. The *glaberrima* specie (Grp1) was well separated from the *sativa* specie as it was previously observed in some genetic diversity studies of rice germplasm using SSR markers (Sow *et al*., 2013). The *Oryza sativa* specie was more represented than the *Oryza glaberrima* in this collection of 480 genotypes. Further, most of these *sativa* specie belonged to the *indica* sub-species probably because they are the more widely cultivated in West Africa. The landraces from Casamance, mainly of *indica* subsp, were clustered differently from the other West African accessions since they were mostly concentred in one of the *O. sativa* subgroup (Grp2-1). Only 4% of the Casamance landraces were identified with the SSR markers as *O. glaberrima* sp. This, regarding the fact that as for some areas in West Africa, the Casamance region traditionally showed high interest in the cultivation of *O. glaberrima* sp (Linares, 2002). This is a general observation in Africa where *O. sativa* is increasingly replacing *O. glaberrima* rice varieties (Sie, 1991; Montcho *et al*., 2013). New technologies including modern high yielding varieties have been intensively introduced in many rice cultivated areas in West Africa to boost rice productivity. However, there are not coordinated actions from government and research institutes for an appropriate conservation of local germplasm. The loss of traditional rice germplasm and landraces is critical for plant breeding and improvement of modern cultivated varieties (Sanni *et al.*, 2008) because local germplasm are valuable sources of adaptation to local conditions.

4.5 Conclusions

The genetic diversity study of the entire collection including rice landraces from Casamance and accessions from West Africa showed an intermediate mean genetic diversity. The landraces were however less diverse than the collection from AfricaRice which was composed mainly of West African accession which presented similar diversity as most of the reported studies on different rice panels. The collection contained more *Oryza sativa* mainly of *indica* type than *Oryza glaberrima*. There were differences in the *indica* landraces from Casamance and those of the collection sourced from AfricaRice genebank. The relatively good genetic diversity of the total collection could be exploited for genetic improvement of cultivated varieties.

CHAPTER FIVE

5.0 ASSESSEMENT OF THE RICE GERMPLASM FOR TOLERANCE TO IRON TOXICITY

5.1 Introduction

Addressing the constraint of iron toxicity should necessarily include the identification of sources of tolerance for the improvement of elite varieties. The use of high yielding varieties performing under different conditions of high Fe content could considerably increase rice productivity and therefore help to achieve self-food sufficiency in many West African such as Senegal. However most of the tolerant varieties so far identified are not currently used in many countries of West Africa. Some of them, specifically the traditional type, present low yielding potential even though they are tolerant (Audebert & Fofana, 2009). Some are not well adapted in West African conditions as the azucena varieties (*japonica* subsp.) which failed to perform in local hot spot lowland ecologies (Dufey *et al*., 2012). In addition to that, the GxE interaction also interferes in the tolerance of some of these genotypes. AfricaRice research centre has, however, recently identified tolerant varieties which are being or released in some West African countries such as Guinea, Burkina Faso and Ghana (AfricaRice, 2017), a lack of widely adapted cultivars is still observed. More sources of tolerance are needed to overcome the constraint of iron toxicity. Rice accessions and cultivars grown in West Africa are supposed to be locally adapted to most of the major biophysical constraints existing in the sub-region due to the rich genetic diversity they hold. The evaluation of these rice germplasm for tolerance to iron toxicity was suggested by Dramé *et al*. (2010) with the fact that they may present different tolerance mechanism which could be interesting for improving rice varieties. Furthermore, the traditional germplasm were reported to have higher tolerance and more consistency in the genetic responses across different iron toxicity environments (Audebert & Fofana, 2009). For more achievement, however, it is important to carry out the evaluations of the germplasm under controlled environment to ward off any extraneous factors. It is equally important to assess the genetic material under their natural habitat.

The objectives of this study were to:

1. assess the genetic variability of the panel of rice germplasm for tolerance to toxic level of iron (Fe^{2+}) in hydroponic and natural hot spot fields; and
2. identify stable and tolerant genotypes as potential donors for ferrous iron tolerance.

5.2 Materials and Methods

5.2.1 Plant material

The plant material comprising 282 genotypes (Appendix 5.1) was selected from the collection of 480 rice genotypes. The selection of these genotypes was based on their distribution and level of genetic diversity detected from the genetic characterization study. The plant material included a collection of 202 rice genotypes sourced from AfricaRice genebank and 80 traditional varieties from Casamance. The collection from AfricaRice genebank was composed for 59% of accessions from West African countries and the rest adapted to West African local conditions. Two standard check varieties, Suakoko 8 as tolerant and Bouake 189 as susceptible (AfricaRice, 2006) were used for the hydroponic screening. For the field evaluation two check varieties DJ 684-D and BG90-2, which are widely cultivated in lowland ecologies in Casamance, were used as local checks in addition to the standard ones.

The 282 genotypes plus the check varieties were evaluated under hydroponic and under field conditions (lowland hot spot field) at Djibelor and Ndeman valley.

5.2.2 Hydroponic screening

The experiment was conducted at AfricaRice Sahel Station (16° 10' N, 16° 11' W) during the dry season of 2015. The screenhouse temperatures varied between 25.1°C and 41.4°C with relative humidity (RH) varying from 41 to 76.7%.

The hydroponic screening method was adopted from Wang *et al.* (2008) where Fe precipitation which can bias the genotypic response, was partially taken into account by increasing the viscosity of the culture solution with agar. Some modifications referring to the iron concentration and the time of screening were performed. Fifteen seeds per genotype were first pre-germinated on a wet paper towel in petri dishes using distilled water. After four days, six uniform seedlings were transferred in one row of six wells (one seedling/well) of a perforated polystyrene container (12 x 8 wells). The genotypes were assigned randomly in the polystyrene and maintained floating in a plastic tank containing distilled water for three days. This was followed by one week growth in a modified full-strength Yoshida culture solution (Yoshida *et al.*, 1976) before the Fe stress treatment was applied. The culture solution was composed of NH_4NO_3 1.41 mM; KH_2PO_4 0.26 mM; K_2HPO_4 56.7 µM; K_2SO_4 0.5 mM; $CaCl_2$ 1 mM; $MgSO_4$ 1.62 mM; $MnCl_2.4H_2O$ 9.3 µM; $(NH_4)_6.MO_7O_{24}.4H_2O$ 0.07 µM; $ZnSO_4.7H_2O$ 0.15 µM; H_3BO_3 18.6 µM; $CuSO_4.5H_2O$ 0.15 µM and 10 mg L^{-1} of Fe ($FeSO4.7H_2O$). The stress conditions consisted of supplementing the Yoshida solution with 1500 mg L^{-1} of Fe^{2+} added as $FeSO4.7H_2O$, whereas the non-stress contained 10 mg L^{-1} of Fe of the Yoshida culture solution. In both Fe treatments (stress and non-stress), agar was added (1000 mg L^{-1}) to the nutrient solution. The agar was first dissolved in boiling deionized water and kept to cool to approximately 60°C before adding it to the trays. The pH of the culture solution was then adjusted to 5.6 using either 1 N HCl or NaOH. The nutrient solution was renewed every week and the pH adjusted once. The experiment was laid out in a randomized complete block design (RCBD) with two replications for the stress and the non-stress condition. The screening period was considered as block.

Data on leaf bronzing score (LBS), plant height (PH) and root length (RL) were recorded on the four middle plants for each genotype after 10 days of Fe screening. Harvested shoot and root samples of these plants were oven dried at 70°C to attain a constant weight, the shoot dry weight (SDW) and root dry weight (RDW) were then determined. The SPAD value considered here as chlorophyll content (CC) of the middle upper face of the youngest fully expanded leaf was measured on four plants using a chlorophyll meter (SPAD 502 Konica model). Leaf bronzing symptom was visually assessed using the Standard Evaluation System for rice (IRRI, 2002) with a scale of 1 to 9 (Appendix 5.4). Shoot and root iron content of the 20 most susceptible and tolerant genotypes were quantified using an Atomic Absorption Spectrophotometer. Figure 5.1 gives an illustration of the screening process.

(a) Effect of iron toxicity on the check varieties

(b) Plant growth in control and stress condition

(c) Symptom of iron toxicity on the leaves "leaf bronzing"

(d) Effect of iron toxicity on root density

Figure 5.1: Effect of Fe stress on leaves and plant growth after 10 days exposure

5.2.3 Field evaluations

The field experiments were conducted during the rainy season of 2016 in two lowland iron toxicity hot spot fields, namely Djibelor (12° 33' N, 16° 18' W) (Southern Senegal) and Ndeman valley located in the village of Somita (13° 12'N, 16° 18' W) (West Coast Region of The Gambia). In each location, one stress experiment was conducted. A non-stress experiment was also established in Djibelor in a less affected field, assumed based on the *in-situ* pH value of ~7. The pH is usually considered as an indicator of possible occurrence of iron toxicity (Fageria *et* *al.*, 2008). Composite soil sampling of the plough layer was done at the fields in Djibelor and in Ndeman. This consisted of collecting soil from each corner and the middle part of the land. Some physico-chemical properties of the soils including particle size analysis, pH, total N and P, cation exchange capacity and exchangeable bases and Fe contents were subsequently determined.

5.2.3.1 Soil characteristics and seasonal conditions

The physical and chemical soil properties of the different fields in Djibelor and Ndeman are summarized in Table 5.1. Variation of *in-situ* water pH of the field experiment at Djibelor, measured every week, and climate data referring to the rainfall, the temperature and the humidity are summarized in Figure 5.2.

Table 5.1: Soil characteristics of the experimental fields at Djibelor and Ndeman

	Djibelor soil characteristics in field stress condition	Ndeman soil characteristics in field stress condition	Djibelor soil characteristics in non-stress field
Clay (%)	20.5	16.9	16.2
Silt (%)	16.2	41.5	21.9
Sand (%)	61.1	38.5	59.2
pH (H2O)	4.75	4.99	4.69
pH (KCl)	4.24	3.97	4.18
N (NO3) (mg kg-1)	0	0.53	0.08
N (NH4) (mg kg-1)	6.05	0.01	22.8
P (mg kg-1)	19.35	6.05	7.79
CEC (cmol kg^{-1})	9.35	5.61	8.27
Ca (g kg-1)	0.01	0.4	0.74
Mg (mg kg-1)	459	534	1413
Na (mg kg-1)	79	53	730
K (mg kg-1)	488	462	1046
Fe (mg kg-1)	25.27	7.83	19.32

CEC Cation Exchange Capacity; Djibelor (Lower Casamance region, Senegal); Ndeman valley (West Coast Region, The Gambia)

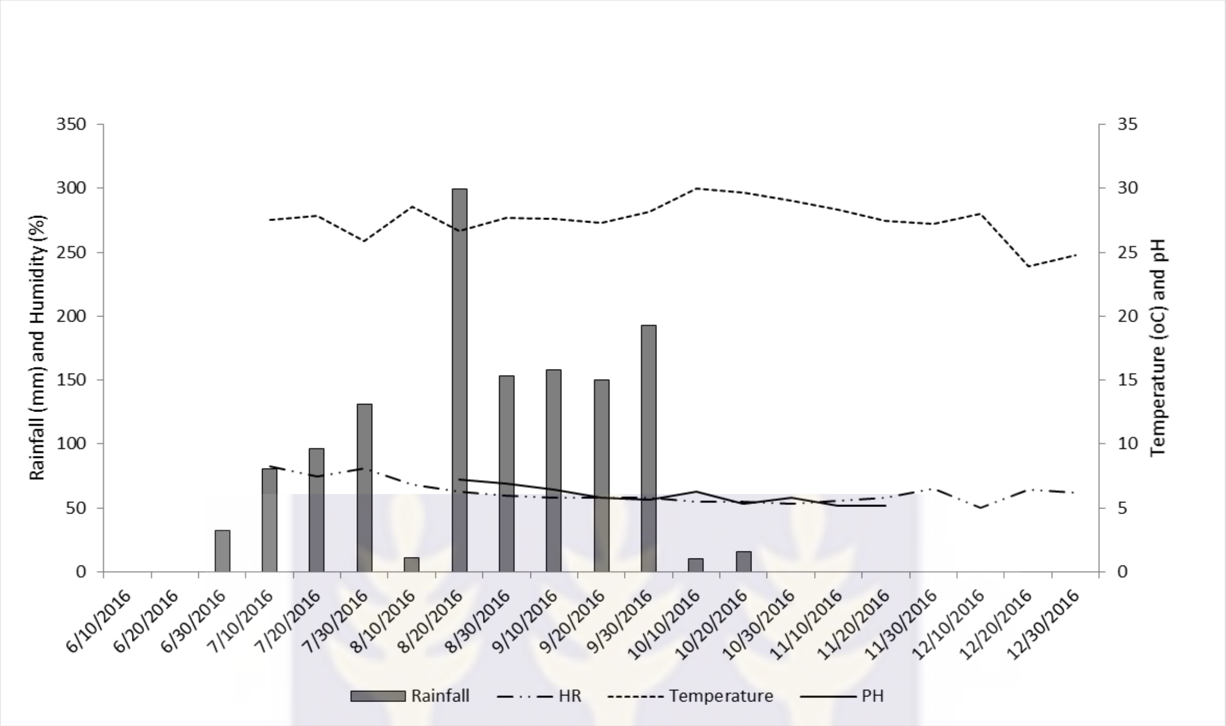


Figure 5.2: Climate conditions and *in-situ* pH of the stress field at Djibelor during 2016 rainy season

5.2.3.2 Experimental design and field managements

For the field experiments, ten out of the 282 rice genotypes did not germinate in the nursery. The trials were therefore laid out in an augmented design (Federer & Raghavarao, 1975) consisting of 16 blocks of 21 plots each. The plots were represented by four rows of 1m with six hills per row. The spacing between rows was 20 cm and 40 cm between plots. The blocks were spaced 40 cm apart. Seedlings of 30 days old were transplanted at a rate of one plant/hill with a spacing of 20 cm after ploughing and flooding. Combination of nitrogen (N) phosphorus (P) and potassium (K) known as NPK (15 15 15) mineral fertilizer was applied at a rate of 200 kg ha^{-1} just after transplanting. Urea was also applied twice as source of nitrogen in a dose of 75 kg ha^{-1} at 20 and 45 days after transplanting (DAT). Manual weeding was done 15, 30 and 45 DAT.

Leaf bronzing symptom was assessed at 45 and 60 DAT following the Standard Evaluation System (IRRI, 2002). Chlorophyll content (CC) at 60 DAT, plant vigour (vigour) and percentage of recovery (PR) were also recorded. Data on agronomic traits such as tiller number (tiller), plant height (PH), flowering and maturing periods, dry biomass production (DBM), yield and yield component including spikelet sterility (STR), 100 grain weight, mean panicle dry weight (PW) and panicle number (PanN) were also recorded.

5.2.4 Data analysis

Data recorded on the different traits were analysed using the SAS version 9.4 software (SAS Institute Inc. 2013). The general linear model was used for the analysis of variance (ANOVA) of data recorded from the hydroponic screening. For the field experiments, the ANOVA was performed for each location using the SAS macro procedure for analysing augmented design. Pooled data were subsequently analysed after performing a chi square test of homogeneity then subjected to an ANOVA analysis using the restricted maximum likelihood (REML) approach. Leaf bronzing scores were transformed using the square root method of transformation. The multivariate analysis, performed on the adjusted mean for each variable, included cluster analysis and Pearson's correlation to define relationships between variables recorded under stress using the XLSTAT 2014 software. The cluster analysis were performed using the Ward methods (Ward, 1963) with dissimilarity based on Euclidian distance.

Selection index on iron tolerance related traits (SI_{ITRT}) was performed following the method of Trouche *et al*. (2011) to identify the best performing genotypes in stress field in Djibelor and Ndeman. Four agronomic traits related to iron toxicity tolerance were considered including GY, DBM, PanN and HI.

$$SI_{ITRT} = \sum_j aj * \left[\frac{x_{ij} - m_j}{s_j} \right]$$

Where: xij is the phenotypic value of the individual i for trait j, m_j the overall mean and s_j the standard deviation of all lines for trait j, aj is the relative weighting of trait j in the index, where score of 4 for GY, 3 DBM, 2 PanN and 1 HI.

Relative reduction of mean value was computed for each trait by calculating the reduction percentage taking into account the performance of the genotype in stress and non-stress conditions.

$$RRT = [(Vc - Vs)/Vc] * 100$$

Where: RRT is the relative reduction of the trait (T); Vc is the value of the trait (T) under control; Vs the value under iron toxicity stress and PR_T the reduction of the trait (T).

To determine the mechanism of Fe uptake and translocation, the bio-concentration factor (BCF) known as iron accumulation in the roots and the translocation factor (TF) were estimated using methods described by Bao *et al.* (2009).

$$BCF\ (\%) = [Fe_{root}/(\ Fe_{shoot} + Fe_{root})] * 100$$

$$TF\ (\%) = [Fe_{shoot}/(Fe_{shoot} + Fe_{root})] * 100$$

Where: Fe_{shoot} and Fe_{root} represented respectively iron concentration (mg kg^{-1}) in the shoot and the root.

The different models underlining the method of analysis of variance for the RCBD with two environments (stress and non-stress hydroponic conditions) (a) and the two location augmented design (b) were defined as follows:

$$\text{(a)}\ \ Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

$$\text{(b)}\ \ Y_{ijk} = \mu + G_i + I_j + G * I_{ij} + \beta_k + e_{ijk}$$

Where:

Y_{ijk} = Plot value of genotype i at environment j in the block k; μ = general mean; α_I = is the effect of i-th line; β_j = the effect of the j-th level of environment; $(\alpha\beta)_{ij}$ = interaction effect; e_{ijk} = residual error; G_i = effect of genotype i; I_j = effect of location j; $G*L_{ij}$ = interaction effect of genotype i with location j; β_k = the effect of the j-th incomplete block.

5.3 Results

5.3.1 Hydroponic screening

5.3.1.1 Effect of the Fe concentration on the genotypes

The concentration of 1500 mg L^{-1} Fe^{2+} used to induce iron toxicity resulted in significant genotype responses for all the parameters assessed (Table 5.2). The variations observed among the different genotypes were significant for all traits except for SDW. The analysis of variance showed differences between the stress and the non-stress conditions for all the variables recorded. The interaction between genotypes and treatments was however not statistically significant for all the recorded traits. Significant reduction was observed in plant performance. SDW and RDW showed the highest reduction percentage with 59% and 53% respectively. Reduction of 32% and 25% were observed for PH and RL respectively while chlorophyll content (CC) was reduced by 20%.

Table 5.2: Effect of the iron treatment and relative reduction in mean performance

Variation	DF	Mean square				
		SDW	RDW	PH	RL	CC
Treatment	1	2.3***	0.13***	77733.9***	2120.6***	8848.2***
Genotype	278	0.0029^{ns}	0.0005**	210.0***	8.9**	12.1***
genotype*treatment	262	0.0019^{ns}	0.0003^{ns}	41.5^{ns}	4.6^{ns}	8.0^{ns}
Reduction %		59	53	32	25	20

SDW shoot dry weight, RDW root dry weight, PH plant height, RL root length, CC chlorophyll content, *** = significant difference at 0.001 level of probability, ** significant at 0.05, ns not significant

5.3.1.2 Relative variation in the performance of the genotypes

Table 5.3 gives a summary of the observed phenotypic variations in non-stress and stress conditions. For each variable, the mean performance of the overall population were compared to the two checks, Suakoko 8 and Bouake 189. The susceptible check Bouake 189 had the highest reduction of SDW (67%) compared to Suakoko 8 (55%) and the overall population (59%). The same observation made in the variation of SDW for Bouake 189, was also noticed for all the other variables when comparing with the overall population and Suakoko 8.

Table 5.3: Agro-morphological variability of the genotypes in stress and non-stress conditions

Variable	Treatment	Min	Max	Adjusted Mean	SD	Suakoko 8	Bouake 189
SDW	Stress	0.006	0.225	0.07	0.03	0.07	0.05
	No stress	0.012	0.45	0.17	0.08	0.16	0.16
RDW	Stress	0.002	0.18	0.02	0.02	0.03	0.02
	No stress	0.003	0.225	0.04	0.03	0.05	0.05
PH	Stress	15.18	68.75	36.92	9.22	31.53	27.47
	No stress	18.9	80.67	54.54	10.46	42.26	43.57
RL	Stress	1.12	19.3	8.86	2.96	9.54	7.31
	No stress	5.6	22.25	11.80	2.27	11.93	11.32
CC	Stress	13.2	32.7	23.82	3.34	25.69	22.33
	No stress	20.4	38	29.82	3.07	31.96	31.97
SQRT LB	Stress	1	3	2.00	0.42	1.8	2.34

SDW shoot dry weight, RDW root dry weight, PH plant height, RL root length, SQRT LB squared root transformation of leaf bronzing, CC chlorophyll content, SD Standard deviation.

The phenotypic variations of the 40 most susceptible and 40 most tolerant genotypes were compared to Bouake 189 and Suakoko 8 (Figure 5.3). The tolerant and the susceptible genotypes were selected based on leaf bronzing score which was highly and negatively correlated to all the traits assessed under stress conditions. The susceptible check, Bouake 189 and the selected susceptible genotype had the lowest mean for RDW, SDW, RL and CC. Score value of leaf bronzing was also higher in Bouake 189 than for Suakoko 8 (tolerant check) and the selected tolerant genotypes. Comparing the tolerant genotypes with Suakoko 8, higher performance was obtained with the tolerant group for SDW and PH. Similar performances were however observed for Suakoko 8 and the tolerant genotypes when considering chlorophyll content, RL and RDW. Suakoko 8 and the tolerant genotypes were observed to be the better performing genotypes for most of the traits evaluated under stress.

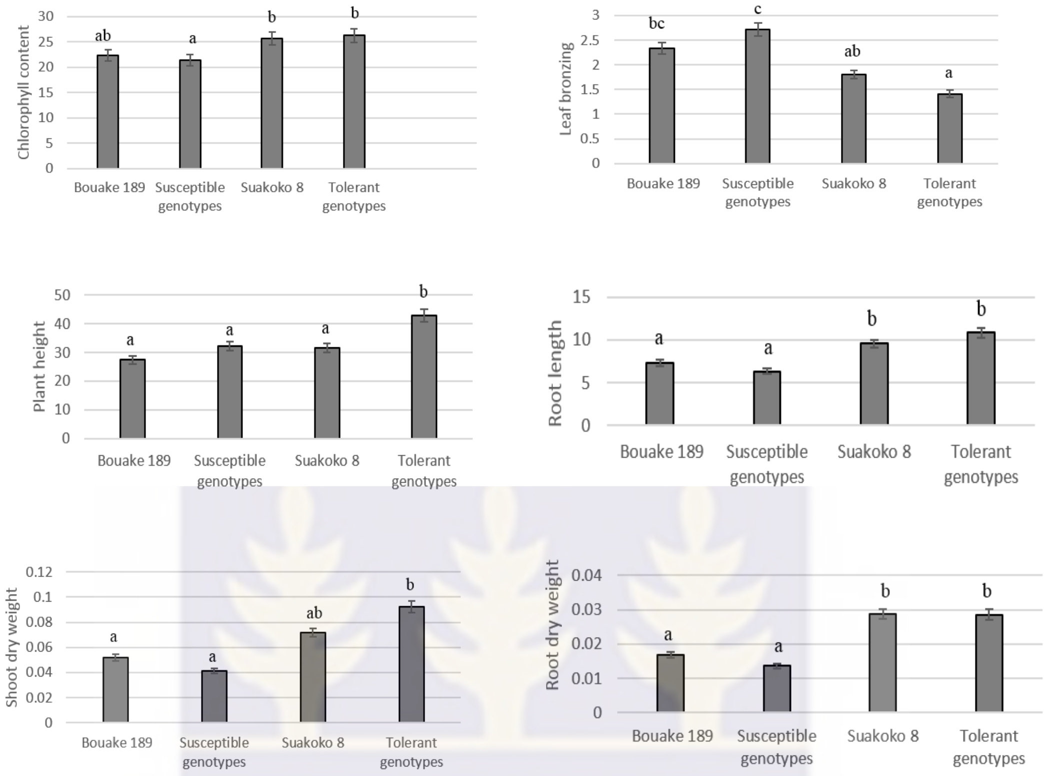


Figure 5.3: Phenotypic performance of the 40 most tolerant and susceptible genotypes compared with the check varieties

Histogram representing the mean with error bars showing the same letter were statistically the same at $p < 0.05$ with the Tukey's HSD test

5.3.1.3 Correlation among variables evaluated under stress conditions

Leaf bronzing score was highly and negatively correlated (*p* value of 5%) with all the variables including shoot dry weight, root dry weight, plant height and chlorophyll content (Table 5.4). Strong and positive correlation also existed among the agro morphological variables of shoot dry weight, root dry weight, root length, plant height and chlorophyll content.

Table 5.4: Relationship between traits in hydroponic stress conditions

	Person correlation Coefficients				
Variables	SDW	RDW	PH	RL	CC
SDW					
RDW	**0.30**				
PH	**0.44**	**0.16**			
RL	**0.57**	**0.28**	**0.30**		
CC	**0.32**	**0.25**	**0.13**	**0.18**	
LBS	**-0.57**	**-0.20**	**-0.19**	**-0.35**	**-0.53**

SDW shoot dry weight, RDW root dry weight, PH plant height, RL root length, CC chlorophyll content, LBS leaf bronzing score. In bold the significant correlation at 5% level of significance

5.3.1.4 Distribution of iron concentration in plant tissues and its relationship with tolerance

Among the top 40 tolerant genotypes selected based on leaf bronzing scores, 20 were subjected to an analysis of shoot and root iron content. The results showed that the tolerant genotypes had a relatively low iron content in the stem and the leaves even though the iron concentration in the roots were significantly higher (Table 5.5). The highest root iron content was recorded with TOS 10526, WAS 20-B-B-1-2-2 and TOS 10490. For shoot Fe concentration, the highest were obtained with Ewintohi and TOS 10867 and the lowest with WAS 21-B-B-20-4, IR 75887-1-3-WAB-1, CK 1 and the landraces Lano, CFA, Kabanfany and Etering. Results from the regression analysis (Figure 5.4) showed that high accumulation of iron in the shoot lead to a decrease in chlorophyll content and shoot dry weight. Shoot iron content significantly and negatively affected CC ($R^2 = 0.20$) and SDW ($R^2 = 0.24$).

LBS showed however a very weak relationship with shoot iron content ($R^2 = 0.07$). It was also observed that shoot iron content was highly influenced by the root iron content ($R^2 = 0.27$).

Table 5.5: Fe content of shoot and root of 20 performing genotypes compared with the checks

Accession/variety name	Species	Shoot Fe mg kg^{-1}	Root Fe mg kg^{-1}	BCF (%)	TF (%)
Etering	*O. sativa*	2229	89141	97.56	2.44
Kabanfany	*O. sativa*	2313	58744	96.21	3.79
CK 1	*O. sativa*	2328	45769	95.16	4.84
CFA	*O. sativa*	2402	87753	97.34	2.66
Lano	*O. sativa*	2512	113368	97.83	2.17
IR 75887-1-3-WAB-1	*O. sativa*	3864	-	-	-
WAS 21-B-B-20-4	*O. sativa*	4022	123726	96.85	3.15
Suakoko 8	***O. sativa***	**4151**	**105665**	**96.22**	**3.78**
Ration	*O. sativa*	4609	138713	96.78	3.22
TOS 876	*O. sativa*	4810	117689	96.07	3.93
TOS 10526	*O. sativa*	5042	176930	97.23	2.77
BEN11-172	*O. sativa*	5457	120958	95.68	4.32
WAS 20-B-B-1-2-2	*O. sativa*	5472	165005	96.79	3.21
DANYI MOLI-A	*O. glaberrima*	5534	103115	94.91	5.09
WAB 2098-WAC 1-FKR 2-4-TGR 1	*O. sativa*	5667	114794	95.3	4.7
TOS 6919	*O. sativa*	5693	115256	95.29	4.71
TOS 10490	*O. sativa*	6255	156074	96.15	3.85
TY 52	*O. glaberrima*	6436	85132	92.97	7.03
Gbangbalony	*O. sativa*	6446	91398	93.41	6.59
TOS 10867	*O. sativa*	7191	148518	95.38	4.62
EWINTOHI	*O. glaberrima*	7518	96628	92.78	7.22
Bouake 189	***O. sativa***	**7691**	**167223**	**95.6**	**4.4**
Mean		4893	115314	95.8	4.2
SE		371.1	7550	0.31	0.31

BCF bio concentration factor, TF translocation factor. The check varieties Suakoko 8(Tolerant), Bouake 189 (Susceptible) are in bold, SE standard error

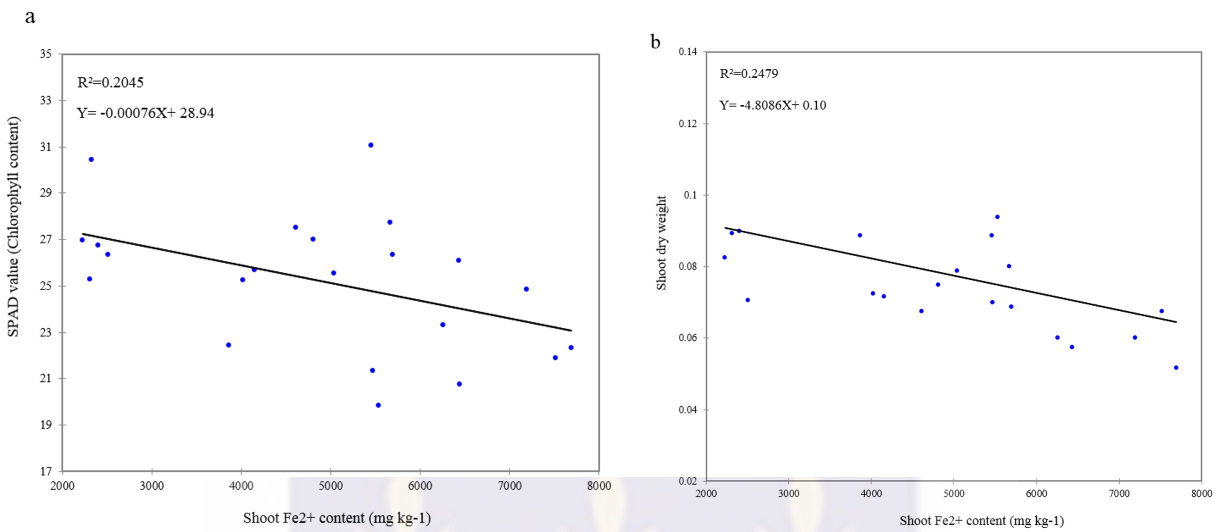


Figure 5.4: Relationship between Fe_{shoot} and SPAD value (a) and Fe_{shoot} and SDW (b)

Fe_{shoot} shoot iron content, SDW shoot dry weight

5.3.2 Field evaluations

5.3.2.1 Phenotypic variation

The analysis of variance showed significant effect of location on the recorded traits except for spikelet sterility which did not vary significantly across the locations (Table 5.6). This high variation was also observed for the different genotypes except in harvest index. Leaf bronzing score was not influenced by the GxE at 45 DAT while the one recorded at 60 DAT was however highly significant. Genotype by environment interaction was significant for flowering time, tillering ability, biomass production, harvest index, panicle number and mean panicle dry weight.

Mean value of each agronomic variable varied across the different field experiments (Table 5.7). The mean squared root of leaf bronzing score was 1.5 in Ndeman and 2.0 in Djibelor at 45 DAT, whereas at 60 DAT it varied between 1.7 and 2 respectively in Ndeman and Djibelor. Plant biomass varied from 365 to 3538 g m^{-2} at Ndeman and 100 to 2917 g m^{-2} at Djibelor in stress conditions. The overall mean of biomass production at Ndeman and Djibelor were for 1660 g m^{-2} and 982.5 g m^{-2} respectively. In the non-stress experiment at Djibelor, dry biomass varied from

200 to 4583 g m^{-2} with a mean of 2221 g m^{-2}. Mean panicle dry weight and spikelet sterility in the stress conditions varied from 886.6 g m^{-2} to 322.6 g m^{-2} and 10.6 to 17.8 respectively in Ndeman and in Djibelor. The earliest and latest flowering time in the experiment at Ndeman were observed in the landraces from Casamance which varied from 62 for the landrace Kosmida (*O. sativa*) to 116 days for Bem Egnou (*O. sativa*). In Djibelor, the number of days from sowing to flowering varied between 72 and 134 days for MAWOU-E (*O. glaberrima*) and Samou gbeli (07) (*O. sativa*) respectively under stress. Under non-stress, the flowering time varied from 78 days to 139 days respectively for IS 692 (*O. sativa*) and Samou gbeli (80) (*O. sativa*). Mean grain yield was 750 g m^{-2} at Ndeman and 296 g m^{-2} at Djibelor for the stress conditions and 682 g m^{-2} in the non-stress field.

Significant decrease was observed in the performance of the genotypes when compared the performance under stress and non-stress conditions at Djibelor. High decrease in plant biomass (55%), mean panicle dry weight (60%) and grain yield (56%) was observed. Plant height, tillering ability and chlorophyll content were also reduced by 20%, 27% and 11% respectively. The tolerant and susceptible checks, Suakoko 8 and Bouake 189, showed a reduction of dry biomass of 69 and 52% while that of the locally adapted checks varieties, DJ 684-D and BG90-2, were reduced by 49 and 39%. Similar response was also observed in the standard checks for the yield related traits (panicle number and grain yield) when compared to the local inbred lines DJ 684-D and BG90-2. Reduction in chlorophyll content was however lower for Suakoko 8 (7%) than for Bouake 189 and DJ 684-D (13%). Frequency distributions of plant vigour and leaf bronzing score at Djibelor and Ndeman are presented in Figure 5.5 and Figure 5.6. This variation was from 1 to 9 (non-transformed values) for both plant vigour and leaf bronzing at 60 DAT in the two locations. Mean leaf bronzing was 3 at Ndeman and 4 at Djibelor.

Table 5.6: Combined analysis of variance of traits recorded under hot spot fields at Djibelor and Ndeman

Effect	DF	F value										
		Flowering	Tillering	PH	DBM	HI	PanN	PW	GY	STR	LBS 45	LBS 60
Location (L)	1	6.7***	2.6***	6.2***	1.9**	1.7**	2.2***	2.1***	1.8**	1.3ns	1.4*	2.5***
Genotype (G)	272	171.0***	19.0**	63.5***	69.4***	0^{ns}	91.9***	108.7***	99.5***	147.5***	63.8***	25.8***
G x L	254	2.5***	1.9**	1.3^{ns}	1.5*	1.5*	1.5*	1.5**	1.3ns	1.3ns	1.2ns	2.2***

DF= degree of freedom, PH plant height, DBM dry biomass production, HI harvesting index, PanN panicle number, PW panicle weight, GY grain yield, STR spikelet sterility, LBS leaf bronzing score 45 and 60 DAT; *, **, ***, Significant at 0.05, 0.01 and 0.001 probability levels, respectively, ns not significant

Table 5.7: Phenotypic variability and relative decrease of mean values in stress and non-stress conditions at Ndeman and Djibelor

	Stress field at Ndeman					Stress field at Djibelor					Non-stress field at Djibelor					
	Mean	Std	SE	Min	Max	Mean	Std	SE	Min	Max	Mean	Std	SE	Min	Max	RV%
Flowering	96.0	8.1	0.5	62.0	116.0	106.0	10.0	0.6	72.0	134.0	103.7	11.0	0.6	77.9	138.7	-
Tillering	176.4	69.2	3.9	50	400.0	153.5	57.5	3.2	33	345.8	212.0	56.6	3.1	60.0	445.8	27.6
PH	107.6	23.1	1.3	52.8	178.4	91.6	22.3	1.2	48.3	160.2	115.8	23.4	1.3	66.3	182.8	20.9
DBM	1660.0	704.2	39.7	365.3	3538.0	982.5	482.6	26.6	100	2917.0	2221.0	854.8	47.5	200.0	4583.0	55.8
HI	0.3	0.1	0.005	0.1	0.5	0.3	0.1	0.005	0.1	0.5	0.3	0.1	0.005	0.008	0.7	5.8
PanN	375.7	151.8	8.6	33.3	858.3	254.1	112.7	6.2	50.0	650.0	459.2	173.6	9.6	75.0	1100.0	44.7
PW	886.6	400.3	22.7	125.0	1800.0	322.6	190.8	10.5	16.6	966.7	811.1	376.6	20.7	25.0	2108.0	60.2
GY	750.4	286.9	16.1	100.0	1000.0	296.0	178.8	9.9	25.0	900.0	682.2	318.6	17.7	8.3	1475.0	56.6
STR	10.6	3.7	0.2	2.0	22.0	17.8	9.8	0.6	2.0	78.0	9.0	4.9	0.3	1.0	35.0	-78.0
CC	-	-	-	-	-	33.8	3.8	0.2	21.5	46.5	38.3	4.1	0.2	25.3	48.8	11.7
LBS 45 DAT	1.5	0.5	0.0	1.0	3.0	2.0	0.4	0.0	1.0	3.0	-	-	-	-	-	-
LBS 60 DAT	1.7	0.3	0.0	1.0	3.0	2.0	0.4	0.0	1.0	3.0	-	-	-	-	-	-

Std standard deviation, SE standard error, Min minimum, Max maximum, RV relative variation percentage, PH plant height, DBM dry biomass, HI harvesting index, PanN panicle number, PW panicle weight, GY grain yield, STR spikelet sterility, LBS leaf bronzing score 45 and 60 DAT

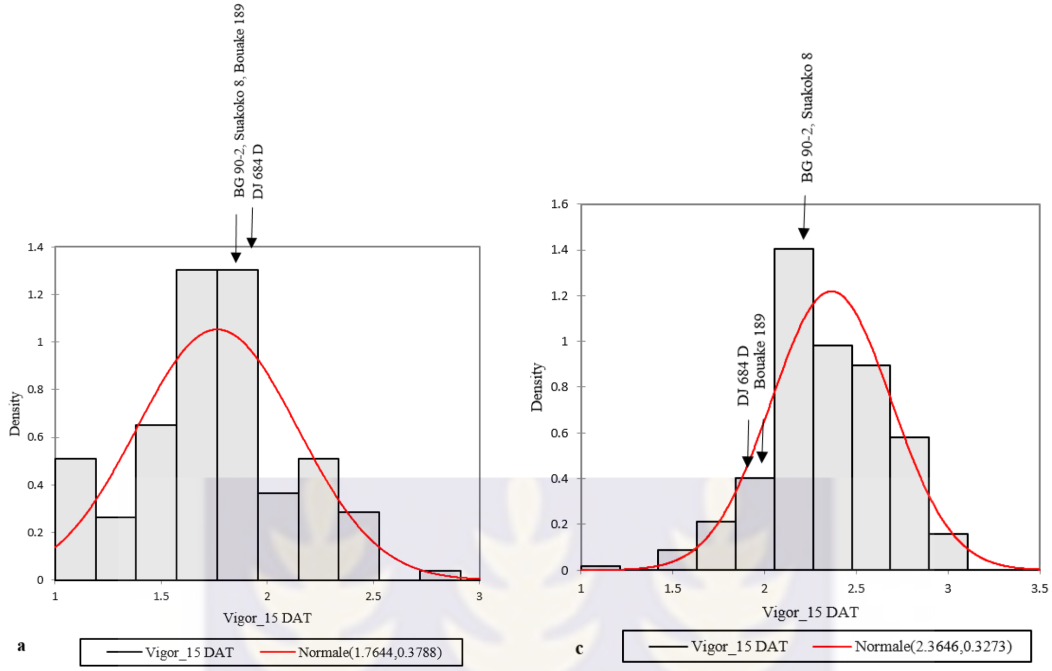


Figure 5.5: Distribution of plant vigour respectively at Ndeman (a) and Djibelor (c)

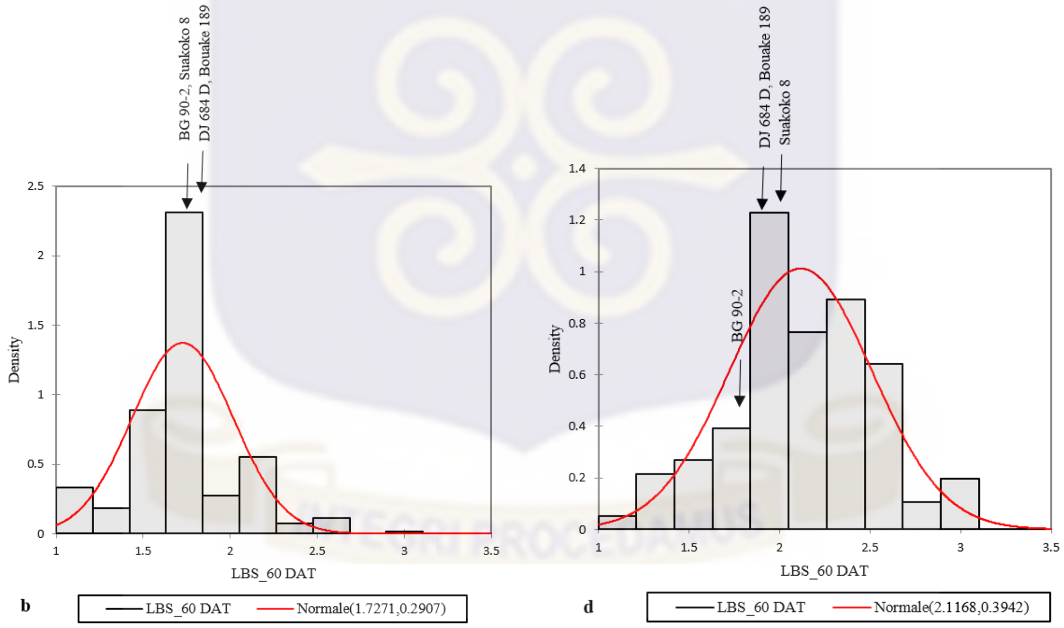


Figure 5.6: Distribution of leaf bronzing score 60 DAT at Ndeman (b) and Djibelor (d)

5.3.2.2 Correlation between variables

The correlation matrix, generated using combined data for the variables evaluated across the two locations, is shown in Table 5.8. The results showed that leaf bronzing score was significantly and negatively correlated with dry biomass production, harvest index, plant height, panicle number, tiller number and flowering time at 0.05% level of significance. Dry biomass was significantly and positively correlated with plant height, panicle number, harvest index, tiller number and flowering time. Spikelet sterility was negatively correlated with plant height and harvest index.

Table 5.8: Relationship between pairs of variables assessed at Djibelor and Ndeman under Fe toxicity

Variables	DBM	HI	PH	PanN	STR	Tiller	FLOWER	LBS
DBM								
HI	**0.3869**							
PH	**0.3744**	0.0290						
PanN	**0.4736**	**0.4025**	**-0.2211**					
STR	-0.0593	**-0.1307**	**-0.1654**	0.0231				
Tiller	**0.4832**	**0.3060**	0.0230	**0.5806**	-0.0663			
FLOWER	**0.2734**	**0.1768**	**0.4513**	**-0.2699**	**-0.2320**	-0.0242		
LBS	**-0.4454**	**-0.1892**	**-0.3169**	**-0.1279**	-0.0382	**-0.2518**	**-0.1567**	

DBM dry biomass, HI harvesting index, PH plant height, PanN panicle number, STR spikelet sterility, FLOWER flowering time, LBS leaf bronzing score at 60 DAT. Significant correlation coefficients at 5% level of significance are shown in bold.

5.3.3 Relationship between the hydroponic and the field evaluations

Similarities were observed in the performance of some genotypes between the hydroponic and the field conditions. Some similarities were also found when computing the analysis across stress fields regardless of the difference in the levels of toxicity. Grain yield of some high performing genotypes identified in hydroponic based on shoot dry weight was confirmed across the two stress fields (Figure 5.7). Even though high variation in grain yield was observed across Djibelor and Ndeman, some of these genotypes showed a mean performance above the average in both locations and better than the tolerant and local checks. These genotypes included BEN11-172, WONKIFON 17, EHAUB, BANDIOUL MANO and IR75887-1-3-WAB1 (ARICA 6).

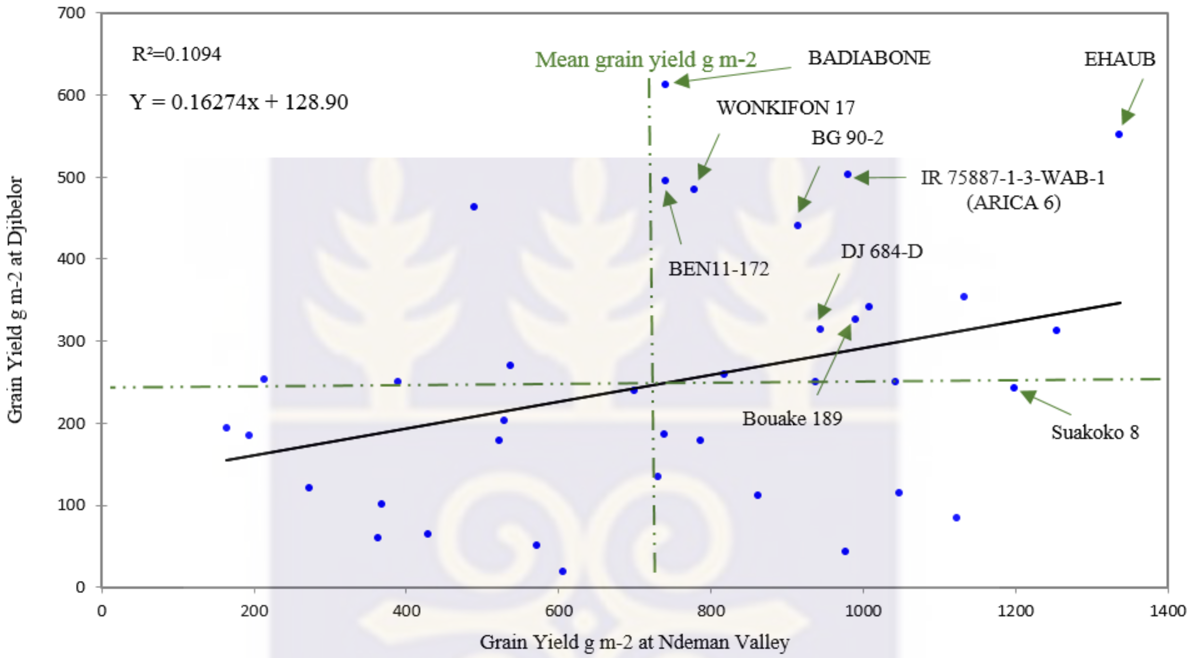


Figure 5.7: Relationship in grain yield under stress at Ndeman and Djibelor of the better performing genotypes identified from the hydroponic based on SDW

SDW shoot dry weight; Ndeman (less iron toxic field); Djibelor (more toxic field); Dot line represented average mean

The cluster analysis revealed three groups in both hydroponic and field evaluations (Figure 5.8). In the hydroponic 107 genotypes were classified as tolerant, while in the field there were 72 (Table 5.9). Among these genotypes, 19 performed under both conditions. Agro-morphological characteristics of the 40 best genotypes identified in stress field in Djibelor based on a selection index using grain yield, plant biomass, panicle number and harvest index are presented in Table 5.10.

Table 5.9: Mean performance of the identified group of genotypes from cluster analysis based on the traits recorded in hydroponic and field evaluations

Cluster	N	Variables evaluated under hydroponic					
		SDW	RDW	PH	RL	CC	LBS
C1	47	0.05	0.02	26.83	7.51	23.19	2.11
C2	124	0.06	0.02	34.15	8.6	23.65	2.01
C3	107	0.08	0.02	44.19	9.78	24.33	1.94

		Variables evaluated in field at Djibelor					
Cluster	N	PH	Tiller	DBM	PanN	CC	LBS
C1	120	96.42	155.72	949.57	247.16	33.69	2.07
C2	84	81.77	104.2	459.93	176.26	32.55	2.42
C3	72	109.26	195.94	1639.09	332.98	33.83	1.79

SDW shoot dry weight, RDW root dry weight, RL root length, PH plant height, tiller, DBM dry biomass, PanN panicle number, STR spikelet sterility, CC chlorophyll content and LBS leaf bronzing score. N represented the number of individual in each cluster

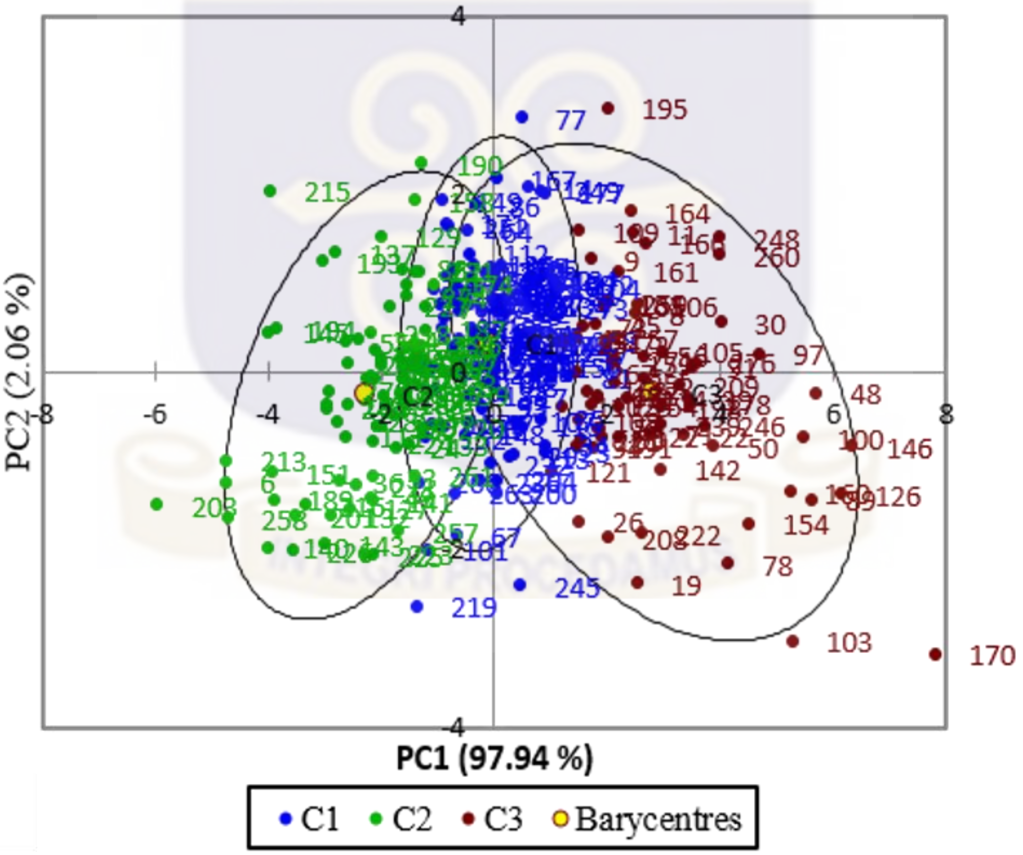


Figure 5.8: Phenotypic distribution of the genotypes based on their mean performance in field at Djibelor

Table 5.10: Top 40 higher performing genotypes under stress field at Djibelor based on selection index

Accession Name	Species	GY	DBM	PanN	HI
SER 5	*O. sativa*	601.3	1360.7	253.5	0.4
Kaolack 1	*O. sativa*	480.5	1466.9	353.5	0.4
SADIA	*O. sativa*	609.6	1760.7	259.8	0.3
***** EHAUB**	***O. sativa***	**551.3**	**1469.0**	**270.2**	**0.4**
ATEBAMA	*O. sativa*	528.4	1889.8	332.7	0.3
**** BANDIOUL MANO**	***O. sativa***	**495.1**	**1279.4**	**347.3**	**0.4**
DJILIBA	*O. sativa*	611.7	2312.8	305.6	0.3
YARAAGNAMBA	*O. sativa*	495.1	1554.4	436.8	0.3
RACIO	*O. sativa*	513.8	1433.6	353.5	0.4
SEFO	*O. sativa*	580.5	1419.0	451.4	0.4
SIMBE	*O. sativa*	661.7	2069.0	203.5	0.4
**** NGHAIMENE**	***O. sativa***	**651.3**	**2269.0**	**293.1**	**0.3**
CT 19298 (27)-1-11-1-2-3 MP	*O. sativa*	647.1	1733.6	478.5	0.4
FAROX 521-176-H1	*O. sativa*	913.8	2041.9	495.2	0.5
WAB 1572-10-B-B-FKR-4-WAC 1-1-TGR 2-WAT10-1	*O. sativa*	861.7	2329.4	363.9	0.4
WAB 1573-22-B-B-FKR 4-2-WAC 1-TGR 3-WAT9-1	*O. sativa*	386.7	2262.8	280.6	0.3
FAROX 521-156-H1	*O. sativa*	686.7	1691.9	307.7	0.4
WAB 2135-WAC B-2-TGR 2-WAT1-1	*O. sativa*	697.1	1652.3	251.4	0.5
**** FAROX 521-356-H1**	***O. sativa***	**611.7**	**1594.0**	**303.5**	**0.5**
*** IR 75887-1-3-WAB-1**	***O. sativa***	**503.4**	**1400.3**	**291.0**	**0.4**
ROK 10	*O. sativa*	786.7	2379.4	428.5	0.3
TOS 6758	*O. sativa*	755.5	2502.3	568.1	0.4
TOS 15383	*O. sativa*	534.6	2144.0	341.0	0.3
Ser 5 (96)	*O. sativa*	620.1	1477.3	236.8	0.4
Samou gbeli (150)	*O. sativa*	886.7	2271.1		0.4
Samou gbeli (07)	*O. sativa*	545.1	1623.2	207.7	0.4
TOS 8613	*O. sativa*	763.8	2835.7	568.1	0.3
TOS 11746	*O. sativa*	536.7	1804.4	299.3	0.4
ITA 252	*O. sativa*	559.6	1404.4	376.4	0.4
***** BEN11-172**	***O. sativa***	**495.1**	**1646.1**	**353.5**	**0.3**
DS4-B1	*O. glaberrima*	486.7	1731.5	507.7	0.4
**** TOS 14650**	***O. sativa***	**495.1**	**1341.9**	**374.3**	**0.4**
EWENTOHI	*O. glaberrima*	380.5	1550.3	703.5	0.3
TOS 13069	*O. sativa*	534.6	1619.0	603.5	0.5
BEN11-13	*O. sativa*	809.6	1912.8	343.1	0.4
Baga malé fikhè	*O. sativa*	570.1	1814.8	599.3	0.4
**** TOS 7090**	***O. sativa***	**613.8**	**1427.3**	**493.1**	**0.5**
TOS 12891	*O. sativa*	584.6	1869.0	226.4	0.4
***** WONKIFON 17**	***O. sativa***	**484.6**	**1460.7**	**320.2**	**0.4**
TOS 7044	*O. sativa*	613.8	1800.3	370.2	0.4
Mean		603.6	1790.2	373.2	0.4
Sdandard deviation		125.1	371.4	119.7	0.1

GY grain yield, DBM dry biomass production, PanN panicle number, HI harvesting index, *** =performing in hydroponic and the two locations, ** = identified in the location, * = identified in hydroponic and stress field in Djibelor. In bold the genotypes also part of 40 performing in Ndeman using the same selection indexes and in hydroponic

5.4 Discussion

The hydroponic screening protocol adopted from Wang *et al*. (2008) reproduced the expected reactions of the checks with Suakoko 8 which showed tolerance while Bouake 189 showed susceptibility as observed in several studies (Sahrawat, 2010; Onaga *et al*., 2013). The hydroponic screening method used in this study has shown clear distinction of the different genotypes based on their level of tolerance. Previous studies also reported differences in phenotypic variations using hydroponic as a technique of evaluation for tolerance of rice germplasm to iron toxicity (Dufey *et al*., 2009; Onaga *et al*., 2013; Elec *et al*., 2013).

The symptom of leaf bronzing appeared in the first week after the iron treatment where the severity of these symptoms increased rapidly in the second week of screening. With the high temperatures observed in the screenhouse (around 40°C), larger Fe uptake and translocation could have happened due to higher evapotranspiration. At high Fe concentration in the media, it was shown that Fe-uptake increased as shown by the intensity and severity of the necrotic leaf spot. Leaf bronzing score which has been used as a key indicator of susceptibility (Asch *et al*., 2005; Engel *et al*., 2012), can however lead to some bias in the identification of tolerance due to its visual and subjective assessment (Shimizu, 2009). In some cases, iron toxicity can cause important decrease in plant performance without much brown spots on the leaves (Onaga *et al*., 2013). Therefore, for an accurate evaluation of tolerance, growth parameters such as root length (RL), plant height (PH), root dry weight (RDW), shoot dry weight (SDW) and chlorophyll content were also considered at seedling stage.

In this study, the high Fe concentration impaired plant growth and chlorophyll content with 59% and 53% decrease in SDW and RDW respectively. These results are in accordance with previous findings by other researches on the effect of excess Fe^{2+} on plant growth (Fageria & Rabelo, 1987; Onaga *et al.*, 2013). The reduction in chlorophyll content (20%) observed in this study is higher than what was observed by Bashir *et al*. (2014) in the transcriptomic analysis of rice varieties exposed to iron toxicity. The forty most tolerant genotypes identified based on leaf bronzing score showed similar performance as Suakoko 8 for root length, root dry weight and SPAD value. These tolerant genotypes performed better than the tolerant check for some traits such as plant height and shoot dry weight, this means that sources of tolerance can effectively be identified in this rice panel. These high performing genotypes showed relatively low iron concentration in the stems and leaves even though retention of iron in the root was high. Onaga *et al*. (2012) reported that tolerant rice genotypes showed high iron content in roots but low in shoot. This tolerance to iron toxicity which could be through avoidance/exclusion mechanism in rice landraces resulting in low grain Fe content was previously reported by Ruengphayak *et al*. (2015).

Three phenotypic groups were identified in the collection of germplasm based on four growth parameters (RDW, SDW, RL and PH), leaf bronzing and chlorophyll content. Considering the first two components, grouping of the genotypes was mainly related to their level of tolerance. Bouake 189 was in the group of highly susceptible genotypes which represented 17% of the panel of germplasm while Suakoko 8 was in the moderately tolerant group of genotypes (45% of the collection). The group of genotypes which was considered as higher performing represented 38% of the collection. They had the highest mean in some of the recorded traits specifically chlorophyll content and lowest mean leaf bronzing symptom. This low mean for leaf bronzing combined with high chlorophyll content was suggested in this study as key traits for selecting tolerant genotypes. It was generally reported that genotypes which can preserve chlorophyll integrity while undergoing stress, are likely to be more efficient in photosynthesis and have better growth. Hence, chlorophyll content was considered as an important secondary trait in indirect selection of high performing genotypes under many oxidative stresses such as boron and iron toxicity (Sikirou *et al*., 2015; Neto *et al*., 2017).

As in the culture solution, differences were also observed among the genotypes in their response to iron toxicity in field conditions. Higher pressure of iron toxicity was however perceived at Djibelor where the clayey soil type combined with low *in-situ* pH and water flooding condition which was observed during the period of the experiment. The low pH in water and high clay content at Djibelor may have played a significant role in contributing to the Fe toxicity. At such low pH, Fe would be very soluble and with the development of more positive charges on the clay surface, there is the likelihood of Fe repulsion from the clay exchange site. These may have culminated a high level of Fe in solution leading to the toxicity. The hot spot field of Djibelor was described earlier by Prade *et al*. (1990) as prone to causes iron toxicity based on its low pH and the presence of sulphate mineral such as jarosite which is associated with the acidic sulphate soil. Apart from the soil type that prevail in lowland ecologies, the occurrence of iron toxicity in Djibelor was also associated with the flooded conditions which promote the reduction of iron Fe^{3+} to Fe^{2+} (Becker & Asch, 2005; Audebert & Fofana, 2009).

Due to the difference in the level of iron toxicity among the two locations, mean value of most of the agro morphological traits were significantly lower in Djibelor than in Ndeman. Mean values of leaf bronzing at 45 DAT was 2 in Ndeman and 4 in Djibelor and varied between 3 and 4 at 60 DAT respectively in these locations. These differences in the mean of leaf bronzing, in addition to the variation of the agronomic traits, reflect the differences in the severity of iron toxicity in Djibelor. In field evaluation, it was reported that variation in leaf bronzing symptoms was not efficient enough for selecting tolerant genotypes, other parameters such as yield and yield related traits need also to be considered (Audebert & Fofana, 2009; Sikirou *et al*., 2015). Nonetheless, in this field evaluation, leaf bronzing showed high relationship with decrease in performance. These results were in contrast to those reported by Onaga *et al*. (2012) while investigating under field conditions, the tolerance of rice germplasm to iron toxicity in relation to other nutrients uptake where foliar symptoms did not correlate with the high decrease observed in performance. Comparing the stress and the non-stress experiments in Djibelor, plant biomass, grain yield, panicle number and mean panicle dry weight presented the highest reduction in their mean values. Dry biomass and grain yield were reduced to 56%. These significant reduction in biomass and yield were previously reported in several studies (Audebert & Fofana, 2009; Engel *et al*., 2012). Onaga *et al*. (2012) observed a reduction in grain yield of 34.2%. Further, pot experiment filled with highland swamp soils from Burundi lead to 50% decrease in grain yield (Genon *et al*., 1994).

As for the phenotypic distribution observed in the hydroponic screening, three groups were also identified in the collection of germplasm based on the different traits assessed in stress field in Djibelor. The higher performing group represented 26% of the genotypes and presented the highest mean for most of the traits. All the check varieties (Suakoko 8, DJ 684 D, BG 90-2 and Bouake 189) were however in the moderately tolerant group with a mean value of squared root of leaf bronzing score of 2.07. As observed in previous studies, the relatively good performance of Bouake 189 as an *indica* cultivar, could be explained by its adaptation to the lowland ecologies as tolerance to iron toxicity likely depends on the growing conditions (Asch *et al*., 2005). High performance of susceptible varieties under hot spot field conditions were previously observed in IR64 (susceptible *indica* variety) when compared to Azucena (tolerant *japonica* variety) which was poor performing (Dufey *et al*., 2012).

Regardless of the problem of inconsistencies due to the environmental effects commonly reported (AfricaRice, 2006; Sikirou *et al*., 2015), some stable performances were observed in the tolerance of some genotypes across the three experimental environments of hydroponic and two field evaluations. Some of the top performing genotypes identified in hydroponic also performed well in both field experiments. Among the 40 best genotypes identified in Djibelor based on grain yield, biomass, panicle number and harvest index, some had similar performance in Ndeman as well as in hydroponic conditions. The identified genotypes included both accessions from Africarice genebank and landraces from Casamance. IR75887-1-3-WAB1 (ARICA 6) was previously identified as tolerant varieties from multi-location field trials by AfricaRice (AfricaRice, 2017). The stability in tolerance to iron toxicity of traditional rice varieties across different locations was highlighted earlier by Audebert & Fofana, (2009). They found in their study that most of the varieties showing stability and tolerance were traditional types.

5.5 Conclusions

A considerable genetic variation for tolerance to iron toxicity in both controlled and natural environments were observed among the different rice genotypes. High relative decrease in the values of the variables was observed under stress. Some genotypes had similar, even better performance than Suakoko 8 under stress conditions. They showed low iron concentration in shoots and high root retention capacity. Some similarities were found on the genotype ranking following their performance under fields and hydroponic conditions. Promising genotypes with stable tolerance across the three screening environments and the main symptomatic traits were identified. The following genotypes BEN11-172, WONKIFON 17, EHAUB, BANDIOUL MANO and IR75887-1-3-WAB1 (ARICA 6) could be used as sources of tolerance to iron toxicity for genetic improvement of cultivated rice varieties.

CHAPTER SIX

6.0 IDENTIFICATION OF CANDIDATE GENES ASSOCIATED WITH TOLERANCE TO IRON TOXICITY

6.1 Introduction

Genes for tolerance to iron toxicity remain unknown despite the numbers of QTL identified. Genetic regions linked to the variation of some morphological and physiological traits and yield parameters were identified by some independent researches (Wu *et al.*, 1997, 1998 ; Wan *et al.*, 2005; Shimizu., 2009; Dufey *et al.*, 2012, 2015). However, due to the complexity of the traits and the limitation observed in most of these approaches, not high achievements were reported in the implication of these QTL in breeding programmes. Superior and highly informative genotyping assays combined with diverse rice germplasm are now being used in the analysis of the genetic mechanisms of iron toxicity tolerance. Depending on the linkage disequilibrium of the population explored, higher QTL resolution may result from using a Genome Wide Association Study (GWAS) due to the unrelated individual genotypes which constitute the genetic material in addition to the multiple SNPs data points used (Famoso *et al.*, 2011; El-soda *et al.*, 2014). For iron toxicity tolerance in rice, association mapping has been used to identify candidate regions for leaf bronzing and K transporter on chromosome 1 and shoot Fe concentration on chromosome 2 (Matthus *et al.*, 2015). These candidate genes were identified by exploring 329 diverse rice accessions of *Oryza sativa* specie collected from 77 countries around the world (Zhao *et al.*, 2011). A total of 22 candidate genes related to ten promising QTLs were also identified by Zhang *et al.* (2017) using a wide collection of 211 *indica* rice genotypes. The candidate genes associated with tolerance to iron toxicity identified so far were reported in diverse panel of rice germplasm including accessions from many countries around the world using hydroponic screening data. None of these candidate loci have been identified in the natural condition of iron toxicity occurrence. Further, none of these studies have been carried out on the existing West African rice genetic material exclusively regarding the fact that these adapted germplasm has an important genetic variation which could contribute to the improvement of the cultivated rice varieties (Dramé *et al*., 2010; Sanni *et al*., 2013). Identifying valuable candidate genes associated with high performance under different conditions of iron toxicity environments among the cultivated rice cultivars will be useful as these genetic resources are mainly adapted to various African local conditions. The objective of this study was to identify candidate genes associated with tolerance to iron toxicity in the collection of rice germplasm mainly composed of existing rice germplasm from West Africa in different conditions of Fe toxicity.

6.2 Materials and methods

6.2.1 Plant material

The plant material was composed of the 282 rice genotypes evaluated for tolerance to iron toxicity in hydroponic and natural conditions of hot spot lowland fields. Additionally, 36 genotypes composed of selected parents of breeding lines developed at AfricaRice research station at Tanzania were added to this panel of germplasm for genotyping.

6.2.2 Genotyping

Seeds of the 318 genotypes (282 genotypes of the GWAS panel plus 36 inbreed lines from Africarice) were grown at Guterman Bioclimatic Laboratory, Cornell University, during the summer of 2016. Leaf samples were collected from a single plant per accession and their DNA extracted using the CTAB method (Murray & Thompson, 1980). Single nucleotide polymorphism (SNP) information was generated using a Genotype-By-Sequencing (GBS) approach with *ApeK1* for digestion. Processing of raw data to generate genotypic information was performed using the GBS informatics pipeline implemented in TASSEL4.0 (Bradbury *et al*., 2007), and sequence reads were aligned using Bowtie2 to MSUv7 genome assembly. A total of 148,934 resultant polymorphic SNPs from the imputation process (Appendix 6.1) were used for the population structure analysis and the association mapping.

6.2.3 Phenotyping

The phenotypic data recorded from the hydroponic and the field experiments described in chapter five were used for the association mapping analysis. For the traits assessed in hydroponic conditions, shoot dry weight was not included in the analysis. For the field experiments, only the phenotypic data recorded at Djibelor were considered and among the traits evaluated, yield and yield related traits were selected for this study.

6.2.4 Data analysis

The genotypic data set consisting of 148,934 SNP markers from the imputed SNPs data at 0.05 minor allele frequency (MAF) and 70% minimum count. Genetic relationship and population structure analysis were performed. For the population structure analysis, genetic information of an additional 51 pure lines were extracted from the McCouch's RiceLab database and used as reference genotypes (subpopulation controls) for each of the five *Oryza sativa* subpopulations. These included the genetic information of 13 *indica* (IND), ten *aus* (Aus), ten temperate *japonica* (TPRJ), 12 tropical *japonica* (TRJ) and six aromatic (ARO) subpopulations. The analysis was performed using fastSTRUCTURE (Raj *et al.*, 2014) with a number of subpopulations set from K=2 to K=5. The genotypes from the panel and the reference inbred lines were assigned to specific group based on an arbitrary ancestry percentage of a minimum of 70%. Subpopulation groups were displayed in plots using Distruct Windows1.1 (Rosenberg, 2004). Phylogenic tree was generated using Geneious program (Kearse *et al.*, 2012) following the method of Neighbour Joining proposed by Saitou & Nei (1987).

Association mapping analysis was performed combining the SNP data and the measurement of the phenotypic variability observed in controlled and natural conditions of occurrence of iron toxicity. The different analysis were performed on the full diversity of the GWAS panel and subsequently on identified subpopulations. Adjusted means obtained from the analysis of variance were used as the phenotypic data. Leaf bronzing scores were first transformed using squared root transformation prior to the association mapping analysis. The Compressed Mixed Linear Model (CMLM) of the Genomic Association and Prediction Integrated Tool, GAPIT

(Zhang *et al.*, 2010) with the default setting of P3D was used for the GWAS analysis. A genetic marker-based Kinship and principal component (PC) matrix generated were jointly used as controls for population structure and family relatedness. Linkage disequilibrium (LD) was measured as R^2 for pair wise makers as integrated in the GAPIT program. To identify SNPs that are associated with variation in response to iron toxicity, a significant threshold was set for $-\log_{10} P > 4.0$ based on previous studies in genome wide association mapping in rice (Storey & Tibshirani, 2003; Wissuwa *et al.*, 2015; Matthus *et al.*, 2015). Two peaks were considered the same when the distance between their physical positions was < 250 kb. In order to determine candidate regions associated with these SNPs, 250 kb was also considered in each side as the starting and the ending position of these different significant SNPs. Linkage Disequilibrium (LD) analysis was performed on regions harbouring significant SNPs with $–\log_{10}$ P>4.0 using Haploview 4.2 (Barrett *et al.*, 2005). A physical distance of 250 kb were considered in each side of the identified significant SNPs to define candidate regions. This distance was selected based on estimated LD in rice (50-500 kb) (Garris *et al*, 2003; Famoso *et al.*, 2011). LD blocks of surrounding significant SNPs were defined using the default program of Haploview (Gabriel *et al.*, 2002) as adopted by Matthus *et al.* (2015). Gene annotations were performed using the MSU7 rice genome database (MSU, 2017).

6.3 Results

6.3.1 Population structure and relatedness

An optimum number of five subpopulations (k=5) was obtained in the total germplasm including the reference genotypes (Figure 6.1). Four subgroup were identified considering only the tested panel of germplasm. These four subpopulations corresponded to *Oryza glaberrima* and the *Oryza sativa*, with further subdivision within *O. sativa* corresponding to *japonica* and *indica* which was divided into two groups, the landraces from Casamance and the collection from AfricaRice genebank. The first subgroup of the *indica* included most of the landraces from Casamance (97%) and part of the accessions and cultivars from AfricaRice (47%). The second subpopulation was composed of mainly the AfricaRice collection which was composed mainly of accessions from the West African countries. The *japonica* group which could include the aromatic, the temperate and tropical *japonica* types was composed of 20 genotypes representing 7.1% of the collection. The *O. glaberrima* specie represented 13.9% of the collection and was composed of 39 accessions. The total individual genotypes of the *indica* subpopulation was composed of 221 genotypes of which 43% were represented by the landraces from Casamance and 57% by the cultivars from AfricaRice. Population relatedness was also assessed using Principal Component (PC) analysis (Figure 6.2) considering the first three components which explained 97.6% of the variation. PC1 (73.9% of the variation) separated the *Oryza sativa* and *O. glaberrima* species and PC2 (22.1%) separated the *japonica* and the *indica* subspecies.

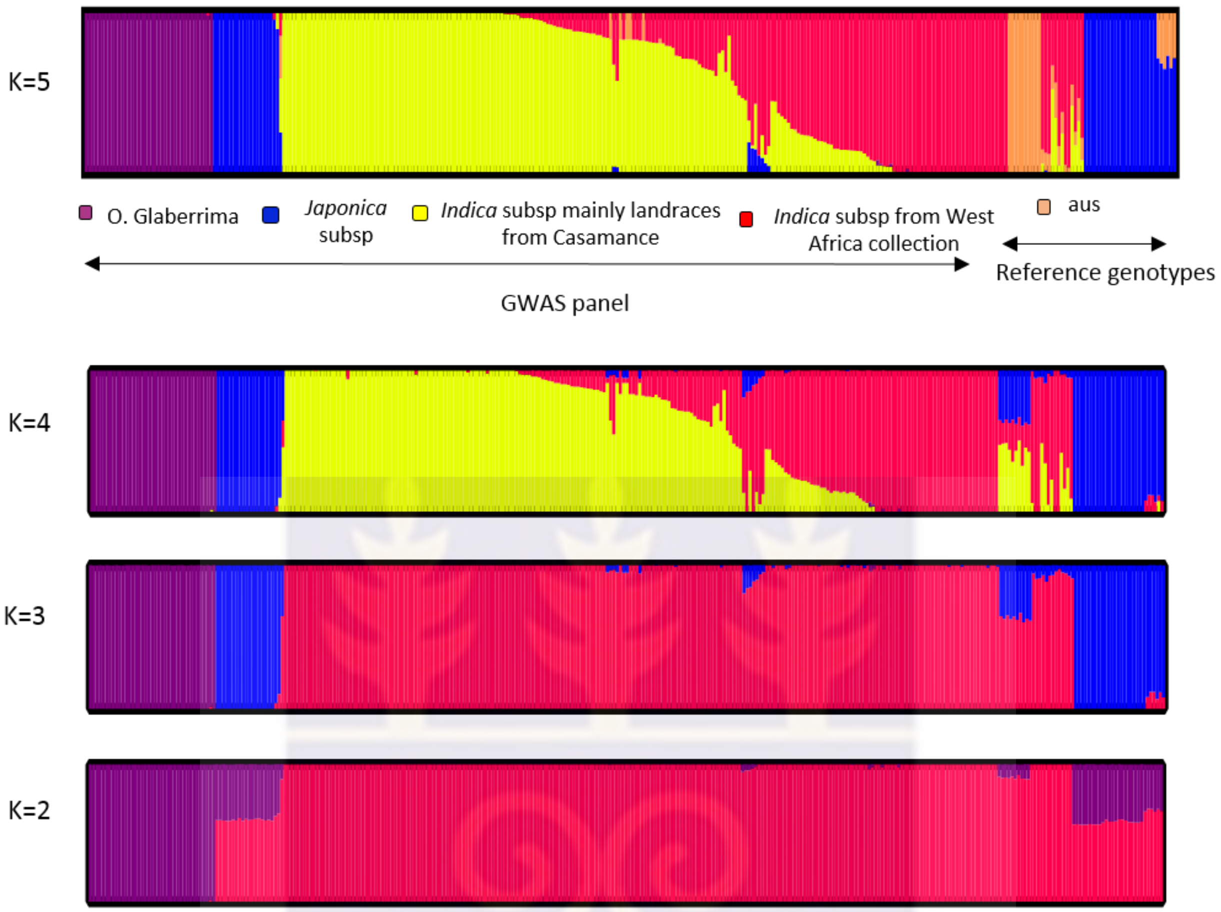


Figure 6.1: Population structure of the panel of germplasm based on 148,934 SNPs markers

Number of subpopulations of K =2 to K=5 of the collection of 331 rice genotypes including the 50 inbreed line of *O. sativa* sub species used as reference genotype GWAS results from analysis performed using the hydroponic data

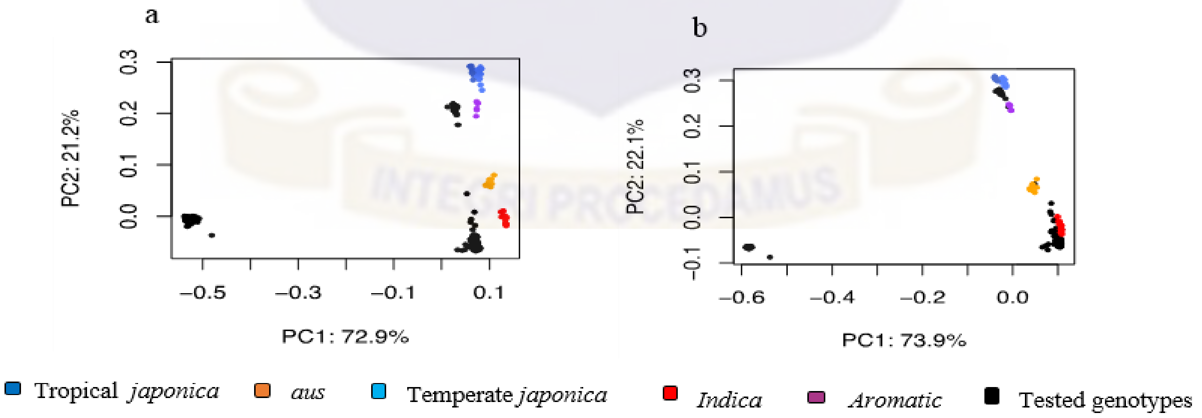


Figure 6.2: Genetic variation of the panel of germplasm through PCA analysis

PCA principal component analysis, PC1 and PC2 of the imputed (a) and non-imputed (b) genotypic data set. Black and coloured dots represent respectively the evaluated genotype and the reference inbred lines

6.3.2 Phenotypic variability across subpopulations in hydroponic conditions

Mean performance of the different subpopulations of *indica*, *japonica* and accessions of the *O. glaberrima* specie in hydroponic conditions are presented in Figure 6.3. These values were compared to the check varieties, Suakoko 8 (tolerant) and Bouake 189 (susceptible). The *indica* subsp and the subpopulation of the *glaberrima* sp had the highest mean for plant height. The different subpopulations had relatively the same mean performance for leaf bronzing, chlorophyll content and root length.

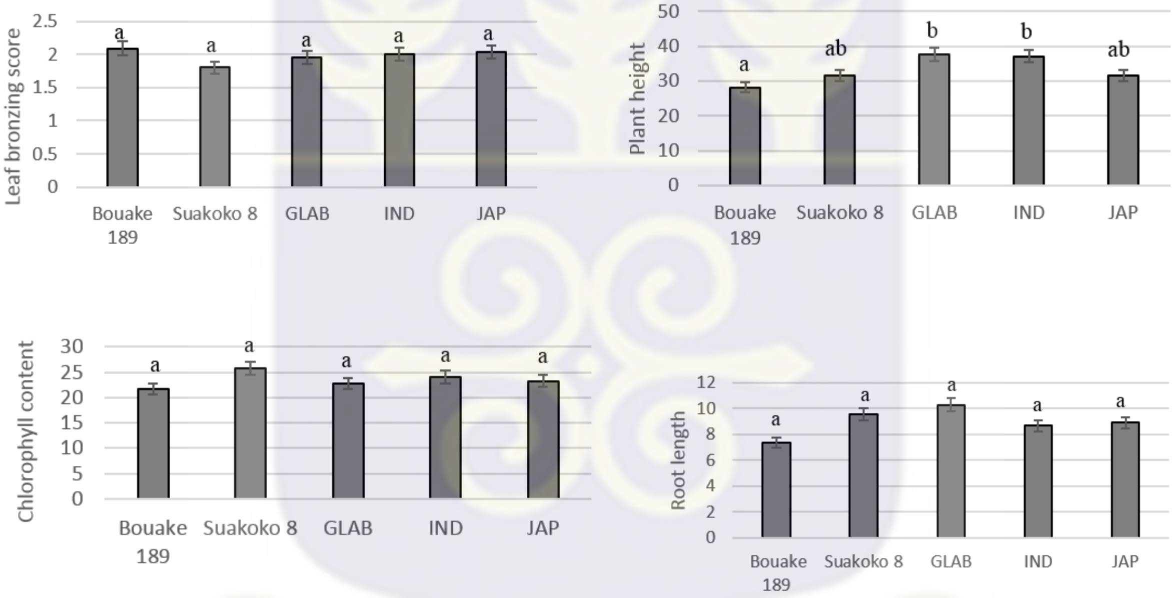


Figure 6.3: Phenotypic variability across subpopulations compared with check varieties

Bouake 189 susceptible check, Suakoko 8 tolerant check, GLAB *glaberrima* sp, IND *Indica* subsp, JAP *Japonica* subsp Histogram representing the mean with error bars showing the same letter were statistically the same at $p < 0.05$ with the Tukey's HSD test

6.3.3 GWAS analysis using phenotypic data from the hydroponic screening

6.3.3.1 GWAS analysis considering the total diversity panel

The association mapping analysis was conducted across 243 genotypes which passed the filtering process and had phenotypic data. This analysis was performed using two PCs separating the *O. sativa* and the *O. glaberrima* specie as well as the *sativa* subspecies *japonica* and *indica*. A total number of 33 significant SNPs with $-\log_{10}$ P>4 were identified considering all selected traits including plant height, root length, root dry weight, chlorophyll content and leaf bronzing score as well as the relative reduction in root dry weight, chlorophyll content and plant height (RDWR, CCR and PHR). The GWAS analysis was then performed on the *indica* subpopulation composed of 186 genotypes.

6.3.3.2 GWAS analysis considering the *indica* subpopulation

The *indica* subpopulation in this panel of germplasm presented a LD decay of 50 kb (Appendix 6.2). The mixed model analysis using plant height, root dry weight, root length, chlorophyll content and leaf bronzing generated nine distinct SNPs with $-\log_{10}$ P>4. Table 6.1 gives a summary of the identified most significant SNPs following the different traits selected. Most of the regions of interest identified in the total panel were confirmed in the *indica* subpopulation. Normal distribution was observed for all traits with quantile-quantile-plot (qq-plot) showing upward deviation from expected value for RL. As indicated in Figure 6.4, significant SNPs were found on chromosomes 4 and 11 for RDW. Candidate SNPs associated with leaf bronzing were identified only on chromosome 4. For PH, all the SNPs with $-\log_{10}$ P>4 were also identified on chromosome 4. Two SNPs on chromosome 8 and one on chromosome 5 were associated with variation of CC. RL was associated with SNPs on chromosomes 3 and 9. Comparing the 12 chromosomes, chromosome 4 presented the highest number of candidate SNPs with threshold exceeding $-\log_{10} P>4$ for traits such as PH and RDW.

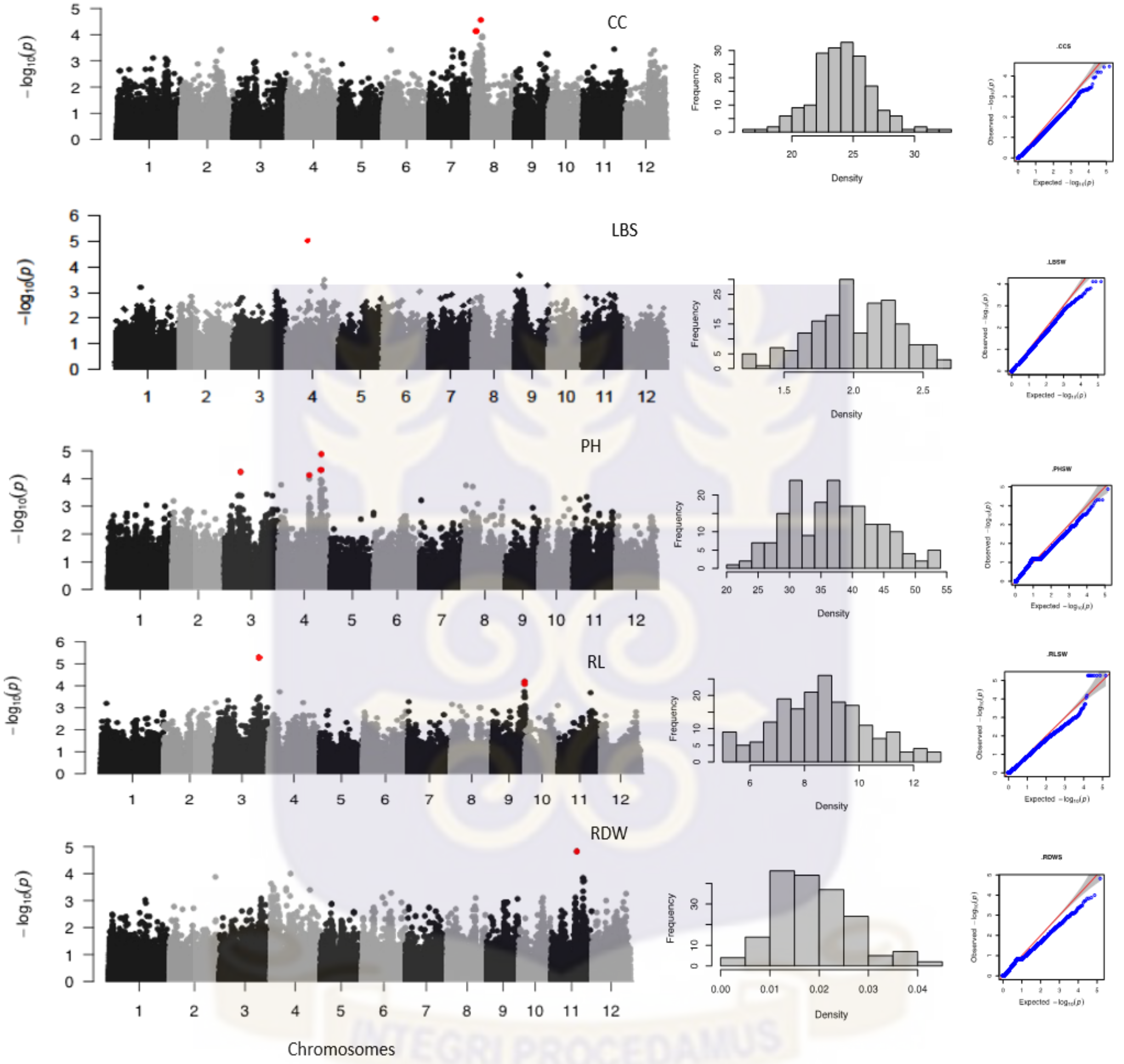


Figure 6.4: Manhattan plots and corresponding phenotypic distribution displayed in histogram and qq-plots of associated SNPs

CC chlorophyll content, PH plant height, RL root length and RDW root dry weight. qq quantile-quantile plots. Red dots in the Manhattan plots represent SNPs with significance of *p* value above the threshold of $-\log_{10} P>4$

Table 6.1: Most significant SNPs ($-\log_{10} P>4$) and LD blocks according to the selected traits

Traits	SNP	Chr.	Position (bp)	P.value	MAF	R^2w/o SNP	R^2w/SNP	FDR	% Phenotype variation	Start LD	End LD
RDW	S11_18164929	11	18164929	1.30E-05	0.314	0.057	0.161	1	10.4	18164929	18174313
RDW	S4_14160399	4	14160399	7.33E-05	0.24	0.057	0.142	1	8.5	14159948	14160399
CC	S5_24384192	5	24384192	3.38E-05	0.067	0.023	0.118	1	9.5	NA	NA
CC	S8_5185287	8	5185287	3.66E-05	0.075	0.023	0.117	1	9.4	NA	NA
CC	S8_2008974	8	2008974	6.41E-05	0.102	0.023	0.111	1	8.8	1996008	2032837
PH	S4_29243126	4	29243126	1.95E-05	0.285	0.431	0.49	1	5.9	29025856	29491873
RL	S3_29880579	3	29880579	5.53E-06	0.038	0.024	0.14	0.09	11.6	29863714	29898248
RL	S9_22433108	9	22433108	4.91E-05	0.056	0.024	0.115	0.73	9.1	NA	NA
LBS	S4_14326453	4	14326453	7.76E-06	0.172	0.045	0.155	1	11	NA	NA

*NA not belong to a LD block, RDW root dry weight, CC chlorophyll content, PH plant height and RL root length, MAF minor allele frequency, Chr chromosome, FDR false discovery rate, All allele, LD linkage disequilibrium

6.3.3.3 Phenotypic variation associated with favourable alleles of the most significant SNPs

The phenotypic variation under iron toxicity conditions in relation with the allelic frequencies of the most significant SNPs allowed the identification of the favourable alleles associated with agro morphological performance. Favourable alleles associated with high mean performance identified in the *indica* subpopulation were then investigated in the *glaberrima* and *japonica* sub populations. Figure 6.5 and Figure 6.6 give an illustration of the LD block, zooming on the most significant associated SNPs and boxplot representation of the phenotypic variation of the traits. For CC, the G allele associated with the highest mean was more frequent in the *indica* group with a percentage frequency of 97%. This allele existed however in low frequencies with only 13% and 11% in the *japonica* subpopulation and *O. glaberrima* specie, respectively. The favourable allele associated with the high mean performance of RL was well represented (89%) in the *glaberrima* specie and did not exist in the *japonica* subspecies. For RDW, the favourable allele was specific to the *indica* subspecies (76%).

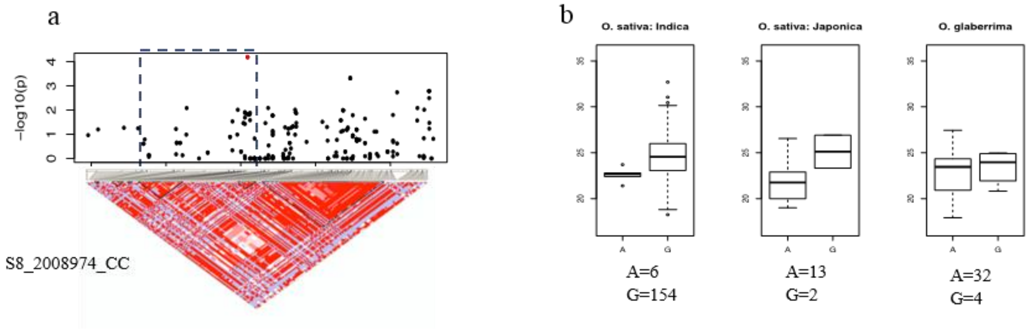


Figure 6.5: Linkage block analysis of candidate region on chromosome 8 shown in triangle frame and zooming of most significant SNPs (a) associated with CC and corresponding phenotypic variation showed in boxplots (b)

CC chlorophyll content

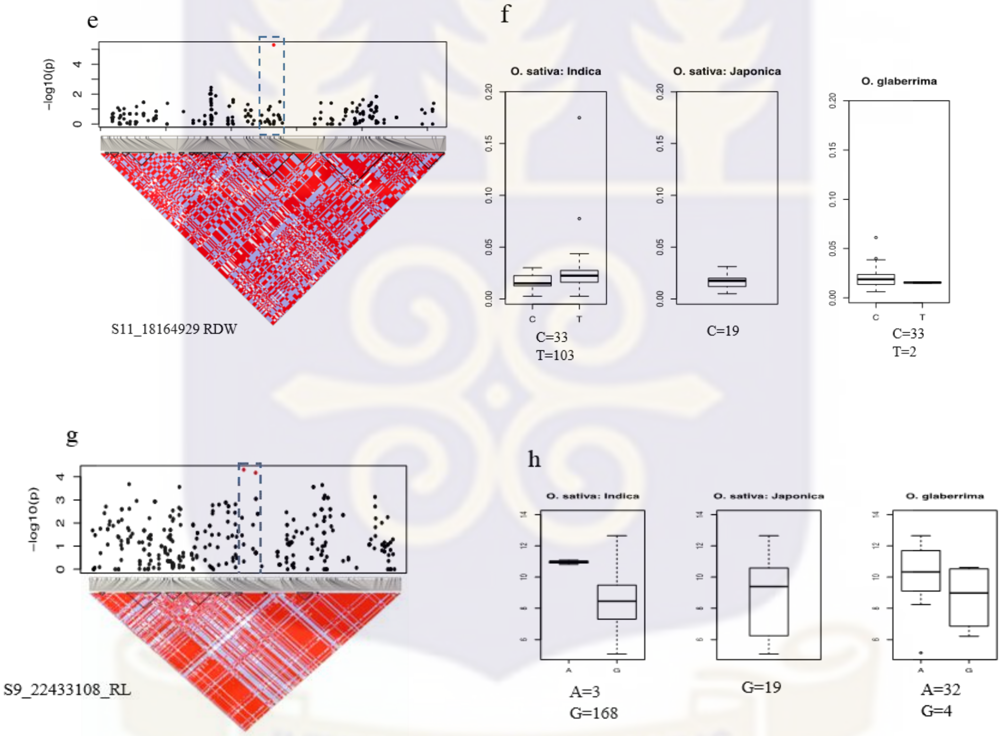


Figure 6.6: Linkage block analysis of candidate region on chromosome 11 and 9 shown in triangle frame and zooming of most significant SNPs associated with RDW (e, f) and RL (g, h) and corresponding phenotypic variation showed in boxplots

RDW root dry weight and RL root length

6.3.4 GWAS results using the phenotypic data from the field experiment

Phenotypic data on chlorophyll content, leaf bronzing, dry biomass, mean panicle dry weight, spikelet sterility, flowering time, panicle number and harvest index were subjected to GWAS analysis using the *indica* subpopulation with the 148,934 SNPs. A total of 60 significant SNPs ($-\log_{10}$ P>4) distributed across the different chromosomes, except for chromosome 11, were obtained. Results from the GWAS performed using the chlorophyll content, harvest index, panicle number and leaf bronzing score are more presented in this study. Table 6.2 gives a summary of the 10 significant SNPs identified with the selected traits. Harvest index and panicle number shared two significant SNPs on chromosomes 1 and 9. Leaf bronzing score shared the same significant SNP with harvest index on chromosome 7 (Figure 6.7). Among the significant SNPs identified in hydroponic, the candidate loci on chromosome 8 for chlorophyll content were confirmed in field conditions, considering the 250 kb window separating the two physical position of the markers (Figure 6.8). The SNPs identified in these regions included S8_5185287 from hydroponic screening and S8_5288795 from the field with a difference in their physical position of less than 250 kb.

Table 6.2: Most significant SNPs (p value = $-\log_{10}$ P>4) identified for HI, CC and LBS

Traits	SNP	Chr	Position	P value	MAF	R^2w/o SNP	R^2w/ SNP	FDR	% Phenotypic variation	start LD	end LD
HI	S7_1781415	7	1781415	1.21E-05	0.04	0.09	0.2	0.24	11	1712647	2029546
HI	S7_2600532	7	2600532	1.37E-05	0.03	0.09	0.19	0.24	10	2453149	2830404
HI	S7_2339564	7	2339564	1.49E-05	0.02	0.09	0.19	0.24	10	NA	NA
HI	**S7_1052475**	**7**	**1052475**	**4.26E-05**	**0.07**	**0.09**	**0.18**	**0.24**	9	**809492**	**1290169**
HI	S9_18449362	9	18449362	1.32E-05	0.02	0.09	0.19	0.24	10	18252470	18693443
HI	S9_18904431	9	18904431	7.86E-05	0.03	0.09	0.18	0.24	9	18658405	19151186
CC	S8_5288795	8	5288795	6.13E-05	0.03	0.06	0.15	0.36	9	5254790	5328855
LBS60	S2_11160651	2	11160651	1.89E-05	0.19	0.07	0.17	1	10	10965339	11407037
LBS60	**S7_919040**	**7**	**919040**	**5.18E-05**	**0.09**	**0.07**	**0.16**	**1**	9	**669122**	**1165134**
LBS60	**S7_1027624**	**7**	**1027624**	**6.34E-05**	**0.07**	**0.07**	**0.16**	**1**	9	**779289**	**1275671**

HI harvesting index, CC chlorophyll content and LBS60 leaf bronzing at 60 day after transplanting. In bold character, the co-localized SNPs between HI and LBS60

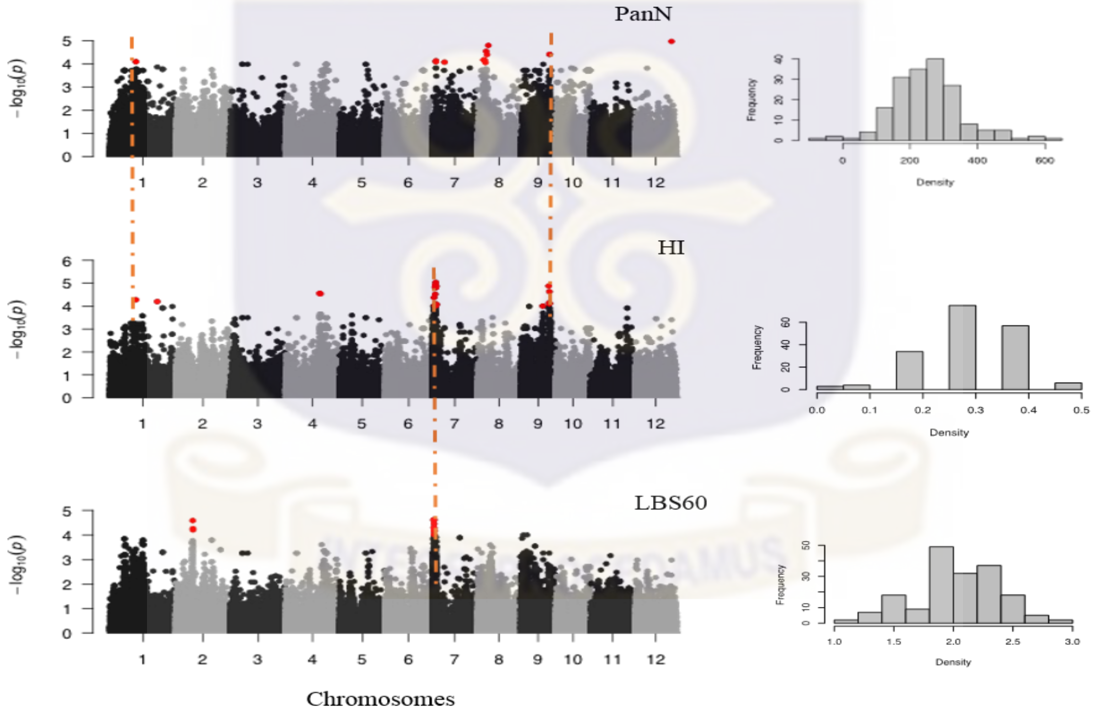


Figure 6.7: Shared significant SNPs between PanN, HI and LBS60 with histograms displaying the phenotypic distributions

PanN panicle number and HI harvest index and HI and LBS60 leaf bronzing score at 60 DAT Red dots in the Manhattan plots represent SNPs with significance of p value above the threshold of $-\log_{10}$ P>4

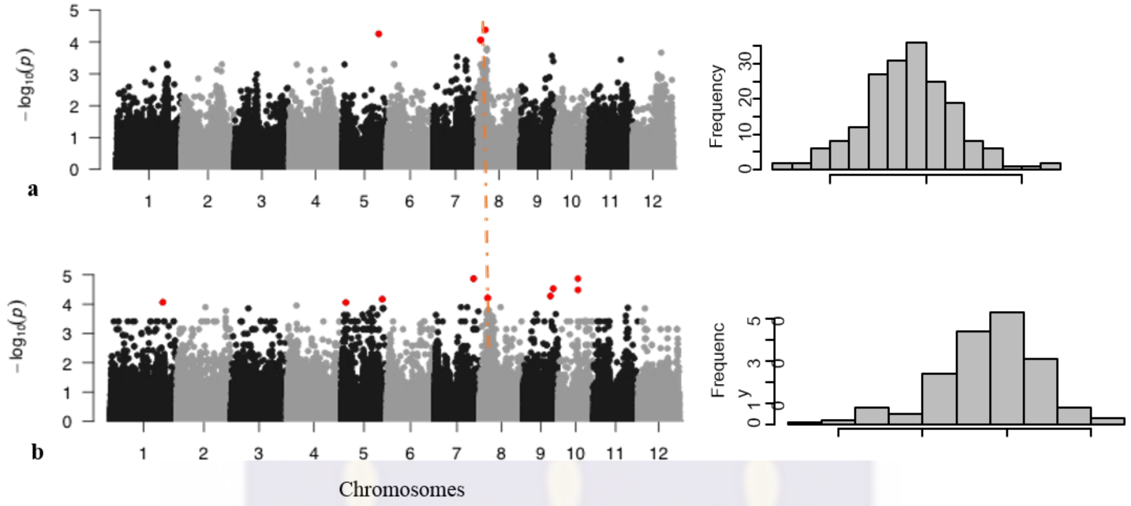


Figure 6.8: Significant SNPs detected in both hydroponic (a) and field conditions (b)

Chr chromosome. Red dots in the Manhattan plots represent SNPs with significance of *p* value above the threshold of $-\log_{10}$ P>4

6.3.5 Linkage disequilibrium analysis and gene annotation of candidate loci identified in hydroponic and field

Analysis of LD blocks harbouring most significant SNPs was followed by the annotation of gene functions associated with the selected candidate loci identified in hydroponic and field conditions. Table 6.3 gives the list of some of the putative candidate loci and annotated gene functions identified in these two experimental conditions. The LD blocks varied between 0.45 to 496 kb for the selected traits in hydroponic and field experiments. In hydroponic, the most significant SNP on chromosome 8 for chlorophyll content (S8_2008974) was in a LD block of 36.8 Kb containing eight gene models associated with seven putative genes. The SNP S5_24384192 was linked to 90 loci. The common candidate loci identified in both hydroponic and field evaluations linked to the SNPs S8_5185287 and S8_5288795 were related to 82 loci which are associated with 22 protein putative gene functions. Among these genes, LOC_Os08g09220 and LOC_Os08g09250 putatively involved in the synthesis of OsFBX262 - F-box domain containing and glyoxalase family protein, respectively. The candidate locus

LOC_Os07g02350 and its annotated gene functions putatively associated with the casein kinase II subunit alpha-2 synthesis was identified on chromosome 7. On the shared peak regions on chromosome 7 for harvest index and leaf bronzing, 129 genes were identified among them the LOC_Os07g05800 which was reported to be associated with the glutathione S-transferase protein express. On chromosome 9, with the harvesting index, 103 genes were identified with the two significant SNPs ($-\log_{10}$ P>4) located, respectively, in LD blocks of 440 and 492 kb. Extracted list of some candidate loci identified in hydroponic and field evaluations are presented in Appendix 6.3 and Appendix 6.4.

Table 6.3: Candidate loci and corresponding putative functions identified in hydroponic and field evaluations

Loc ID	Start position	End position	Gene function putative express	Associated traits in the GWAS study	Experimental condition
LOC_Os11g31190	18171677	18174478	nodulin MtN3 family protein	Root dry weight	Hydroponic
LOC_Os05g41440	24271076	24275174	cytochrome P450, putative	Chlorophyll content	Hydroponic
LOC_Os08g09250	**5373215**	**5377152**	**glyoxalase family protein**	**Chlorophyll content**	**Hydroponic /Field**
LOC_Os08g09060	5260151	5261299	Cupin domain containing protein	Chlorophyll content	Hydroponic /Field
LOC_Os08g09220	**5345884**	**5347221**	**OsFBX262 - F-box domain containing protein**	**Chlorophyll content**	**Hydroponic /Field**
LOC_Os08g04180	**2029731**	**2033180**	**tryptophan synthase beta chain 1**	**Chlorophyll content**	**Hydroponic**
LOC_Os08g04110	1994482	1998899	transporter, putative	Chlorophyll content	Hydroponic
LOC_Os08g04120	2000176	2005227	expressed protein	Chlorophyll content	Hydroponic
LOC_Os08g04130	2007743	2011765	copine-6, putative	Chlorophyll content	Hydroponic
LOC_Os08g04140	2011999	2013407	X8 domain containing protein	Chlorophyll content	Hydroponic
LOC_Os08g04150	2013235	2015563	RNA polymerase II complex component SRB7	Chlorophyll content	Hydroponic
LOC_Os08g04170	**2019180**	**2026643**	**zinc finger C-x8-C-x5-C-x3-H type family protein**	**Chlorophyll content**	**Hydroponic**
LOC_Os04g49360	**29458213**	**29459808**	**OsFBX149 - F-box domain containing protein**	**Plant height**	**Hydroponic**
LOC_Os03g52040	29858904	29864596	OsSCP19 - Putative Serine Carboxypeptidase homologue	Root length	Hydroponic
LOC_Os09g38560	22181586	22183290	OsRCI2-11 - Hydrophobic protein OSR8	Root length	Hydroponic
LOC_Os04g32920	19886673	19892492	potassium transporter	Plant height	Hydroponic
LOC_Os04g32980	19940101	19942156	protein binding protein	Plant height	Hydroponic
LOC_Os10g32070	16847404	16849151	GATA zinc finger domain	Shoot dry weight	Hydroponic
LOC_Os10g31040	16225410	16238853	citrate transporter protein	Shoot dry weight	Hydroponic
LOC_Os10g33040	17312260	17317922	receptor-like protein kinase precursor	Shoot dry weight	Hydroponic
LOC_Os07g05800	**2453148**	**2830403**	**glutathione S-transferase**	**Harvesting index**	**Field**
LOC_Os07g05810	2453148	2830403	glycine-rich protein, putative, expressed	Harvesting index	Field
LOC_Os07g02350	**669121**	**1165133**	**casein kinase II subunit alpha-2, putative, expressed**	**Leaf bronzing**	**Field**

Gene putative functions were sourced from the MSU Rice genome annotation database (MSU, 2017). In bold, the discussed candidate loci and annotated gene functions

6.4 Discussion

Findings from the GWAS analysis demonstrated a relatively good quality of the data recorded in controlled conditions. Extensive screening in hydroponic conditions of diverse rice germplasm was previously demonstrated to provide some results with GWAS analysis (Matthus *et al.*,2015). The agro morphological traits assessed in hydroponic conditions and used in the GWAS analysis included plant height, root length, root dry weight, chlorophyll content (SPAD value) and leaf bronzing. These traits have been widely assessed in hydroponic for tolerance to iron toxicity (Asch *et al.*, 2005; Onaga *et al.*, 2013; Sikirou *et al.*, 2015) and were commonly targeted in many QTLs mapping studies. Shoot dry weight was not included in the GWAS analysis due to the fact that the genotypic effect was not significant. For the field evaluations only the location of Djibelor was considered, this was justified by a higher presence of iron toxicity symptom in this field. For the field, more results were presented for panicle number, harvest index, chlorophyll content and leaf bronzing score which were more relevant after mixed model analysis. The SNPs data used in this study (148,934 SNPs) resulted from imputation. SNPs dataset from imputation have been used by some authors for performing GWAS analysis (Wang *et al.*, 2015).

In the association mapping analysis, population structure and relatedness which is known to generate false positive (Famoso *et al.*, 2011) was determined and taken into account by adopting the method of using PCs as covariates proposed by Zhao *et al.* (2011). The structure analysis performed with the set of 148,934 SNPs generated four subpopulations represented by the *Oryza glaberrima* specie, the *japonica* and the *indica* subspecies which were clearly divided into the *indica* genotypes from the Casamance and those from the collection from AfricaRice. In addition to the use of PCs, kinship matrix integrated in the mixed linear model of GAPIT (Zhang *et al.*, 2010) also helped to balance the effects of population stratification. The results showed that some significant marker-trait associations were primarily explained by the effect of population structure when the analysis was performed on the all diversity panel of 243 rice genotypes including all subpopulations. The mixed model, considerably reduced the false positive rate in rice when used within subpopulations (Yu *et al.*, 2006; Atwell *et al.*, 2010). However, it was established that the mixed model may induce false negatives when the analysis are performed across subpopulations with an overcorrection of the population structure (Zhao *et al.*, 2011). The *indica* subpopulation composed of 186 genotypes was most used in the association mapping analysis.

LD decay was estimated at 50 kb compared to the LD variation in rice which has been estimated between 50 and 500 kb (Garris *et al*., 2003). The LD was therefore considered as relatively rapid and could provide high resolution as assumed by Matthus *et al*. (2015) in the case of rapid decay of LD in rice. With the total number of markers used, the Bonferroni test at 5% suggested a threshold *p* value = $3.6*10^{-7}$ which was too stringent in this study. Since the false positive discoveries due to population structure and relatedness was taken into account by the mixed model as proposed by Zhao *et al*. (2011), the level of association suggested by Chan *et al.* (2010) previously used in *Arabidopsis*, barley and pearl millet (Chan *et al.*, 2010; Pasam *et al.*, 2012; Gemenet *et al*., 2015) was adopted. In this approach where the 0.1% distribution of *p* value was considered, a threshold of $-\log_{10} P = 3$ was obtained with several significant SNPs (*p* value = $-\log_{10} P = 3$ and >4) for most of the traits. Significance of SNPs identified in this study as associated with variation of the different traits was therefore fixed at a minimum of $-\log_{10} P>4$ (Storey & Tibshirani, 2003; Wissuwa *et al.*, 2015; Matthus *et al.*, 2015). Nine SNPs were identified with five traits assessed in hydroponic and 60 SNPs for the eight variables recorded in the field evaluations. The identified LD block varied from < 1 kb to 496 kb. The extent of LD of less than 1 kb found in this study was previously reported by Ueda *et al.* (2015) in the genetic study of ozone tolerance in *O. sativa* rice specie. This large variability found in LD blocks was similar to the finding of Matthus *et al.* (2015). With these variations, the use of fixed window in the definition of candidate loci was suggested by Guo *et al.* (2009) and Matthus *et al.* (2015).

Gene annotation from the MSU7 rice genome database (MSU, 2017) was adopted to identify gene model and putative functions associated with the loci. Non-annotated genes could be obtained for some loci which prevented the identification of gene information linked to the associated phenotypic variation. The LOC_Os08g09220 and LOC_Os08g09250 identified in hydroponic and under field conditions are putatively involved, respectively, in the synthesis of OsFBX262 - F-box domain and a glyoxalase family protein (MSU, 2017). The F-box family are involved during panicle and seed development in rice and essential for the regulation of protein degradation in plant. Some of the F-box protein encoding genes have also been found to be regulated in a different way in rice, particularly at seedling stage, when subjected to abiotic stress (Jain *et al.*, 2007). These findings are interesting in that this study investigated the effect of iron toxicity in rice at seedling stage and the corresponding genes could be important in the mechanism of tolerance. The glyoxalase pathway has been reported to be linked with stress tolerance including salinity and heavy metal stress (Kaur *et al.*, 2017). Some glyoxalase genes (OsGLYII-2) in rice are thought to be highly involved in stress tolerance (Ghosh, *et al.*, 2014). The OsGLYI-11.2 was also reported to enhance adaptation to abiotic stress and anti-oxidant production in tobacco under salinity (Mustafiz *et al.*, 2014). Association of the identified loci with the SPAD value of chlorophyll content could be of interest considering the role of chlorophyll in the photosynthetic process and its variation in stress conditions. SPAD value has been widely investigated as being related to tolerance of rice to mineral toxicity such as salinity, boron, aluminium and iron toxicity (AfricaRice, 2006; Bashir *et al.*,2014; Neto *et al.,* 2017). This study showed that the stress experiment in hydroponic and hot spot evaluation also affected considerably the chlorophyll content. Using this biochemical variable as a measure of tolerance to iron toxicity in indirect selection could help in the identification of sources of tolerance since it is highly and negatively correlated to leaf bronzing. Some candidate loci associated with natural variation of chlorophyll content in rice using SPAD value were identified with the approach of genome wide association mapping (Wang *et al.*, 2015).

The other most valuable candidate loci identified in hot spot field experiments included the LOC_Os07g05800, putatively associated with the biosynthesis of glutathione S-transferase proteins (MSU, 2017). The glutathione acts as an antioxidant ROS scavenging enzyme, it reduced the negative effect of iron toxicity coming from $FeSO_4$ when tested on rice leaves (Fang *et al.*, 2001). Further, it was suggested that shoot and root based tolerance mechanism could result from antioxidant activities such as glutathione (Majerus *et al.*, 2007; Wu *et al.*, 2017). Candidate genes associated with the synthesis of glutathione S-transferase for leaf bronzing were also identified on chromosome 1 (LOC_Os01g49710 and LOC_Os01g49720) through genome wide association mapping (Matthus *et al.,* 2015). Glutathione S-transferase genes variously regulated under Fe toxicity in rice were also reported by Wu *et al.* (2017). Implications of the identified candidate genes in the biosynthesis of these antioxidant proteins and their relation to the leaf bronzing described earlier by Matthus *et al.* (2015) could be of interest for further investigations. The candidate locus LOC_Os07g02350 and its annotated gene functions putatively associated with the casein kinase II subunit alpha-2 synthesis was confirmed in this study. This protein was identified as part of the twenty rice predicted protein-protein interactions (Ho *et al.*, 2012) involved in many important biological functions. Orthologous genes were also identified in plant models such as *Arabidopsis*, maize and sorghum (MSU, 2017). Protein Kinase II named CK2 exists in all eukaryotes and play a role in rice developmental stage and response to stress (Takahashi *et al*., 2001; Mulekar & Huq, 2014).

6.5 Conclusions

A total of 69 SNPs significantly associated with variation of the different traits and various candidate loci reported to be putatively involved in the tolerance of rice to many oxidative stresses including iron toxicity were detected. The candidate loci LOC_Os08g09220 and LOC_Os08g09250 identified for SPAD value in both hydroponic and field evaluations could be of interest since they are putatively involved in the synthesis of proteins which are variously regulated under oxidative stress conditions. LOC_Os07g05800 and LOC_Os07g02350 putatively associated with the biosynthesis of glutathione S-transferase protein and casein kinase II subunit alpha-2 could be valuable as they were previously suggested as relevant for tolerance to iron toxicity. The locus LOC_Os07g02350 which was previously predicted as involved in the most conservative group of protein-protein interactions in rice could be an interesting candidate gene for tolerance to iron toxicity. These identified genes could be good candidates for further study including their validation and the characterization of their function for proper use in breeding programmes. Furthermore, this study showed that favourable alleles associated with most of the significant SNPs are present in the *indica* subpopulation which facilitates population development through intraspecific crosses.

CHAPTER SEVEN

7.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

7.1 General conclusion

Iron toxicity in rice is ranked among the main constraints of lowland ecosystem causing important yield losses and unproductive lands in the Casamance area of Senegal. Farmers in Casamance zone were conscious that the incidence of iron toxicity was associated with the soil properties and the flooded conditions of their fields. Their perception of this oxidative stress and traditional techniques they employed were mainly in accordance with literature and results from previous PRA studies conducted in Southern Benin. Low adoption of the improved varieties was observed in this study and most of the farmers continued to grow traditional cultivars which meet their preferences. They were however disposed to adopt new varieties if they expressed high grain yield and earliness in their field conditions in addition to a moderately tall plant type and tolerance to iron toxicity. This study showed that farmers' preferred traits and traditional knowledge are valuable for the improvement and development of high yielding varieties which could withstand toxic levels of iron.

The genetic diversity study of the collection of rice germplasm with SSR markers showed a high mean genetic diversity of the collection from AfricaRice including mainly accessions from West African countries. This study also revealed that the landraces from Casamance presented a low genetic diversity. A moderate genetic diversity was observed among the 480 rice genotypes which composed the total collection. This entire collection was divided into three distinct groups represented by the two cultivated rice species and subspecies of *Oryza sativa* and *Oryza glaberrima*. In the *sativa* group which was divided into two sub groups, two groups of *indica* subspecies represented by the landraces from Casamance and those from the AfricaRice collection were observed. This was confirmed by the population structure analysis performed on the subset of germplasm of used as GWAS panel with the 148,934 SNPs dataset.

The assessment of the GWAS panel (282 rice genotypes) for tolerance to iron toxicity showed an important phenotypic variation for most of the traits assessed in hydroponic and under field conditions. Secondary traits such as leaf bronzing and chlorophyll content (SPAD value) also gave a good estimate of the genotypic variation in response to iron toxicity in both controlled and natural conditions. It was observed in the field evaluations that environments had significant effects on most of the traits evaluated in the two locations of Djibelor and Ndeman. An important reduction of the performance of the genetic material for all traits was observed under the stress conditions of iron toxicity. The chlorophyll content was reduced to 12% in the stress compared to the non-stress field at Djibelor and 20% in hydroponic. Large decreases in biomass and grain yield (56%) was also observed in the field evaluation. Based on their level of tolerance, three different groups of genotypes were identified in both hydroponic and field evaluations. Some accessions were as tolerant as or better than the tolerant check Suakoko 8 and had a high iron root retention capacity. Consistency was found in the genetic variation of some genotypes under both hydroponic and field experiments. These promising genotypes included some accessions from the West African accessions (BEN11-172 and WONKIFON 17), some previously identified tolerant varieties such as IR75887-1-3-WAB1 (ARICA 6) and landraces from Casamance (EHAUB and BANDIOUL MANO). These genotypes could be used as sources of tolerance to iron toxicity for the genetic improvement of cultivated rice varieties.

The variation observed in this panel of rice germplasm for tolerance to iron toxicity in hydroponic and field conditions could provide an important source of genetic diversity for adaptive breeding programmes in West Africa particularly in the Casamance area in Senegal. Promising candidate loci were identified with the association mapping analysis. The candidate loci LOC_Os08g09220 and LOC_Os08g09250 identified for SPAD value in both hydroponic and field condition were putatively involved in the biosynthesis of OsFBX262- F-box domain and glyoxalase family proteins. These proteins may play a key role in the tolerance of rice to iron toxicity since they were reported to be variously regulated when rice seedlings were exposed to oxidative stresses. Candidate loci associated with the biosynthesis of glutathione S-transferase protein previously reported as relevant in the tolerance of rice to iron toxicity and the casein kinase II subunit alpha-2 which is involved in many biological functions were also identified. These loci were respectively LOC_Os07g05800 associated with variation of harvest index and LOC_Os07g02350 for leaf bronzing. The locus LOC_Os07g02350 was previously identified and reported as part of the twenty rice predicted protein-protein interactions which are involved in key biological functions. The different loci identified and discussed in this study could be good candidates for further studies including functional characterisation. The other key result from this study was that favourable alleles associated with most of the significant SNPs were identified in the *indica* subpopulation. Since most of the cultivated rice varieties belong to the *sativa* group these findings could be important for further population development through intraspecific crosses.

7.2 Recommendations

- Local constraints and farmers' preferred varietal traits should imperatively be considered for large adoption of newly developed lowland rice varieties. Assisting farmers for enhancing their knowledge could have a huge impact for boosting rice productivity of lowland ecologies developing toxic level of iron;

- The genetic characterization of the panel of rice germplasm used in this research activity revealed the existence of important diversity in the collection from AfricaRice mainly composed of West African rice accessions that could constitute useful sources of diversity for breeding programmes;

- The genotypic data points generated as well as the panel of germplasm could be used in other research activities;

- The genotypes that showed stability and high performance in both controlled and natural conditions of incidence of iron toxicity are potential sources of tolerance and could be used as donor parents in breeding programmes for varietal improvement;

- The promising candidate loci, based on their annotated functions, identified in this research activity need further investigation about their functions and full implication in iron toxicity tolerance mechanism for proper use in breeding programmes.

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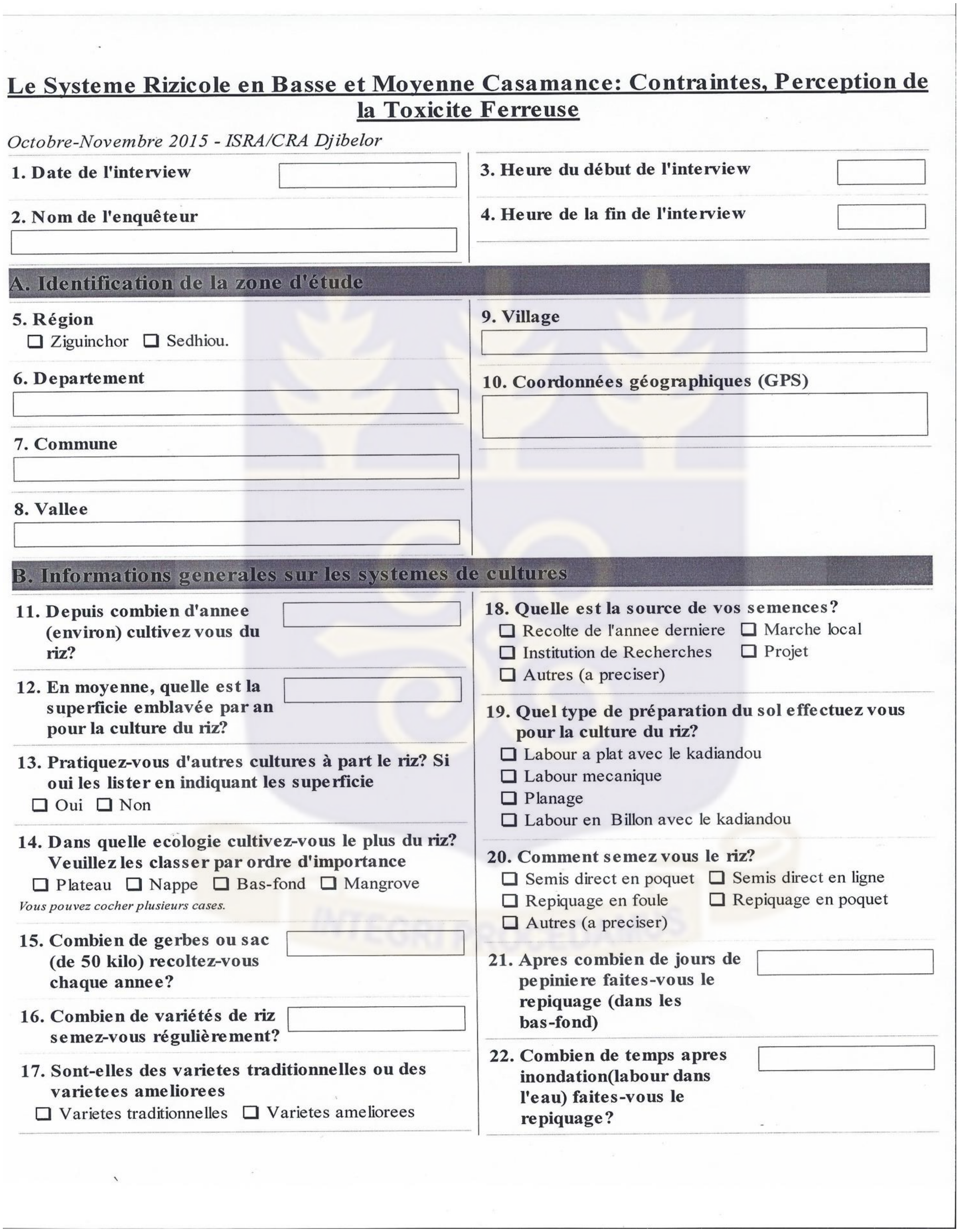
APPENDICES

Appendix 3.1: Structured questionnaire administered to farmers during the PRA



Le Systeme Rizicole en Basse et Moyenne Casamance: Contraintes, Perception de la Toxicite Ferreuse

Octobre-Novembre 2015 - ISRA/CRA Djibelor

1. Date de l'interview

2. Nom de l'enquêteur

3. Heure du début de l'interview

4. Heure de la fin de l'interview

A. Identification de la zone d'étude

5. Région
☐ Ziguinchor ☐ Sedhiou.

6. Departement

7. Commune

8. Vallee

9. Village

10. Coordonnées géographiques (GPS)

B. Informations generales sur les systemes de cultures

11. Depuis combien d'annee (environ) cultivez vous du riz?

12. En moyenne, quelle est la superficie emblavée par an pour la culture du riz?

13. Pratiquez-vous d'autres cultures à part le riz? Si oui les lister en indiquant les superficie
☐ Oui ☐ Non

14. Dans quelle ecologie cultivez-vous le plus du riz? Veuillez les classer par ordre d'importance
☐ Plateau ☐ Nappe ☐ Bas-fond ☐ Mangrove
Vous pouvez cocher plusieurs cases.

15. Combien de gerbes ou sac (de 50 kilo) recoltez-vous chaque annee?

16. Combien de variétés de riz semez-vous régulièrement?

17. Sont-elles des varietes traditionnelles ou des varietees ameliorees
☐ Varietes traditionnelles ☐ Varietes ameliorees

18. Quelle est la source de vos semences?
☐ Recolte de l'annee derniere ☐ Marche local
☐ Institution de Recherches ☐ Projet
☐ Autres (a preciser)

19. Quel type de préparation du sol effectuez vous pour la culture du riz?
☐ Labour a plat avec le kadiandou
☐ Labour mecanique
☐ Planage
☐ Labour en Billon avec le kadiandou

20. Comment semez vous le riz?
☐ Semis direct en poquet ☐ Semis direct en ligne
☐ Repiquage en foule ☐ Repiquage en poquet
☐ Autres (a preciser)

21. Apres combien de jours de pepiniere faites-vous le repiquage (dans les bas-fond)

22. Combien de temps apres inondation(labour dans l'eau) faites-vous le repiquage?

23. Inondation des parcelles
☐ Periodique ☐ Permanente ☐ frequente
☐ Rare ☐ Inexistante

24. Avez-vous la possibilite de drainer vos parcelles de temps en temps
☐ Oui ☐ Non

25. Comment jugez vous la fertilite des sols de bas-fonds?
☐ Fertile ☐ Moyennement Fertile ☐ Pauvre

26. Quelle type d'ammendement utilisez-vous?
☐ Cendre ☐ paille
☐ composte ☐ fumier
☐ Autres (a preciser)

27. Quelle est la dose d'application (nombre de panier ou de seau)

28. Utilisez vous de l'engrais mineral (NPK, Uree) pour la culture du riz? Si oui quel type ?

29. Quelles sont les periodes d'application et les doses?

30. Comment gerez-vous les mauvaises herbes?

C. Contraintes à la production du riz

31. Que considérez-vous comme principales contraintes à la production du riz dans votre zone
☐ Toxicite ferreuse
☐ Salinite
☐ Insectes
☐ Secheresse
☐ Pauvrete des sols
☐ Acces aux Semences de qualite
☐ Enherbement
☐ Ensablement
☐ Autres (a preciser)

32. Veuillez classer ces contraintes par ordre d'importance;
☐ Toxicite ferreuse
☐ Salinite
☐ Insectes
☐ Maladies
☐ Secheresse
☐ Pauvrete des sols
☐ Acces aux Semences de qualite
☐ Enherbement
☐ Ensablement
☐ Autres (a preciser)

D. Perception paysanne de la toxicite ferreuse

33. Connaissez vous la toxicite ferreuse (Photos pour illustrer) ?
☐ Oui ☐ Non.

34. Comment reconnaissez-vous la toxicite ferreuse par rapport aux autres contraintes. A Decrire

35. Dans quelles vallees pensez vous que la toxicite ferreuse est plus presente

36. Avez-vous abandonne des parcelles a cause de la toxicite ferreuse
☐ Oui ☐ Non

37. Si oui, pouvez-vous estimer la superficie

38. Depuis quand cultivez-vous vos parcelles actuelles

39. Avez-vous la toxicité ferreuse durant ces cinq (05) dernieres annees dans ces parcelles?
☐ Oui ☐ Non.

40. Indiquer le stade de developpement de la plante ou les symptomes apparaissent
☐ Avant epiaison ☐ Apres epiaison ☐ Maturite
☐ tout le cycle

41. Decrire les symptomes

42. Comment estimez-vous l'importance des pertes causees par la toxicite ferreuse
☐ Sans importance ☐ Peu important ☐ Important
☐ Tres Important.

43. Que faites vous pour prevenir la toxicite ferreuse.

44. Que faites vous quand la toxicite ferreuse apparait dans votre parcelle.

E. Préférences variétales

45. Pourquoi le choix des varietes que vous utilisez? Mettre une note selon leur importance
☐ Rendement ( )
☐ Tallage ( )
☐ Hauteur ( )
☐ Cycle ( )
☐ Remplissage en grain ( )
☐ Qualite grain ( )
☐ Tolerante a la toxicite ferreuse ( )
☐ Tolerante a la salinite ( )
☐ Resistante aux maladies ( )
☐ Resistante aux insectes ( )
☐ Autres (a preciser)

Vous pouvez cocher plusieurs cases (10 au maximum).

46. Selon vous, quels sont les caracteres importants pour le choix d'une variete? Mettre une note selon leur importance(/10)
☐ Rendement ( )
☐ Tallage ( )
☐ Hauteur ( )
☐ Cycle ( )
☐ Remplissage en grain ( )
☐ Qualite grain ( )
☐ Tolerante a la toxicite ferreuse ( )
☐ Tolerante a la salinite ( )
☐ Resistante aux maladies ( )
☐ Resistante aux insectes ( )
☐ Autres (a preciser)

Vous pouvez cocher plusieurs cases (10 au maximum).

47. Voulez vous une autre variete de riz differente de celles que vous cultivez presentement?
☐ Oui ☐ Non

48. Quelles caracteristiques voudriez vous voir sur cette nouvelle variete? Veuillez les classer par ordre d'importance

☐ Haut rendement ( )
☐ Tolerante a la toxicite ferreuse ( )
☐ Resistante aux maladies ( )
☐ Resistante aux insectes ( )
☐ Tolerante aux mauvaises herbes ( )
☐ Tolerante a la secheresse( )
☐ Tolerante aux sols pauvres( )
☐ Autres (a preciser)

Vous pouvez cocher plusieurs cases.

F. Identification du producteur

49. Avez-vous d'autres occupations en dehors de l'agriculture ?

☐ Oui ☐ Non

50. Si oui laquelle

☐ Elevage ☐ Peche ☐ Commerce
☐ Transport ☐ Autres

51. Etes-vous le proprietaire de l'exploitation

☐ Oui ☐ Non

52. Etes vous membre d'un groupement de producteur ?

☐ Oui ☐ Non

53. Si oui, lequel?

54. Nom

55. Prenom

56. Telephone

Le code a 10 caractères.

57. Classe d'âge

☐ 18-24 ☐ 25-34 ☐ 35-49 ☐ 50-64 ☐ 65+

58. Sexe

☐ Masculin ☐ Féminin

59. Ethnie

☐ Diola ☐ Manjacque ☐ Soce
☐ Mandingue ☐ Baïnouck ☐ Mancagne
☐ Wolof ☐ Serere ☐ Al pulaar
☐ Autres

60. Quelle est votre situation matrimoniale ?

☐ Célibataire ☐ Marié(e) ☐ Veuf(ve)
☐ Divorcé(e)

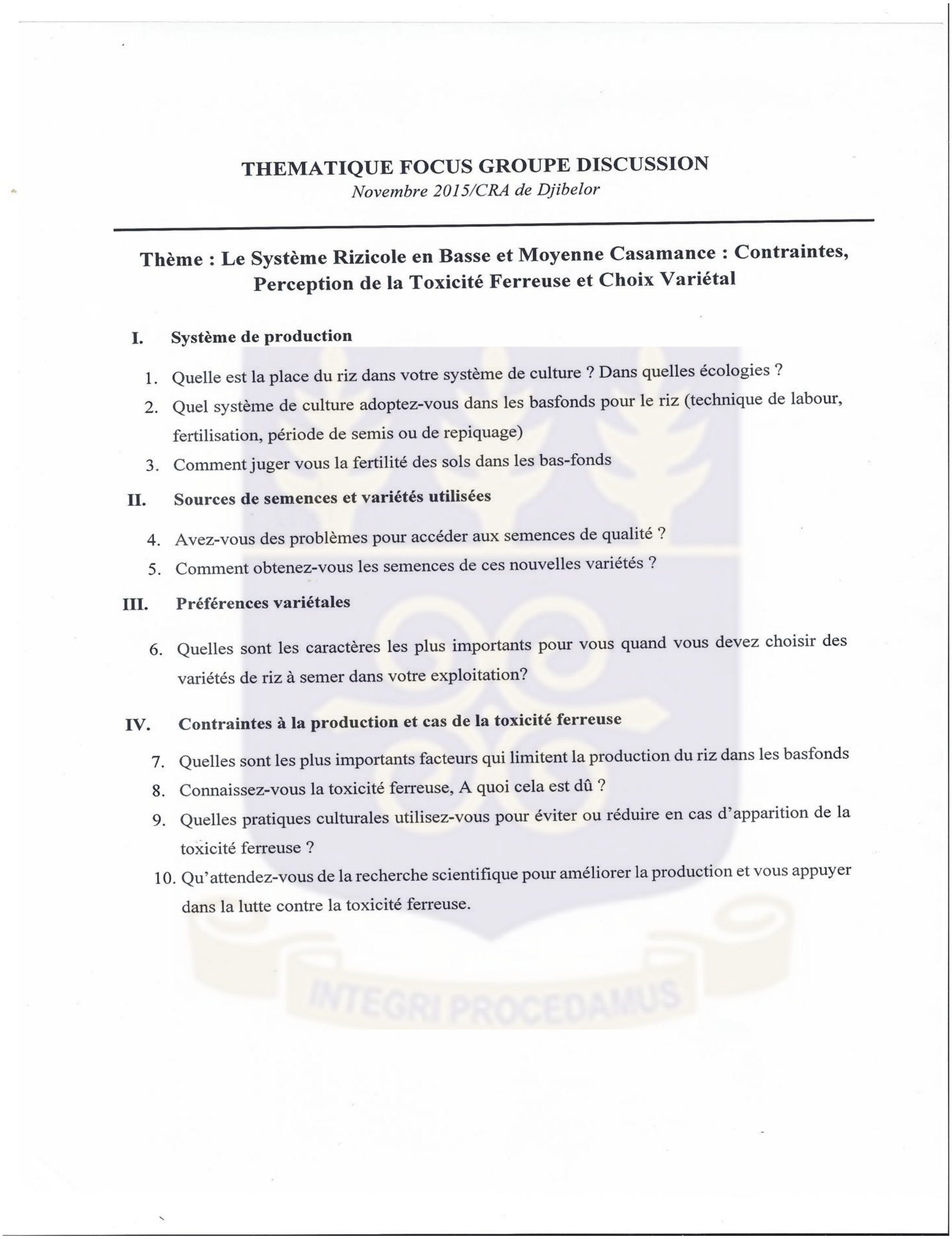
61. Etes-vous le (la) Chef de famille?

☐ Oui ☐ Non

62. Quel est votre niveau d'instruction ?

☐ Non instruit ☐ Alphabetise en langue locale
☐ Formation coranique ☐ Etudes primaires
☐ Etudes secondaires ☐ Etudes superieures
☐ Autres.

Appendix 3.2: Main point discussed during focus group discussions



THEMATIQUE FOCUS GROUPE DISCUSSION

Novembre 2015/CRA de Djibelor

Thème : Le Système Rizicole en Basse et Moyenne Casamance : Contraintes, Perception de la Toxicité Ferreuse et Choix Variétal

I. Système de production

1. Quelle est la place du riz dans votre système de culture ? Dans quelles écologies ?
2. Quel système de culture adoptez-vous dans les basfonds pour le riz (technique de labour, fertilisation, période de semis ou de repiquage)
3. Comment juger vous la fertilité des sols dans les bas-fonds

II. Sources de semences et variétés utilisées

4. Avez-vous des problèmes pour accéder aux semences de qualité ?
5. Comment obtenez-vous les semences de ces nouvelles variétés ?

III. Préférences variétales

6. Quelles sont les caractères les plus importants pour vous quand vous devez choisir des variétés de riz à semer dans votre exploitation?

IV. Contraintes à la production et cas de la toxicité ferreuse

7. Quelles sont les plus importants facteurs qui limitent la production du riz dans les basfonds
8. Connaissez-vous la toxicité ferreuse, A quoi cela est dû ?
9. Quelles pratiques culturales utilisez-vous pour éviter ou réduire en cas d'apparition de la toxicité ferreuse ?
10. Qu'attendez-vous de la recherche scientifique pour améliorer la production et vous appuyer dans la lutte contre la toxicité ferreuse.

Appendix 4.1: Genetic diversity among the collection from AfricaRice mainly composed of West African rice accessions and characteristics of the SSR markers used

Marker	Chr	Repeat type and length	Annealing Temp (°C)	PCR product size range	Allele Frquency	Allele No.	GD	PIC
RM105	9	(CCT) 6	55	131-140	0.47	4	0.68	0.63
RM206	11	(CT) 21	55	128-202	0.35	5	0.76	0.72
RM212	1	(CT) 24	55	112-134	0.58	5	0.61	0.58
RM216	10	(CT) 18	55	127-143	0.64	4	0.54	0.50
RM218	3	(TC)24 (ACT)5(GT)11	55	120-148	0.34	5	0.75	0.71
RM241	4	(CT) 31	55	102-142	0.39	5	0.70	0.65
RM248	7	(CT) 25	55	80-104	0.37	5	0.72	0.68
RM249	5	(AG)5A2 (AG) 14	55	154-174	0.47	5	0.66	0.61
RM253	6	(GA) 25	55	125-143	0.60	4	0.57	0.52
RM277	12	(GA) 11	55	118-124	0.65	3	0.51	0.45
RM279	2	(GA)16	55	148-174	0.37	5	0.74	0.69
RM310	8	(GT) 19	55	85-120	0.23	6	0.81	0.79
Mean					0.46	5	0.67	0.63

Appendix 4.2: Genetic diversity among the landraces from Casamance and characteristics of the SSR markers used

Marker	Chr	Repeat type and length	Annealing Temp (°C)	PCR product size range	Allele Frquency	Allele No	GD	PIC
RM105	9	(CCT) 6	55	131-140	0.59	4	0.58	0.53
RM206	11	(CT) 21	55	128-202	0.63	5	0.57	0.54
RM212	1	(CT) 24	55	112-134	0.87	5	0.24	0.23
RM216	10	(CT) 18	55	127-143	0.84	4	0.28	0.27
RM218	3	(TC)24 (ACT)5(GT)11	55	120-148	0.61	5	0.58	0.53
RM241	4	(CT) 31	55	102-142	0.65	5	0.53	0.48
RM248	7	(CT) 25	55	80-104	0.40	5	0.75	0.71
RM249	5	(AG)5A2 (AG) 14	55	154-174	0.82	5	0.31	0.30
RM253	6	(GA) 25	55	125-143	0.82	4	0.32	0.30
RM277	12	(GA) 11	55	118-124	0.83	3	0.30	0.28
RM279	2	(GA)16	55	148-174	0.51	5	0.67	0.62
RM310	8	(GT) 19	55	85-120	0.32	6	0.79	0.76
Mean					0.64	5	0.50	0.47

Appendix 5.1: List of the rice germplasm

Number	**Djibelor Code**	**Acc. Number**	**Name**	**species name**	**Origin**
1	SN44	WAB0014215	DISSI	*O. sativa*	Burkina Faso
2	SN37	WAB0019811	TOS 8229	*O. sativa*	Benin
3	SN38	WAB0025976	TOS 6954	*O. sativa*	Sierra Leone
4	SN42	WAB0031015	MBAH 1-1	*O. sativa*	Benin
5	SN46	WAB0007523	IS 692	*O. sativa*	Cote d'Ivoire
6	SN49	WAB0026958	TOS 12542	*O. sativa*	Senegal
7	SN50	WAB0010251	TOS 8901	*O. glaberrima*	Nigeria
8	SN51	WAB0009191	TOS 7090	*O. sativa*	Sierra Leone
9	SN54	WAB0012261	TOS 12891	*O. sativa*	Senegal
10	SN59	WAB0031072	XLETIETOE	*O. glaberrima*	Togo
11	SN56	WAB0029381	DANYI-MOLI	O. glaberrima	Togo
12	SN57	WAB0013763	TOG 14936	*O. glaberrima*	Togo
13	SN58	WAB0013761	TOG 14931	*O. glaberrima*	Togo
14	SN67	WAB0003916	IDSA 62	*O. sativa*	Côte d'Ivoire
15	SN71	WAB0000036	IDSA 46	*O. sativa*	Côte d'Ivoire
16	SN84	WAB0007847	EX GIDAN GALADIMA	*O. sativa*	Nigeria
17	SN86	WAB0015523	DE GAULLE (FARO 24)	*O. sativa*	Nigeria
18	SN87	WAB0007848	EX SHENDAM	*O. sativa*	Nigeria
19	SN93	WAB0007472	IS 533	*O. sativa*	Cote d'Ivoire
20	BL16	NA	WITA 4	*O. sativa*	Côte d'Ivoire
21	SN94	WAB0030559	WONKIFON 17	*O. sativa*	Guinea
22	BL7	NA	Missi missi 3	*O. sativa*	Guinea
23	SN95	WAB0030572	MISSI MISSI 2	*O. sativa*	Guinea
24	BL5	NA	Kaolack gbeli	*O. sativa*	Guinea
25	SN96	WAB0014131	TOS 15508	*O. sativa*	Burkina Faso
26	SN97	WAB0017289	SALI FORE	*O. sativa*	Guinea
27	SN98	WAB0009867	TOS 8233	*O. sativa*	Benin
28	SN99	WAB0009153	TOS 7044	*O. sativa*	Sierra Leone
29	SN102	WAB0004150	ROK 10	*O. sativa*	Sierra Leone
30	SN103	WAB0011161	TOS 10818	*O. sativa*	Guinea-Bissau
31	SN104	WAB0024993	TOS 6691	*O. sativa*	Nigeria
32	SN108	WAB0025457	TOS 10484	*O. sativa*	Guinea-Bissau
33	SN109	WAB0024846	TOS 12821	*O. sativa*	Côte d'Ivoire
34	SN110	WAB0011052	TOS 10651	*O. sativa*	Ghana
35	SN111	WAB0025509	TOS 10511	*O. sativa*	Guinea-Bissau
36	SN112	WAB0014132	TOS 15509	*O. sativa*	Burkina Faso
37	SN113	WAB0025586	TOS 8608	*O. sativa*	Benin
38	SN114	WAB0009052	TOS 6913	*O. sativa*	Sierra Leone
39	SN116	WAB0010200	TOS 8835	*O. sativa*	Benin
40	SN117	WAB0009324	TOS 7481	*O. sativa*	Ghana
41	SN118	WAB0024962	TOS 9029	*O. sativa*	Nigeria
42	SN120	WAB0008969	TOS 6798	*O. sativa*	Nigeria
43	SN121	WAB0030584	CK 21-1	*O. sativa*	Guinea
44	SN122	WAB0025179	TOS 10863	*O. sativa*	Liberia
45	SN124	WAB0025626	TOS 8833	*O. sativa*	Benin
46	SN125	WAB0011019	TOS 10543	*O. sativa*	Guinea-Bissau
47	SN127	WAB0010201	TOS 8836	*O. sativa*	Benin
48	SN128	WAB0030590	CK 90-3	*O. sativa*	Guinea
49	SN129	WAB0009260	TOS 7356	*O. sativa*	Liberia
50	SN133	WAB0025239	TOS 6758	*O. sativa*	Nigeria
51	SN138	WAB0025058	TOS 10654	*O. sativa*	Ghana

52	SN139	WAB0009054	TOS 6919	*O. sativa*	Sierra Leone
53	SN140	WAB0009868	TOS 8234	*O. sativa*	Benin
54	SN141	WAB0014059	TOS 15386	*O. sativa*	Burkina Faso
55	SN142	WAB0009328	TOS 7488	*O. sativa*	Sierra Leone
56	SN143	WAB0025480	TOS 10675	*O. sativa*	Senegal
57	SN146	WAB0011053	TOS 10656	*O. sativa*	Ghana
58	SN147	WAB0014057	TOS 15383	*O. sativa*	Burkina Faso
59	SN149	WAB0025369	TOS 10495	*O. sativa*	Guinea-Bissau
60	SN150	WAB0009753	TOS 8083	*O. sativa*	Sierra Leone
61	SN151	WAB0025482	TOS 10867	*O. sativa*	Liberia
62	SN189	WAB0015215	Meada	*O. sativa*	Benin
63	SN190	WAB0015877	Sabougnouma	*O. sativa*	Guinea
64	SN191	WAB0019891	Gambiaka Sébéla	*O. sativa*	Mali
65	SN193	WAB0019994	Gambiaka L41	*O. sativa*	Mali
66	SN163	WAB0031746	Tamba yêguéti 2	*O. sativa*	Guinea
67	SN162	WAB0031745	Tamba yeguéti 1	*O. sativa*	Guinea
68	SN157	WAB0031732	Samou gbeli (80)	*O. sativa*	Guinea
69	BL10	NA	Wonkifon fikhè (162)	*O. sativa*	Guinea
70	SN156	WAB0031731	Samou gbeli (150)	*O. sativa*	Guinea
71	SN166	WAB0031752	Wonkifon 3	*O. sativa*	Guinea
72	SN159	WAB0031739	Solima 2	*O. sativa*	Guinea
73	BL8	NA	Missi missi monchon	*O. sativa*	Guinea
74	SN155	WAB0031735	Ser 5 (96)	*O. sativa*	Guinea
75	SN158	WAB0031738	Solima 1 (56)	*O. sativa*	Guinea
76	SN165	WAB0031759	Samou gbeli (07)	*O. sativa*	Guinea
77	SN154	WAB0031715	Konyi khakate 3	*O. sativa*	Guinea
78	SN172	WAB0011011	TOS 10530	*O. sativa*	Guinea-Bissau
79	SN173	WAB0010053	TOS 8615	*O. sativa*	Benin
80	SN175	WAB0027224	TOS 8613	*O. sativa*	Benin
81	SN180	WAB0007537	IS 737	*O. sativa*	Cote d'Ivoire
82	SN181	WAB0018372	BEN11-171	*O. sativa*	Benin
83	SN380	WAB0034911	TOS 3440	*O. sativa*	Sierra Leone
84	SN194	WAB0031129	TOS 876	*O. sativa*	Sierra Leone
85	SN197	WAB0019830	TOS 11746	*O. sativa*	Senegal
86	BL13 (T1)	NA	SUAKOKO 8 (ROK 24)	*O. sativa*	Liberia
87	SN206	WAB0005011	BEN11-175	*O. sativa*	Benin
88	SN210	WAB0006668	BEN11-2	*O. sativa*	Benin
89	SN214	WAB0006821	BEN11-134	*O. sativa*	Benin
90	SN216	WAB0006758	ITA 252	*O. sativa*	Nigeria
91	SN221	WAB0004587	MK 3-87	*O. sativa*	Togo
92	SN222	WAB0004588	MK 9-87	*O. sativa*	Togo
93	SN226	WAB0004591	NIARIS 12	*O. sativa*	Benin
94	SN227	WAB0004592	NIARIS 13	*O. sativa*	Benin
95	SN230	WAB0004382	CK 1	*O. sativa*	Guinea
96	SN234	WAB0004323	KOGONI 91-1	*O. sativa*	Mali
97	SN261	WAB0013760	OUMOLI	*O. glaberrima*	Togo
98	SN262	WAB0029575	TOS 12806	*O. sativa*	Côte d'Ivoire
99	SN272	WAB0029378	Ekoudzi	*O. glaberrima*	Togo
100	SN301	WAB0013762	AMEYIBOMELI	*O. glaberrima*	Togo
101	SN302	WAB0002104	DS4-B1	*O. glaberrima*	Niger
102	SN238	WAB0033364	TOS 8736-A (M)	*O. sativa*	Nigeria
103	SN237	WAB0027213	TOS 835	*O. sativa*	Senegal
104	SN242	WAB0033431	MB 9B	*O. sativa*	Mali
105	SN250	WAB0011035	TY 72	*O. sativa*	Niger
106	SN253	WAB0009710	TY 71	*O. glaberrima*	Niger

107	SN257	WAB0009870	TOS 8236	*O. sativa*	Benin
108	SN263	WAB0002107	IG 36	*O. sativa*	Cote d'Ivoire
109	SN266	WAB0016848	ORYLUX 1	*O. sativa*	Togo
110	SN275	WAB0016848	BEN11-172	*O. sativa*	Benin
111	SN279	WAB0034177	DS 47-B	*O. sativa*	Niger
112	SN277	WAB0034169	DS11-A	*O. glaberrima*	Niger
113	SN281	WAB0034196	EWENTO YIBO	*O. glaberrima*	Togo
114	SN283	WAB0025834	TOS 15385	*O. sativa*	Burkina Faso
115	SN284	WAB0014058	TOS 15384	*O. sativa*	Burkina Faso
116	SN288	WAB0014063	TOS 15391	*O. sativa*	Burkina Faso
117	SN286	WAB0025795	TOS 15392	*O. sativa*	Burkina Faso
118	SN285	WAB0014061	TOS 15388	*O. sativa*	Burkina Faso
119	SN290	WAB0014133	TOS 15511	*O. sativa*	Burkina Faso
120	SN298	WAB0034442	TY 73	*O. sativa*	Niger
121	SN303	WAB0011903	TOG 12367	*O. glaberrima*	Guinea-Bissau
122	SN304	WAB0029376	Venutohe	*O. glaberrima*	Togo
123	SN305	WAB0029379	Ewinto moli	*O. glaberrima*	Togo
124	SN306	WAB0029377	Ewemoli	*O. glaberrima*	Togo
125	SN308	WAB0032367	DANYI MOLI-A	*O. glaberrima*	Togo
126	SN309	WAB0030266	MAWOU-C	*O. glaberrima*	Togo
127	SN310	WAB0030442	XLETIETOE EVENTOHI	*O. glaberrima*	Togo
128	SN311	WAB0029381	TY 7-C	*O. glaberrima*	Niger
129	SN313	WAB0032588	MAWOU-D	*O. glaberrima*	Togo
130	SN315	WAB0029512	TOS 12818	*O. sativa*	Côte d'Ivoire
131	SN317	WAB0029521	TOS 11698	*O. sativa*	Senegal
132	SN318	WAB0029587	TOS 14650	*O. sativa*	Nigeria
133	SN319	WAB0029375	EWENTOHI	*O. glaberrima*	Togo
134	SN323	WAB0032613	MOLI YIBO CEWE MOLI	*O. glaberrima*	Togo
135	SN320	WAB0029374	EWINTOHI	*O. glaberrima*	Togo
136	SN321	WAB0032615	MOLI VIBO CEWEMOLI	*O. glaberrima*	Togo
137	SN324	WAB0030267	MAWOU-E	*O. glaberrima*	Togo
138	SN327	WAB0029373	EWINTO YIBO	*O. glaberrima*	Togo
139	SN329	WAB0029561	TOS 12914	*O. sativa*	Côte d'Ivoire
140	SN338	WAB0008279	TOS 1106	*O. sativa*	Mauritania
141	SN342	WAB0029528	TOS 12825	*O. sativa*	Côte d'Ivoire
142	SN344	WAB0024751	TOS 10650	*O. sativa*	Ghana
143	SN346	WAB0009051	TOS 6912	*O. sativa*	Sierra Leone
144	SN347	WAB0010986	TOS 10490	*O. sativa*	Guinea-Bissau
145	SN348	WAB0012173	TOS 12788	*O. sativa*	Nigeria
146	SN349	WAB0008377	TOS 3365	*O. sativa*	Liberia
147	SN350	WAB0019961	TOS 13089	*O. sativa*	Guinea
148	SN351	WAB0033005	DS 34-B	*O. sativa*	Niger
149	SN353	WAB0011429	TOS 11522	*O. sativa*	Mali
150	SN362	WAB0008036	TY 74	*O. sativa*	Niger
151	SN363	WAB0033209	TOS 13069	*O. sativa*	Guinea
152	SN364	WAB0033230	Marowile	*O. sativa*	Burkina Faso
153	SN366	WAB0033296	TY 7-D	*O. sativa*	Niger
154	SN367	WAB0009865	TOS 8230	*O. sativa*	Benin
155	BL3	NA	SER 6	*O. sativa*	Guinea
156	SN385	WAB0034966	BEN11-13	*O. sativa*	Benin
157	SN387	WAB0035077	BEN11-111-A	*O. sativa*	Benin
158	SN389	WAB0035114	Baga malé fikhè	*O. sativa*	Guinea
159	SN391	WAB0035133	Conté malé (1)	*O. sativa*	Guinea
160	SN392	WAB0035147	Gbangbalony	*O. sativa*	Guinea
161	BL4	NA	Kaolack 1	*O. sativa*	Guinea

162	SN396	WAB0035166	Kock	*O. sativa*	Guinea
163	SN395	WAB0035171	Konyi khakate 1	*O. sativa*	Guinea
164	SN397	WAB0035174	Saliforeh	*O. sativa*	Guinea
165	BL2	NA	SER 5	*O. sativa*	Guinea
166	BL14	NA	FARO 2 (TOS 7672)	*O. sativa*	Nigeria
167	BL15	NA	FARO 23 (IR 5)	*O. sativa*	Philippines
168	C1	NA	BEM EGNOU	*O. sativa*	Casamance
169	C8	NA	SIMBE	*O. sativa*	Casamance
170	C10	NA	DIAMISSA	*O. sativa*	Casamance
171	C19	NA	TUMLOUYE	*O. sativa*	Casamance
172	C20	NA	BANDIOUL MANO	*O. sativa*	Casamance
173	C21	NA	LIMBI LARBA	*O. sativa*	Casamance
174	C23	NA	TOUMBOUR	*O. sativa*	Casamance
175	C24	NA	KOSMIDA	*O. sativa*	Casamance
176	C27	NA	DJILIBA	*O. sativa*	Casamance
177	C28	NA	CAUMO MANO	*O. sativa*	Casamance
178	C31	NA	TOUMBOUR	*O. sativa*	Casamance
179	C32	NA	AMISSA EDIOUGUEUK	*O. sativa*	Casamance
180	C34	NA	DJIBELOR	*O. sativa*	Casamance
181	C35	NA	COULCOULBA KAYO	*O. sativa*	Casamance
182	C40	NA	DJIBABOUYA	*O. sativa*	Casamance
183	C41	NA	MIDJI	*O. sativa*	Casamance
184	C42	NA	YARAAGNAMBA	*O. sativa*	Casamance
185	C47	NA	RACIO	*O. sativa*	Casamance
186	C48	NA	BRIKISA	*O. sativa*	Casamance
187	C49	NA	KAMISSA	*O. sativa*	Casamance
188	C52	NA	BARRAGE	*O. sativa*	Casamance
189	C53	NA	EBOUKITELO	*O. sativa*	Casamance
190	C54	NA	ABENTOUL	*O. sativa*	Casamance
191	C56	NA	CHINOIS	*O. sativa*	Casamance
192	C57	NA	MAN KANN	*O. sativa*	Casamance
193	C58	NA	DIAMISSE	*O. sativa*	Casamance
194	C60	NA	FOULEGUE	*O. sativa*	Casamance
195	C61	NA	TENBOUYI	*O. sativa*	Casamance
196	C62	NA	THIERO	*O. sativa*	Casamance
197	C63	NA	AMISSAYAFITE	*O. sativa*	Casamance
198	C64	NA	DEMBO	*O. sativa*	Casamance
199	C65	NA	ZALANG SAN	*O. sativa*	Casamance
200	C67	NA	SEFO	*O. sativa*	Casamance
201	C68	NA	NIOCOMBA	*O. sativa*	Casamance
202	C71	NA	BETYA	*O. sativa*	Casamance
203	C72	NA	BARASSE	*O. sativa*	Casamance
204	C73	NA	YANGHOLAL	*O. sativa*	Casamance
205	C74	NA	EGNOMOULAYE	*O. sativa*	Casamance
206	C76	NA	BADIABONE	*O. sativa*	Casamance
207	C80	NA	CANLCANLBA	*O. sativa*	Casamance
208	C81	NA	YAGHOLAL	*O. sativa*	Casamance
209	C83	NA	DJIFERO	*O. sativa*	Casamance
210	C86	NA	KHARISSARISSE	*O. sativa*	Casamance
211	C87	NA	DIAMDONE	*O. sativa*	Casamance
212	C89	NA	COULANLBA	*O. sativa*	Casamance
213	C90	NA	CANALINE	*O. sativa*	Casamance
214	C91	NA	MALIBA	*O. sativa*	Casamance
215	C93	NA	TITILO	*O. sativa*	Casamance
216	C94	NA	ETOUKAL	*O. glaberrima*	Casamance

217	C96	NA	NDIOL	*O. sativa*	Casamance
218	C98	NA	NGHAIMENE	*O. sativa*	Casamance
219	C99	NA	KASSEL	*O. sativa*	Casamance
220	C101	NA	KARANFANY	*O. sativa*	Casamance
221	C102	NA	BONTY	*O. sativa*	Casamance
222	C104	NA	ETOUKAL	*O. glaberrima*	Casamance
223	C110	NA	FRANCOUNDA	*O. sativa*	Casamance
224	C113	NA	DIAMINE	*O. sativa*	Casamance
225	C114	NA	SADIA	*O. sativa*	Casamance
226	C115	NA	ERAGOUAYE	*O. sativa*	Casamance
227	C116	NA	BADIOURE	*O. sativa*	Casamance
228	C117	NA	EHAUB	*O. sativa*	Casamance
229	C126	NA	EFELHOR	*O. sativa*	Casamance
230	C131	NA	CFA	*O. sativa*	Casamance
231	C132	NA	SEBOR	*O. sativa*	Casamance
232	C133	NA	FOULEGUE YAMEUK	*O. sativa*	Casamance
233	C136	NA	MANSA	*O. sativa*	Casamance
234	C137	NA	ETOUMOREUL	*O. sativa*	Casamance
235	C138	NA	ATEBAMA	*O. sativa*	Casamance
236	C140	NA	NGAIMENE	*O. sativa*	Casamance
237	C142	NA	NIGUI	*O. sativa*	Casamance
238	C145	NA	BALANTA MANO	*O. sativa*	Casamance
239	C147	NA	KASSILAYE	*O. sativa*	Casamance
240	C148	NA	BASSITE	*O. sativa*	Casamance
241	C151	NA	DIAKON	*O. sativa*	Casamance
242	C154	NA	KAYURA	*O. sativa*	Casamance
243	C156	NA	KOULOU KOUBA	*O. sativa*	Casamance
244	C157	NA	NGONKOUMA	*O. sativa*	Casamance
245	C158	NA	KAOUROU	*O. sativa*	Casamance
246	C159	NA	OUBAUME	*O. sativa*	Casamance
247	C160	NA	GLORY	*O. sativa*	Casamance
248	KV 1	NA	CT 19298 (27)-1-11-1-2-3 MP	*O. sativa*	AfricaRice
249	KV 10	NA	IR 06A 150	*O. sativa*	AfricaRice
250	KV 11	NA	FAROX 521-83-H1	*O. sativa*	AfricaRice
251	KV 13	NA	WAB 2151-TGR 1-WAT B4	*O. sativa*	AfricaRice
252	KV 14	NA	FAROX 527-101-H1	*O. sativa*	AfricaRice
253	KV 12	NA	CT 18838-1-1-2-1SR-2P	*O. sativa*	AfricaRice
254	KV 15	NA	FAROX 521-176-H1	*O. sativa*	AfricaRice
255	KV 17	NA	WAC 11-TGR 6	*O. sativa*	AfricaRice
256	KV 18	NA	WAB 1572-10-B-B-FKR-4-WAC 1-1-TGR 2-WAT10-1	*O. sativa*	AfricaRice
257	KV 19	NA	IR 84649-21-15-1-B	*O. sativa*	AfricaRice
258	KV 2	NA	WAB 1529-7-B-B-FKR 4-WAC 1-2-TGR 2-WAT7-1	*O. sativa*	AfricaRice
259	KV 20	NA	WAB 1573-22-B-B-FKR 4-2-WAC 1-TGR 3-WAT9-1	*O. sativa*	AfricaRice
260	KV 21	NA	FAROX 521-82-H1	*O. sativa*	AfricaRice
261	KV 22	NA	FAROX 521-156-H1	*O. sativa*	AfricaRice
262	KV 23	NA	WAB 2135-WAC B-2-TGR 2-WAT1-1	*O. sativa*	AfricaRice
263	KV 24	NA	FAROX 521-357-H1	*O. sativa*	AfricaRice
264	KV 25	NA	CT 18491-7-3-1-4-4P	*O. sativa*	AfricaRice
265	KV 26	NA	FAROX 521-288-H1	*O. sativa*	AfricaRice
266	KV 27	NA	CT 21407-9P-5P-4SR-1	*O. sativa*	AfricaRice
267	KV 28	NA	CT 19558-2-17-4P-3-1-1-M	*O. sativa*	AfricaRice
268	KV 29	NA	WAB 2098-WAC 1-FKR 2-4-TGR 1	*O. sativa*	AfricaRice
269	KV 3	NA	WAC 18-WAT 65-1-1	*O. sativa*	AfricaRice

270	KV 30	NA	FAROX 521-356-H1	*O. sativa*	AfricaRice
271	KV 5	NA	WAC 13-WAT21-2-1	*O. sativa*	AfricaRice
272	KV 9	NA	SAHEL 108	*O. sativa*	AfricaRice
273	KV 8	NA	ORYLUX 6	*O. sativa*	AfricaRice
274	KV 16	NA	SAHEL 134	*O. sativa*	AfricaRice
275	KV 31	NA	ARICA 2	*O. sativa*	AfricaRice
276	SG 1	NA	WAT 1046-B-43-2-2	*O. sativa*	AfricaRice Sahel
277	SG 2	NA	IR 75866-2-7-1-WAB-1	*O. sativa*	AfricaRice Sahel
278	SG 3	NA	WAS 122-IDSA-1-WAS-2-B-1-TGR123 (NL16)	*O. sativa*	AfricaRice Sahel
279	SG 4	NA	WAS 21-B-B-20-4	*O. sativa*	AfricaRice Sahel
280	SG 6	NA	WAS 20-B-B-1-2-2	*O. sativa*	AfricaRice Sahel
281	SG 7	NA	IR 75887-1-3-WAB-1	*O. sativa*	AfricaRice Sahel
282	SG5	NA	ROCK 25	*O. sativa*	AfricaRice Sahel
283	DJ 684-D	NA	DJ 684-D	*O. sativa*	Djibelor
284	BG 90-2	NA	BG 90-2	*O. sativa*	Djibelor
285	SUAKOKO 8 (TC)	NA	SUAKOKO 8	*O. sativa*	AfricaRice Sahel
286	BOUAKE 189 (SC)	NA	BOUAKE 189	*O. sativa*	AfricaRice Sahel

Appendix 5.2: Lamda Wilk test showing *p*-value of the discriminant traits associated with the phenotypic variation in hydroponic conditions

Variable	Lambda	F	p-value
Shoot dry weight	0.805	33.204	< 0.0001
Root dry weight	0.970	4.197	0.016
Plant height	0.232	454.650	< 0.0001
Root length	0.841	25.985	< 0.0001
Chlorophyll content	0.971	4.136	0.017
Leaf bronzing score	0.967	4.753	0.009

Appendix 5.3: Lamda Wilk test showing *p*-value of the discriminant traits associated with the phenotypic variation in stress field experiment in Djibelor

Variables	Lambda	F	p-value
Flowering	0.9292	10.4025	< 0.0001
Plant height	0.7670	41.4688	< 0.0001
Tillering ability	0.6678	67.9153	< 0.0001
Biomass	0.2190	486.6851	< 0.0001
Panicle number	0.7577	43.6554	< 0.0001
harvesting index	0.8267	28.6145	< 0.0001
Spikelet Sterility	0.9796	2.8381	0.0603
Chlorophyll content	0.9770	3.2113	0.0418
Leaf bronzing score 60 DAT	0.6927	60.5525	< 0.0001

Appendix 5.4: Scoring of iron toxicity tolerance as described in the Standard Evaluation System for Rice (SES, IRRI, 2002)

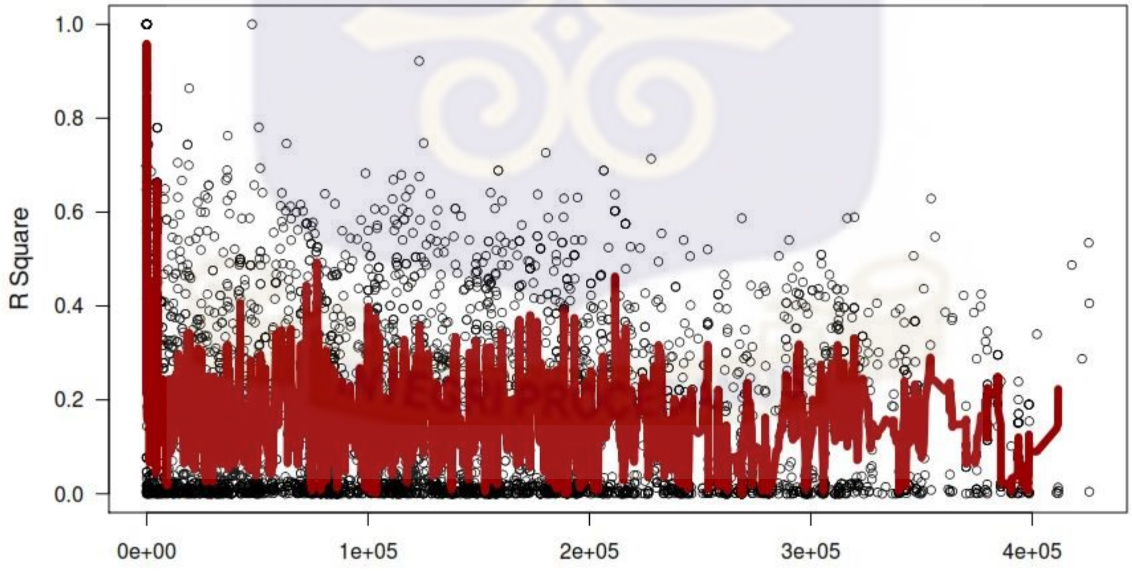
Score	**Observation**	**Tolerance**
1	Normal growth, no leaf symptoms	Highly tolerant
2	Normal growth; reddish-brown spots or orange discoloration on the tips of old leaves	Tolerant
3	Nearly normal growth; older leaves reddish-brown, purple, or orange yellow	
5	Growth retarded; many leaves discolored	Moderately tolerant
7	Complete cessation of growth; most leaves dry; some plants dying	Susceptible
9	Almost all plants dead or drying	Highly susceptible

Appendix 6.1: Imputation and SNPs processing

No of test	%min count	equivalent No	minor allele frequency (MAF)	number of sites (markers)
1	80	271	0.01	19198
2	70	237	0.01	32804
3	50	170	0.01	67019
4	80	271	0.05	10857
5	70	237	0.05	19504
6	50	170	0.05	43635
After imputation				
1	80	271	0.01	158408
2	70	237	0.01	186828
3	50	170	0.01	205379
4	80	271	0.05	124970
5	70	237	0.05	148934
6	50	170	0.05	164821

We select markers from the line highlighted this because the MAF is 0.05, which for 100 markers we can 5% rare allele. 70% of min count means markers which work for 70%

Appendix 6.2: LD decay of the *indica* subpopulation



Appendix 6.3: List of some extracted loci identified in hydroponic screening

ROOT DRY WEIGHT

Chrom	region_start	region_end	region_name	gene_start	gene_end	gene_name
chr11	18164928	18174312	region_S11_18164929	18171677	18174478	LOC_Os11g31190.1
chr11	22615823	22626855	region_S11_22626856	22614876	22616875	LOC_Os11g38140.1
chr11	22615823	22626855	region_S11_22626856	22618818	22624351	LOC_Os11g38150.1
chr11	22615823	22626855	region_S11_22626856	22625529	22627579	LOC_Os11g38160.1
ROOT LENGTH						
chr3	29863713	29898247	region_S3_29880579	29858904	29864596	LOC_Os03g52040.1
chr3	29863713	29898247	region_S3_29880579	29858904	29864596	LOC_Os03g52040.2
chr3	29863713	29898247	region_S3_29880579	29858904	29864596	LOC_Os03g52040.3
chr3	29863713	29898247	region_S3_29880579	29871084	29875766	LOC_Os03g52070.1
chr3	29863713	29898247	region_S3_29880579	29871084	29875767	LOC_Os03g52070.3
chr3	29863713	29898247	region_S3_29880579	29871166	29878401	LOC_Os03g52070.2
chr3	29863713	29898247	region_S3_29880579	29883091	29887239	LOC_Os03g52080.1
chr3	29863713	29898247	region_S3_29880579	29889953	29910917	LOC_Os03g52090.1
chr9	22036639	22536639	region_S9_22286640	22036956	22040688	LOC_Os09g38310.1
chr9	22036639	22536639	region_S9_22286640	22041966	22045393	LOC_Os09g38320.1
chr9	22036639	22536639	region_S9_22286640	22048463	22057029	LOC_Os09g38330.1
chr9	22036639	22536639	region_S9_22286640	22064453	22070450	LOC_Os09g38340.2
chr9	22036639	22536639	region_S9_22286640	22064453	22070492	LOC_Os09g38340.3
chr9	22036639	22536639	region_S9_22286640	22064453	22070751	LOC_Os09g38340.1
chr9	22036639	22536639	region_S9_22286640	22082636	22084897	LOC_Os09g38350.1
chr9	22036639	22536639	region_S9_22286640	22090278	22091358	LOC_Os09g38360.1
chr9	22036639	22536639	region_S9_22286640	22092163	22095301	LOC_Os09g38370.1
chr9	22036639	22536639	region_S9_22286640	22096083	22097379	LOC_Os09g38380.1
PLANT HEIGHT						
chr4	13930606	14430606	region_S4_14180607	13934206	13936022	LOC_Os04g24310.1
chr4	13930606	14430606	region_S4_14180607	13936563	13937522	LOC_Os04g24319.1
chr4	13930606	14430606	region_S4_14180607	13937942	13939152	LOC_Os04g24328.1
chr4	13930606	14430606	region_S4_14180607	13949075	13950277	LOC_Os04g24340.1
chr4	13930606	14430606	region_S4_14180607	13952456	13956426	LOC_Os04g24350.1
chr4	13930606	14430606	region_S4_14180607	13957782	13958408	LOC_Os04g24360.1
chr4	13930606	14430606	region_S4_14180607	13972779	13980162	LOC_Os04g24370.1
chr4	13930606	14430606	region_S4_14180607	13981496	13982153	LOC_Os04g24380.1
chr4	13930606	14430606	region_S4_14180607	13984191	13986892	LOC_Os04g24390.1
chr4	13930606	14430606	region_S4_14180607	13987529	13988039	LOC_Os04g24400.1

CHLOROPHYLL CONTENT

chr5	24134191	24634191	region_S5_24384192	24132440	24134899	LOC_Os05g41210.1
chr5	24134191	24634191	region_S5_24384192	24137574	24141002	LOC_Os05g41220.1
chr5	24134191	24634191	region_S5_24384192	24137574	24141002	LOC_Os05g41220.2
chr5	24134191	24634191	region_S5_24384192	24149268	24151542	LOC_Os05g41230.1
chr5	24134191	24634191	region_S5_24384192	24153732	24158054	LOC_Os05g41240.1
chr5	24134191	24634191	region_S5_24384192	24153732	24158054	LOC_Os05g41240.3
chr5	24134191	24634191	region_S5_24384192	24153732	24158056	LOC_Os05g41240.2
chr5	24134191	24634191	region_S5_24384192	24171333	24171900	LOC_Os05g41250.1
chr5	24134191	24634191	region_S5_24384192	24175472	24178191	LOC_Os05g41270.1
chr5	24134191	24634191	region_S5_24384192	24180354	24182187	LOC_Os05g41280.1
chr8	1598091	1603744	region_S8_1603421	1597233	1599568	LOC_Os08g03410.1
chr8	1598091	1603744	region_S8_1603421	1600715	1603803	LOC_Os08g03420.3
chr8	1598091	1603744	region_S8_1603421	1600715	1604906	LOC_Os08g03420.1
chr8	1598091	1603744	region_S8_1603421	1600715	1604906	LOC_Os08g03420.2
chr8	1598091	1603744	region_S8_1603421	1600715	1604906	LOC_Os08g03420.4
chr8	1996007	2032836	region_S8_2008974	1994482	1998899	LOC_Os08g04110.1
chr8	1996007	2032836	region_S8_2008974	1994521	1998899	LOC_Os08g04110.2
chr8	1996007	2032836	region_S8_2008974	2000176	2005227	LOC_Os08g04120.1
chr8	1996007	2032836	region_S8_2008974	2007743	2011765	LOC_Os08g04130.1
chr8	1996007	2032836	region_S8_2008974	2007743	2011765	LOC_Os08g04130.2

Appendix 6.4: List of some extracted loci identified in field conditions

Leaf bronzing score

Chrom	region_start	region_end	region_name	gene_name
chr2	10965338	11407036	region_S2_11160651	LOC_Os02g18810.1
chr2	10965338	11407036	region_S2_11160651	LOC_Os02g18810.2
chr2	10965338	11407036	region_S2_11160651	LOC_Os02g18820.1
chr2	10965338	11407036	region_S2_11160651	LOC_Os02g18820.2
chr2	10965338	11407036	region_S2_11160651	LOC_Os02g18830.1
chr2	10965338	11407036	region_S2_11160651	LOC_Os02g18840.1
chr2	10965338	11407036	region_S2_11160651	LOC_Os02g18850.1
chr2	10965338	11407036	region_S2_11160651	LOC_Os02g18860.1
chr2	10965338	11407036	region_S2_11160651	LOC_Os02g18870.1
chr2	10965338	11407036	region_S2_11160651	LOC_Os02g18880.1
chr7	669121	1165133	region_S7_919040	LOC_Os07g02110.1
chr7	669121	1165133	region_S7_919040	LOC_Os07g02120.1
chr7	669121	1165133	region_S7_919040	LOC_Os07g02130.1
chr7	669121	1165133	region_S7_919040	LOC_Os07g02140.1
chr7	669121	1165133	region_S7_919040	LOC_Os07g02150.1
chr7	669121	1165133	region_S7_919040	LOC_Os07g02170.1
chr7	669121	1165133	region_S7_919040	LOC_Os07g02180.1
chr7	669121	1165133	region_S7_919040	LOC_Os07g02192.1
chr7	669121	1165133	region_S7_919040	LOC_Os07g02200.1
chr7	669121	1165133	region_S7_919040	LOC_Os07g02210.1
chr7	779288	1275670	region_S7_1027624	LOC_Os07g02330.1
chr7	779288	1275670	region_S7_1027624	LOC_Os07g02340.1
chr7	779288	1275670	region_S7_1027624	LOC_Os07g02350.1
chr7	779288	1275670	region_S7_1027624	LOC_Os07g02360.1
chr7	779288	1275670	region_S7_1027624	LOC_Os07g02370.1
chr7	779288	1275670	region_S7_1027624	LOC_Os07g02380.1
chr7	779288	1275670	region_S7_1027624	LOC_Os07g02390.1
chr7	779288	1275670	region_S7_1027624	LOC_Os07g02400.1
chr7	779288	1275670	region_S7_1027624	LOC_Os07g02410.1
chr7	779288	1275670	region_S7_1027624	LOC_Os07g02420.1
Harvest index				
chr7	1712646	2029545	region_S7_1781415	LOC_Os07g04040.1
chr7	1712646	2029545	region_S7_1781415	LOC_Os07g04050.1
chr7	1712646	2029545	region_S7_1781415	LOC_Os07g04060.1
chr7	1712646	2029545	region_S7_1781415	LOC_Os07g04070.1

chr7	1712646	2029545	region_S7_1781415	LOC_Os07g04080.1
chr7	1712646	2029545	region_S7_1781415	LOC_Os07g04090.1
chr7	1712646	2029545	region_S7_1781415	LOC_Os07g04100.1
chr7	1712646	2029545	region_S7_1781415	LOC_Os07g04110.1
chr7	1712646	2029545	region_S7_1781415	LOC_Os07g04120.1
chr7	1712646	2029545	region_S7_1781415	LOC_Os07g04130.1
chr7	2089563	2589563	region_S7_2339564	LOC_Os07g04700.1
chr7	2089563	2589563	region_S7_2339564	LOC_Os07g04700.2
chr7	2089563	2589563	region_S7_2339564	LOC_Os07g04700.3
chr7	2089563	2589563	region_S7_2339564	LOC_Os07g04700.5
chr7	2089563	2589563	region_S7_2339564	LOC_Os07g04700.6
chr7	2089563	2589563	region_S7_2339564	LOC_Os07g04720.1
chr7	2089563	2589563	region_S7_2339564	LOC_Os07g04730.1
chr7	2089563	2589563	region_S7_2339564	LOC_Os07g04740.1
chr7	2089563	2589563	region_S7_2339564	LOC_Os07g04750.1
chr7	2089563	2589563	region_S7_2339564	LOC_Os07g04770.1
chr7	2453148	2830403	region_S7_2600532	LOC_Os07g05360.1
chr7	2453148	2830403	region_S7_2600532	LOC_Os07g05360.2
chr7	2453148	2830403	region_S7_2600532	LOC_Os07g05365.1
chr7	2453148	2830403	region_S7_2600532	LOC_Os07g05370.1
chr7	2453148	2830403	region_S7_2600532	LOC_Os07g05380.1
chr7	2453148	2830403	region_S7_2600532	LOC_Os07g05390.1
chr7	2453148	2830403	region_S7_2600532	LOC_Os07g05400.1
chr7	2453148	2830403	region_S7_2600532	LOC_Os07g05410.1
chr7	2453148	2830403	region_S7_2600532	LOC_Os07g05420.1
chr7	2453148	2830403	region_S7_2600532	LOC_Os07g05430.1
chr7	809491	1290168	region_S7_1052475	LOC_Os07g02360.1
chr7	809491	1290168	region_S7_1052475	LOC_Os07g02370.1
chr7	809491	1290168	region_S7_1052475	LOC_Os07g02380.1
chr7	809491	1290168	region_S7_1052475	LOC_Os07g02390.1
chr7	809491	1290168	region_S7_1052475	LOC_Os07g02400.1
chr7	809491	1290168	region_S7_1052475	LOC_Os07g02410.1
chr7	809491	1290168	region_S7_1052475	LOC_Os07g02420.1
chr7	809491	1290168	region_S7_1052475	LOC_Os07g02430.1
chr7	809491	1290168	region_S7_1052475	LOC_Os07g02440.1
chr7	809491	1290168	region_S7_1052475	LOC_Os07g02450.1
chr9	18252469	18693442	region_S9_18449362	LOC_Os09g30000.1
chr9	18252469	18693442	region_S9_18449362	LOC_Os09g30010.1
chr9	18252469	18693442	region_S9_18449362	LOC_Os09g30020.1

chr9	18252469	18693442	region_S9_18449362	LOC_Os09g30030.1
chr9	18252469	18693442	region_S9_18449362	LOC_Os09g30040.1
chr9	18252469	18693442	region_S9_18449362	LOC_Os09g30050.1
chr9	18252469	18693442	region_S9_18449362	LOC_Os09g30060.1
chr9	18252469	18693442	region_S9_18449362	LOC_Os09g30070.1
chr9	18252469	18693442	region_S9_18449362	LOC_Os09g30090.1
chr9	18252469	18693442	region_S9_18449362	LOC_Os09g30100.1
chr9	18658404	19151185	region_S9_18904431	LOC_Os09g30980.1
chr9	18658404	19151185	region_S9_18904431	LOC_Os09g30990.1
chr9	18658404	19151185	region_S9_18904431	LOC_Os09g31000.1
chr9	18658404	19151185	region_S9_18904431	LOC_Os09g31019.1
chr9	18658404	19151185	region_S9_18904431	LOC_Os09g31025.1
chr9	18658404	19151185	region_S9_18904431	LOC_Os09g31031.1
chr9	18658404	19151185	region_S9_18904431	LOC_Os09g31031.2
chr9	18658404	19151185	region_S9_18904431	LOC_Os09g31031.3
chr9	18658404	19151185	region_S9_18904431	LOC_Os09g31040.1
chr9	18658404	19151185	region_S9_18904431	LOC_Os09g31050.1

Chlorophyll Content

chr8	5254789	5328854	region_S8_5288795	LOC_Os08g09040.1
chr8	5254789	5328854	region_S8_5288795	LOC_Os08g09050.1
chr8	5254789	5328854	region_S8_5288795	LOC_Os08g09060.1
chr8	5254789	5328854	region_S8_5288795	LOC_Os08g09080.1
chr8	5254789	5328854	region_S8_5288795	LOC_Os08g09080.2
chr8	5254789	5328854	region_S8_5288795	LOC_Os08g09090.1
chr8	5254789	5328854	region_S8_5288795	LOC_Os08g09100.1
chr8	5254789	5328854	region_S8_5288795	LOC_Os08g09110.1
chr8	5254789	5328854	region_S8_5288795	LOC_Os08g09120.1
chr8	5254789	5328854	region_S8_5288795	LOC_Os08g09130.1